33rd
WORLD VETERINARY CONGRESS
2017 인천 세계수의사대회
Incheon Songdo Convensia, KOREA
August 27(SUN) – 31(THU), 2017
“One Health, New Wave”

PROCEEDINGS
33rd WORLD VETERINARY CONGRESS
2017 인천 세계수의사대회
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“One Health, New Wave”

PROCEEDINGS
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Dear Friends and Colleagues,

It is our great pleasure and honor to introduce the proceedings of the 33rd World Veterinary Congress which will be held in Incheon, Korea, from August 27 to 31, 2017. A number of distinguished veterinarians and valuable participants have contributed to making the Congress successful. Under the theme “One Health, New Wave,” we endeavor to promote and raise awareness of One Health, a concept which recognizes that the health of humans is connected to that of animals and provides an unprecedented platform reflecting the latest trends.

The scientific programs of the 33rd World Veterinary Congress are composed of Plenary Lectures, Invited Lectures, and E-Poster Sessions. Various and detailed invited lectures feature insightful sessions categorized into five sub-specialties (Companion Animal, Farm Animal, Large Animal, Public Health / Welfare / Ecology and Education) and 24 streams. A total of 255 lectures will be presented to the participants by 95 distinguished experts. They will share their expertise on diverse topics including diagnosis and treatment of veterinary diseases of all animals, prevention of epidemics, veterinary education, and others. Also, a total of 307 E-Posters covering the most current issues and topics in the veterinary field will be displayed on monitors during the Congress. The lecture notes of invited lectures and abstracts for E-Posters are available in the proceedings. An integrated author index is expected to allow an easy reference to all of the lecture notes and E-Posters.

We would like to take advantage of this opportunity to pay our profound gratitude to all invited speakers and E-Poster presenters. It is our pleasant duty to acknowledge Scientific and Publication committee members as well as many colleagues and friends for their contributions to preparing the Congress. They have all joined us with continued interest and participation in the success of the Scientific Program and this substantial Proceeding of the 33rd World Veterinary Congress.

We believe that 33rd World Veterinary Congress will make a significant contribution to the advancement of scientific knowledge and technological development of the veterinary field.

Sincerely,

Jae-Hong KIM  
Chair of Organizing Committee  
WVC Incheon, KOREA 2017  
Professor, Seoul National University

Kangmoon SEO  
Chair of Scientific and Publication Committee  
WVC Incheon, KOREA 2017  
Professor, Seoul National University
CONGRESS OVERVIEW

Title: 33rd World Veterinary Congress (WVC Incheon, KOREA 2017)
Period: August 27(Sun) ~ 31(Thu), 2017
Venue: Incheon Songdo ConvensiA, KOREA
Official Language: English
Hosted by: Korean Veterinary Medical Association (KVMA)
World Veterinary Association (WVA)
Organized by: 33rd World Veterinary Congress Organizing Committee
Sponsored by: Ministry of Agriculture, Food and Rural Affairs
Incheon Metropolitan City
Website: www.wvc2017korea.com
Facebook: www.facebook.com/2017.incheon.wvc

Theme: “One Health, New Wave”

The 33rd World Veterinary Congress is aimed at promoting and raising awareness about One Health, an emerging concept that encompasses the health of humans, animals and their environment, across the globe as well as sharing New Wave of the New Technologies in all disciplines of clinical veterinary medicine and creating a new trend in the field.
WVC INCHEON, KOREA 2017
ORGANIZING COMMITTEE

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René A. CARLSON, President of WVA
Johnson CHIANG, President Elect of WVA
Kechrid FAOUZI, Immediate Past President of WVA

Local Advisory Boards
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Do-Kyung RA
Hye Jin WEE
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Jong Young Kim
Sejoon AHN
SeungWon SUH
Tae Ho HAN

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Dongchul ROH
Geun Seog SONG
Hwa Soon KANG
In-Joong YOON
Jong Ho KIM
Jung Hyang SUR
Seol Ryung JEONG
SungHwan WEE
Yoyun LEE

Doo KIM
Hong Sik MOON
Hyun NAMKOONG
Jaehoon SOHN
Jong Young KIM
Sangmin LEE
Seong Cheol MOON
Sung Phil KIM

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Heeyoung JANG
Kang-Wook CHO
Seung You KANG
YunKuok WANG

Kyu Seok LEE
Eun-Jung AHN
Jai-Gyu LEE
Kyung Mi KIM
Tae Ho HAN

CONGRESS EMBLEM

Five colors representing five continents are used to welcome veterinarians from around the world. Superimposing silhouettes of humans with animals and some oriental pattern of clouds symbolizing the environment embody the Congress theme of One Health that the health of humans is connected to the health of animals and the environment.
01 INVITED LECTURES

Daily Scientific Program
Lecture Notes
# DAILY SCIENTIFIC PROGRAM

**Day 1 - August 28 (Mon)**

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<tr>
<td>Stream</td>
<td>Surgery: Aquatic Animal Medicine</td>
<td>Porcine Medicine</td>
<td>Ophthalmology</td>
<td>One Health</td>
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**AM**

- **Opening Ceremony** (10:00 – 10:15, Premier Ballroom A/C)
- **Plenary Lecture** (10:20 – 11:00, Premier Ballroom A/B) *Kenzo BAN*
- **Exhibition Opening Ceremony** (11:45 – 12:10, Exhibition Hall)

**Lunch / Exhibition / Poster Visit (12:10 – 13:45)**

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<td>Kei HAYASHI</td>
<td>Harmish RODGER</td>
<td>Kyunggun J. YOON</td>
<td>Gillian MCELLEN</td>
<td>Alan A. MONAVARI</td>
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<tr>
<td>Diagnosis of Early Canine Cranial Ligament Disease</td>
<td>Applications and the Use of Molecular Diagnostics in Aquaculture and Aquatic Animal Medicine</td>
<td>Small Animal Coronavirus (PEDV, PDCV, TGEV) – Pathogenesis, Impact, Prevention and Control</td>
<td>Ophthalmic Imaging: Seeing the Unseen</td>
<td>Zoology: The Health Benefits of Companion Animals and an Essential Contributor to One Health in the Community</td>
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**PM**

- **Coffee Break / Poster Visit (15:25 – 16:20)**

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<td>Kei HAYASHI</td>
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<td>Kyunggun J. YOON</td>
<td>David WILKIE</td>
<td>Alan A. MONAVARI</td>
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<tr>
<td>Patellar Laxation in Young Dogs</td>
<td>Orthopedic Surgery: 10 Key Aspects for Successful Health Management</td>
<td>Oral Fluid Sampling and Testing – A Paradigm Shifting for Disease Monitoring in Pig Populations</td>
<td>The Future of Phacoemulsiﬁcation and Cataract Surgery</td>
<td>Interprofessional Collaboration to Mitigate the Four Categories of Zoonotic Risk in Families and the Community</td>
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<td>David WILKIE</td>
<td>Alan A. MONAVARI</td>
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<tr>
<th>SU-04 (17:15 – 18:00) (Orthopedic Surgery)</th>
<th>AA-04 (17:15 – 18:00)</th>
<th>PM-04 (17:15 – 18:00)</th>
<th>OP-04 (17:15 – 18:00)</th>
<th>OH-04 (17:15 – 18:00)</th>
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<tbody>
<tr>
<td>Han-Roong LEE</td>
<td>Harmish RODGER</td>
<td>Joaquim SEGALÉS</td>
<td>Gillian MCELLEN</td>
<td>Alan A. MONAVARI</td>
</tr>
<tr>
<td>Treatment for Complications of Medial Patellar Laxation</td>
<td>Emerging Gill Health Challenges in Finfish Aquaculture: Causes, Effects and Control</td>
<td>Emerging Infectious Diseases of Pigs: What’s Next?</td>
<td>Tools and Techniques in Aquaculture from Clinical Signs to Treatment</td>
<td>Opportunities and Activities for One Health in Veterinary Care in the Community</td>
</tr>
</tbody>
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**Welcome Reception** (19:00 – 21:00, Premier Ballroom ABC) *Reception: 18:30*
## INVITED LECTURES

*The Daily Scientific Program is subject to change without prior notice.*

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<thead>
<tr>
<th>Time</th>
<th>Speaker(s)</th>
<th>Title</th>
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<tbody>
<tr>
<td>10:00 – 10:30</td>
<td>Tobias Schwarz</td>
<td>Abdominal and Thoracic CT Angiography in the Dog and Cat</td>
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<tr>
<td>11:30 – 12:00</td>
<td>Paolo Marcelli</td>
<td>Introduction to Zoological Medicine</td>
</tr>
<tr>
<td>12:00 – 12:30</td>
<td>Philippe Hennet</td>
<td>Piezoelectric Bone Cutting Devices in Oropharyngolaryngological Surgery: Advantages and Clinical Use</td>
</tr>
<tr>
<td>13:30 – 14:00</td>
<td>Kerstin Elisabeth Müller</td>
<td>Abdominal Disorders in Dogs: Diagnosis and Treatment</td>
</tr>
<tr>
<td>14:30 – 15:00</td>
<td>Christopher Riggs</td>
<td>Common Fractures in Thoroughbred Racehorses: Prevention, Diagnosis and Treatment</td>
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<td></td>
<td>Kent Lloyd</td>
<td>Mouse Modeling to Inform Precision Medicine</td>
</tr>
<tr>
<td>15:00 – 15:30</td>
<td>Silke Hecht</td>
<td>Imaging of Canine and Feline Cytology</td>
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<tr>
<td>16:00 – 16:30</td>
<td>Paolo Marcelli</td>
<td>Cytocan Medicine with Emphasis on the Importance of Operant Conditioning</td>
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<tr>
<td>16:30 – 17:00</td>
<td>Philippe Hennet</td>
<td>Periodontal Treatment: How can Ultrasonic Devices Make Treatment Easier</td>
</tr>
<tr>
<td>17:00 – 17:30</td>
<td>Kerstin Elisabeth Müller</td>
<td>Hypoalbuminemia in Dairy Cows: Importance, Biopathogenesis and Treatment</td>
</tr>
<tr>
<td>17:30 – 18:00</td>
<td>Christopher Riggs</td>
<td>Guarding the Welfare of the Thoroughbred Racehorse</td>
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<tr>
<td>18:00 – 18:30</td>
<td>Colin McKirle</td>
<td>Extending Availability and Enhancing Accessibility of Mouse Resources Worldwide</td>
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<tr>
<td>19:00 – 21:00</td>
<td>Colin Fletcher</td>
<td>Large-Scale Pathology Phenotyping</td>
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### Coffee Break / Poster Visit (15:30 – 16:30)

### Welcome Reception (19:00 – 21:00, Premier Ballroom ABC)
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<tr>
<td><strong>Streams</strong></td>
<td><strong>Diagnostic Imaging</strong></td>
<td><strong>Internal Medicine</strong></td>
<td><strong>Surgery</strong></td>
<td><strong>Repro</strong></td>
<td><strong>Oncology</strong></td>
<td><strong>Ophthalmology</strong></td>
<td><strong>One Health</strong></td>
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<tr>
<td><strong>DI 05 08:30 – 09:15</strong></td>
<td><strong>Tobias SCHWARZ</strong></td>
<td><strong>Jörgen STEINER</strong></td>
<td><strong>Jörgen STEINER</strong></td>
<td><strong>SU 05</strong></td>
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<td><strong>Orthopaedics</strong></td>
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<td><strong>Vit &amp; Rep</strong></td>
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<td><strong>Day 06 09:25 – 10:10</strong></td>
<td><strong>Tobias SCHWARZ</strong></td>
<td><strong>Jörgen STEINER</strong></td>
<td><strong>Jörgen STEINER</strong></td>
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<td><strong>AM</strong></td>
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<td><strong>DI 07 10:30 – 11:15</strong></td>
<td><strong>Silke HECHT</strong></td>
<td><strong>MRD of Inflammatory</strong></td>
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<td><strong>Brain Diseases</strong></td>
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<td><strong>DI 08 11:25 – 12:10</strong></td>
<td><strong>Silke HECHT</strong></td>
<td><strong>MRD of Congenital</strong></td>
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<td><strong>DI 09 13:45 – 14:30</strong></td>
<td><strong>Tobias SCHWARZ</strong></td>
<td><strong>Oncology</strong></td>
<td><strong>Oncology</strong></td>
<td><strong>SU 09</strong></td>
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<td><strong>Diagnosis &amp; Treatment</strong></td>
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<td><strong>DI 10 14:40 – 15:25</strong></td>
<td><strong>Tobias SCHWARZ</strong></td>
<td><strong>Oncology</strong></td>
<td><strong>Oncology</strong></td>
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<td><strong>DI 11 16:20 – 17:05</strong></td>
<td><strong>Silke HECHT</strong></td>
<td><strong>MRI of the Spine</strong></td>
<td><strong>MRI of the Spine</strong></td>
<td><strong>SU 11</strong></td>
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<td><strong>Diagnosis &amp; Treatment</strong></td>
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<td><strong>DI 12 17:15 – 18:00</strong></td>
<td><strong>Silke HECHT</strong></td>
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*The Daily Scientific Program is subject to change without prior notice.*
## Day 3 - August 30 (Wed)

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<td><strong>WVA Global Conference on Quality Assurance in Veterinary Education</strong></td>
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<td><strong>(09:00 – 09:20)</strong></td>
<td>Remi CARLSON, Pan Dong Ryo</td>
<td>Welcome and Opening</td>
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<td><strong>(09:20 – 10:00)</strong></td>
<td>Andrew T. MACABE</td>
<td>Introduction of the Topic: The Importance of a Reliable Quality Assurance in Veterinary Education</td>
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<td><strong>(10:30 – 11:30)</strong></td>
<td>Chin Cheng CHOI, Patricia TURNER, Nourouddeillé TSLOU</td>
<td>Self-Assessment of VEEs: Experiences with Self-Assessment of Establishments for Veterinary Education</td>
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<td><strong>(11:30 – 12:30)</strong></td>
<td>Aaron WIGNE, Pierre LEKEUX</td>
<td>Accreditation of VEEs: Experiences with Accreditation of Establishments for Veterinary Education</td>
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<td><strong>(13:30 – 14:00)</strong></td>
<td>Pierre-Marie BORNE</td>
<td>Private and Academic Sectors: A Potential Synergistic Combination for Veterinary Education</td>
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<td><strong>(14:00 – 14:45)</strong></td>
<td>Norman WILLIAMSON</td>
<td>GAP: Analyses What is Missing?</td>
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<td><strong>(14:45 – 15:45)</strong></td>
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<td><strong>(16:15 – 16:45)</strong></td>
<td>Remi CARLSON</td>
<td>Next Steps</td>
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<td><strong>(16:45 – 17:15)</strong></td>
<td>Remi CARLSON</td>
<td>Conclusions, Recommendations</td>
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<td><strong>(17:15 – 18:00)</strong></td>
<td>Remi CARLSON</td>
<td>Gala Dinner (19:30 – 21:30, Paradise City Hotel) / Reception: 19:00</td>
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### Schedule Overview
- **09:00 – 09:20**: Welcome and Opening by Remi CARLSON and Pan Dong Ryo.
- **09:20 – 10:00**: Introduction by Andrew T. MACABE on the importance of reliable quality assurance in veterinary education.
- **10:30 – 11:30**: Presentation by Chin Cheng CHOI, Patricia TURNER, and Nourouddeillé TSLOU on self-assessment of VEEs.
- **11:30 – 12:30**: Presentation by Aaron WIGNE and Pierre LEKEUX on accreditation of VEEs.
- **13:30 – 14:00**: Discussion by Pierre-Marie BORNE on the potential synergistic combination for veterinary education.
- **14:00 – 14:45**: GAP analysis by Norman WILLIAMSON.
- **14:45 – 15:45**: Panel discussion.
- **16:15 – 16:45**: Next steps presented by Remi CARLSON.
- **16:45 – 17:15**: Conclusions and recommendations by Remi CARLSON.

Gala Dinner and Reception conclude the evening activities.
### INVITED LECTURES

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<td>Douglas DEBOER</td>
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<td>Taming Canine Atopic Dermatitis: Allergen-Specific Immunotherapy &amp; Sublingual Immunotherapy in Theory and Practice</td>
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<td>Mark MCKWOOD</td>
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<td>Andrea FASCIETI</td>
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<td>Geraldine BLANCHARD</td>
<td>NU-07 (16:20 ~ 17:05)</td>
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<td>For Canine Patients: Nutritional Management and Homemade Recipe</td>
<td>Shinshu KANAZONO</td>
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<td>Intestinal Pressure Elevation: How Dangerous? What to do?</td>
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<td>17:15 ~ 18:00</td>
<td>Geraldine BLANCHARD</td>
<td>NU-08 (17:15 ~ 18:00)</td>
<td>Brainstorming</td>
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The Pharmacology of the Different Anesthetics
(Inhalant and Injectable)

Kirby PASLOSKE

Jurox Inc., Research & Development, Canada

Inhalant Anesthetics (not scheduled)
Modern anesthesia is said to have occurred on October 16th, 1846 in public display at Massachusetts General Hospital using diethyl ether. Today molecular modeling based on structurally homologous proteins has been used to identify putative anesthetic binding sites for inhalant anesthetics in GABAA and glycine receptors1.

GABAergic Injectable Anesthetics
The majority of the anesthetic injectable drugs work by interacting at the GABAA receptor in the CNS. Figure 1 (Belelli D2) shows the GABAA receptor and the ligands that interact with it.

Barbiturates (schedule III)
The first injectable anesthetic to be used in a clinical setting was the thiobarbiturate sodium thiopental (Pentothal® Sodium, Abbott, USA) in 19342. Dr. John Lundy (Mayo Clinic) was the first physician to publish research on sodium thiopental and is credited with its rapid integration into anesthesia practice. The drug was also approved for use in veterinary species and has a long legacy in veterinary medicine.

Alfaxalone (schedule IV)
Alfaxalone, a neurosteroid, was first registered in the formulation Saffan® for cats and primates in the early 1970s by Glaxo UK. To remove the side effects (histamine release) observed with drug carriers such as Cremophor® EL, groups like Brewster et al found that alfaxalone and other drugs could be solubilized in safe carriers like 2-hydroxypropyl-β-cyclodextrin (2-HPCD)3,4. Since 2000, alfaxalone in 2-HPCD (Alfaxon®) has been registered as an intravenous anesthetic in dogs and cats.

Etomidate (not scheduled)
Etomidate, a carboxylated imidazole, was developed by Janssen Pharmaceutica and first discussed in the literature in 19655. It was registered for human use and first introduced into clinical practice in 1972. There is currently no registration of etomidate for anesthesia in any veterinary species. The current product Amidate® (Hospira Inc.) contains 2 mg mL⁻¹ of etomidate in 35% v/v propylene glycol.

Propofol (not scheduled)
Propofol, a phenol derivative, was discovered at Imperial Chemical Industries (ICI) Pharmaceuticals and launched as Diprivan® (by ICI now Astrazeneca) in 1986. The currently available emulsion formulation with label claims for dog ± cat contains 10 mg mL⁻¹ propofol ± benzyl alcohol as a preservative.

N-methyl-D-aspartate (NMDA) Injectable Anesthetics
Figure 2 (Li Jih-Heng8) shows the NMDA protein receptor (a glutamate receptor). The injectable anesthetics that interact and antagonize the NMDA receptor and produce a cataleptic type state are:
**Ketamine (schedule III)**
Ketamine was first discovered in 1962 by Parke Davis. In 1970 the drug was approved by the FDA for use in humans and in the cat and subhuman primate through IM injection. Pioneer ketamine HCl (Ketaset®; Zoetis), pH 3.5-4.5, contains a preservative making it a multi-dose vial after broaching with a 4 month shelf life.

**Tiletamine (schedule III)**
Tiletamine, another phencyclidine congener, in combination with the benzodiazepine zolazepam HCl (Telazol®; Zoetis) was first approved by the FDA in 1982 for anesthetic use in cats and dogs. The drug combination is reconstituted in 5 mL sterile water resulting in 50 mg mL\(^{-1}\) of both actives in an aqueous solution (pH 2-3.5). Shelf life is 4 days at room temperature and 14 days when refrigerated.

---

**Figure 1** GABA\(_A\) receptor and known ligands

**Figure 2** The NMDA receptor and known ligands
References
(9) Ketaset (Zoetis) NADA 043-304 drug insert (USA).
(10) Tiletamine-Zolazepam (Putney) ANADA 200-557 drug insert (USA).
The Pharmacology of the Sedatives and Analgesics Used Before, During and After Anesthesia

Kirby PASLOSKE
Jurox Inc., Research & Development, Canada

Sedatives

Phenothiazines (not scheduled)
This drug class was first used in people in the 1950s as an anti-psychotic where sedation was required (e.g. chlorpromazine; Thorazine®); however, a small population of patients would seizures at higher dose rates. The principle mechanism of action is the blockade of excitatory dopaminergic receptors (D2). This leads to decreased motor activity and sedation. There is no good evidence to show that acepromazine decreases seizure threshold in dogs. With respect to Boxer dogs there is a UK lineage that may be more susceptible to the effects of acepromazine. Breeds heterozygous for the multi drug resistance P-glycoprotein transporter protein in the CNS are more susceptible to the effects of acepromazine and include mostly herding and sighthound breeds. Acepromazine is approved as a sedative by the FDA for use in dogs, cats and horses.

Benzodiazepines (scheduled IV)
In 1955 Hoffman-La Roche identified the first benzodiazepine (BZ), chlordiazepoxide (Librium®). A more potent derivative, diazepam, followed in 1963. The mechanism of action is through allosteric modulation of the GABA$_A$ receptor coupled with binding of the natural inhibitory peptide gamma aminobutyric acid (GABA). CNS effects include antianxiety, anticonvulsant, sedation and centrally-mediated muscle relaxation. The two most common BZs administered by injection in veterinary medicine are diazepam and midazolam; however, neither drug has been approved for use in veterinary species by the FDA and can be harmful. Indeed long term administration of diazepam is contraindicated in cats because of slow biotransformation and toxicity to the liver.

$\alpha$-2 adrenergic receptor agonists (not scheduled)
The $\alpha$-2 adrenergic receptors are pre-junctional inhibitory receptors within the sympathetic nervous system and are found in multiple tissue types. The first of the drugs developed for this receptor subtype was xylazine. Xylazine was first developed as an antihypertensive agent by Bayer AG in 1962; however, the FDA did not approve it for people because of severe depressant effects on the CNS. Xylazine was developed and approved for veterinary use as a sedative, analgesic and muscle relaxant in dogs, cats, horses, deer and elk. More potent and selective $\alpha$-2 drugs have been developed and registered with the FDA including medetomidine (Domitor®), an equal mixture of levo- and dex- medetomidine enantiomers and the dexametomidine, dexmedetomidine (Dexdomitor®).

Opioid Analgesics (schedule II to IV)
The opium poppy, *Papaver somniferum*, is the source of the opium alkaloids including morphine. Morphine is the prototype opioid to which all other opioids are compared. In 1973 Pert and Snyder published the breakthrough research demonstrating opium receptors in the brain. Opioids can be categorized into sub groups based on their affinity for different CNS receptors: namely $\mu$ (mu), $\kappa$ (kappa) or $\delta$ (sigma). Table
1 compares and contrasts the opioids available in the USA.

**Anticholinergics (not scheduled)**

The atropine alkaloid interferes with muscarinic, cholinergic transmission of the parasympathetic nervous system. The synthetic quaternary nitrogen compound glycopyrrolate has similar pharmacological effects as atropine although it does not appear to penetrate the blood brain barrier and produce CNS effects (e.g. sedation, amnesia) at clinical dose rates.

**Table 1** Opioids used in veterinary medicine (note potency is an approximation)

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Potency (mg kg(^{-1})) versus morphine</th>
<th>Receptor binding agonist/antagonist</th>
<th>CVM drug approval</th>
<th>FDA drug approval</th>
<th>CVM drug approval example</th>
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<tr>
<td>morphine</td>
<td>1</td>
<td>μ agonist</td>
<td>No</td>
<td>Yes</td>
<td>none</td>
</tr>
<tr>
<td>methadone</td>
<td>1</td>
<td>μ agonist</td>
<td>No</td>
<td>Yes</td>
<td>none</td>
</tr>
<tr>
<td>buprenorphine</td>
<td>3-5</td>
<td>partial μ agonist, high affinity, low activity</td>
<td>Yes</td>
<td>Yes</td>
<td>Simbadol® (cats, SC)</td>
</tr>
<tr>
<td>butorphanol</td>
<td>4-7</td>
<td>μ antagonist/κ agonist</td>
<td>Yes</td>
<td>Yes</td>
<td>Torbugesic® (dogs, cats, horses; IV, SC, PO)</td>
</tr>
<tr>
<td>oxymorphone</td>
<td>5-10</td>
<td>μ agonist</td>
<td>Yes</td>
<td>Yes</td>
<td>Numorphan® (dogs and cats; IV, SC, IM)</td>
</tr>
<tr>
<td>hydromorphone</td>
<td>5-10</td>
<td>μ agonist</td>
<td>No</td>
<td>Yes</td>
<td>none</td>
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<tr>
<td>fentanyl</td>
<td>100</td>
<td>μ agonist</td>
<td>Yes</td>
<td>Yes</td>
<td>Recuvyra® (dogs, topical)</td>
</tr>
<tr>
<td>remifentanil</td>
<td>100</td>
<td>μ agonist</td>
<td>No</td>
<td>Yes</td>
<td>none</td>
</tr>
<tr>
<td>sufentanil</td>
<td>500-1000</td>
<td>μ agonist</td>
<td>No</td>
<td>Yes</td>
<td>none</td>
</tr>
<tr>
<td>carfentanil</td>
<td>10,000</td>
<td>μ agonist</td>
<td>Yes</td>
<td>No</td>
<td>Wildnil® (deer, elk, moose)</td>
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**Keywords:** Pre-Anesthetics, Sedation, Analgesia

**References**

Perioperative Pain Control in Dogs and Cats

Kazuto YAMASHITA

Department of Small Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Japan

Summary
The ability to experience pain is universally shared by all mammals, including companion animals, and as members of the veterinary healthcare team it is our moral and ethical duty to mitigate this suffering to the best of our ability. However, despite advances in the recognition and treatment of pain, there remains a gap between its occurrence and its successful management. The inability to accurately diagnose pain and limitations in the available analgesic modalities remain root causes. Based on this background, the World Small Animal Veterinary Association-Global Pain Council (WSAVA-GPC) was established and charged with the task of developing pain assessment and treatment guidelines having universal relevance, taking into account regional differences in attitude, education and available analgesic modalities [1]. In this lecture, an outline of perioperative pain control based on the WSAVA-GPC guidelines will be introduced.

Physiology and pathophysiology of pain
Pain is a subjective emotion that can be enhanced or abolished by a wide range of behavior including fear and memory. Adaptive ‘physiological’ pain announces the presence of a potentially harmful stimulus and has an essential protective function. Maladaptive pain represents malfunction of neurological transmission leading to chronic syndromes in which pain itself may become the primary disease. Conscious perception of pain represents the final product of a complex neurological information-processing system, resulting from the interplay of facilitatory and inhibitory pathways throughout the periphery and central nervous systems.

Pain pathway: The conscious experience of acute pain resulting from a noxious stimulus is mediated by a high-threshold nociceptive sensory system. Nociceptors represent the free endings of primary sensory neurons that their cell bodies located in the dorsal root. The primary afferent nerve fibers consist of unmyelinated C-fibers and myelinated Aδ-fibers, and carry information from the free nerve endings to their central location. The Aδ-fibers conduct impulses more quickly and contribute to the rapid ‘stab’ of the acute pain response and function primarily as a warning (protective) resulting in rapid withdrawal from the stimulus. Delay of withdrawal results in C-fiber activation, the intensity of which is dependent on injury. There is also a population of so-called ‘silent’ nociceptors, which may become active during inflammation or tissue damage. Primary afferent fibers carrying sensory information from nociceptors synapse in the spinal dorsal horn of the spinal cord. The fibers of ‘nociceptive’ responsive cells of the spinal cord are projected to their original higher centers. Several spinal-brainstem-spinal pathways are activated simultaneously when a noxious stimulus occurs, providing widespread positive and negative feedback loops by which information relating to noxious stimulation can be amplified or diminished (descending inhibitory pathways). The cerebral cortex is the seat of conscious experience of pain and exerts top-down control to modulate the sensation of pain.

Peripheral and central sensitization: The nociceptive sensory system is an inherently plastic system. When tissue injury or inflammation occurs, the sensitivity of an injured region is enhanced so that both noxious and normally innocuous stimuli are perceived as painful. Hyperalgesia and allodynia are a consequence of peripheral
and central sensitization. Hyperalgesia is an exaggerated and prolonged response to a noxious stimulus, while allodynia is a pain response to a low-intensity normally innocuous stimulus (e.g. light touch to the skin or gentle pressure). Peripheral sensitization is the result of changes in the environment bathing nociceptor terminals as a result of tissue injury or inflammation. Chemical mediators released by damaged cells directly activate nociceptors or sensitize the nerve terminals. This results in long-lasting changes in the functional properties of peripheral nociceptors. Trauma and inflammation can also sensitize nociceptor transmission in the spinal cord to produce central sensitization. This requires a brief but intense period of nociceptor stimulation such as surgical procedure. As a result, the response threshold of the central neurons falls, their responses to subsequent stimulation are amplified and their receptive fields enlarge to recruit additional previously ‘sleeping’ afferent fibers into nociceptive transmission. Inflammatory pain is usually responsible for acute postoperative pain, until the wound has healed. It has a rapid onset and, in general, its intensity and duration are related directly to the severity and duration of tissue damage.

**Recognition and assessment of postoperative pain**

The most important step in managing postoperative pain is to actively assess the animals for signs of pain on a regular basis, and use the outcomes of these assessments (through observation and interaction) along with knowledge of the disease/surgical status and history of the animal to make a judgment on the pain state. The postoperative pain can be presented from very mild to very severe and expected to be from a few hours to several days. Postoperative pain is generally well managed by the use of analgesic drugs. The effective management of pain relies on the ability of the veterinarian, animal health technician and veterinary nurse to recognize pain, and assess and measure it in a reliable manner. When the animal is discharged home, owners should be given guidance on signs of pain and how to treat it. Objective measurements including heart rate, arterial blood pressure and plasma cortisol and catecholamine levels have been associated with acute pain in animals. However, they are unreliable as stress, fear and anesthetic drugs affect them. Thus, evaluation of pain in animals is primarily subjective and based on behavioral signs.

**Pain recognition in cats:** The cat should be observed from a distance then, if possible, the caregiver should interact with the cat and palpate the painful area to fully assess pain. A good knowledge of the cat’s normal behavior is very helpful as changes in behavior (e.g. absence of normal behaviors such as grooming and climbing into the litter box) and presence of new behaviors (e.g. a friendly cat becoming aggressive, hiding or trying to escape) may provide helpful clues. Some cats may not display clear overt behavior indicative of pain, especially in the presence of human beings, other animals or in stressful situations. Cats should not be awakened to check their pain status. Rest and sleep are good signs of comfort but one should ensure the cat is resting or sleeping in a normal posture (relaxed, curled up). Some cats may feign sleep when stressed or may remain very still because they are afraid or it is too painful to move. Facial expressions and postures can be altered in cats experiencing pain. Furrowed brow, orbital squeezing (squinted eyes) and a hanging head (head down) can be indicators of pain. Following abdominal surgery a hunched position or a tense abdomen is indicative of pain. Abnormal gait or shifting of weight and sitting or lying in abnormal positions may reflect discomfort and protection of injured area. Comfortable cats should display normal facial expressions, postures and movement after successful analgesic therapy. Behavioral changes such as reduced activity, loss of appetite, quietness, hiding, hissing and growling, excessive licking of a specific area of the body involving surgical wounds, guarding behavior, cessation of grooming, tail flicking and aggression may be associated with acute pain in cats. Cats in severe pain are usually depressed, immobile and silent. They will appear tense and distant from their environment. Thrashing, restlessness and continuous activity can be signs of severe pain in cats. However, these can also be related to dysphoria. Dysphoria is associated with poor anesthetic recovery after inhalant anesthesia, ketamine administration or high doses of opioids. Hyperthermia associated with administration of opioids may lead to anxiety and signs of agitation in cats.

**Pain recognition in dogs:** Behavioral expression of pain is species-specific and is influenced by age, breed, individual temperament and the presence of additional stressors such as anxiety or fear. Debilitating disease
can dramatically reduce the range of behavioral indicators of pain that the dog would normally show (e.g. dogs may not vocalize and may be reluctant to move to prevent worsening pain). Therefore, when assessing a dog for pain a range of factors should be considered, including the type, anatomical location and duration of surgery, the medical problem, or extent of injury. It is helpful to know the dog’s normal behavior. However, this is not always practical and strangers, other dogs, and analgesic and sedatives may inhibit the animal's normal behaviors. Behavioral signs of pain in dogs include change in posture or body position, change in demeanor, vocalization, altered reaction to touch, altered interaction with people (e.g. reduced interaction, aggression), altered mobility (e.g. lameness, reluctance to move), and reduction in appetite.

**Pain measurement tools:** In dogs and cats, few of fully validated pain scales are available such as simple uni-dimensional scales (e.g. the Numerical Rating Scale, the Visual Analogue Scale), the Simple Descriptive Scale, composite scales (e.g. the Glasgow Composite Measure Pain Scale and its short form, the French Association for Animal Anesthesia and Analgesia pain scoring system), scales combined the numerical rating scale with composite behavioral observation (e.g. the Colorado State University acute pain scale for the dog and for the cat, Japanese Society of Study for Animal Pain Canine Acute Pain Scale), and a scale combined physiologic data with behavioral responses(e.g. the University of Melbourne Pain Scale).

**Assessing response to pain treatment:** Assessing the response to pain treatment strategies is a fundamental aspect of effective perioperative pain management. Too often dogs and cats are given one-off doses of analgesic drugs without effective follow-up. Dogs and cats should be assessed on a regular basis following surgery, in the early recovery period every 15-30 min (depending on the surgical procedure) and on a hourly basis thereafter for the first 6-8 hours after surgery. Thereafter, if pain is well controlled, 3-6 hourly assessments are recommended. The exact time interval depends on the severity of the surgery, the type of drugs used to manage pain and other factors relating to the animal's physical status. If in doubt about pain status, the animal is reassessed in 15 min. When an animal is judged to be in pain, treatment should be given immediately to provide relief.

**Perioperative pain management**
Perioperative pain extends beyond 24 hr and should be managed accordingly. There are four key time-points when the choice of analgesic strategy will influence a patient’s postoperative pain status. These are the preoperative, intraoperative, immediate postoperative ('in hospital'), and later postoperative ('at home') periods. The most important periods are preoperative and intraoperative periods. Postoperative pain can be prevented or reduced via the concept of preventive and multimodal analgesia. Postoperative pain treatment should continue until the inflammatory response is minimal.

**Preventive and multimodal analgesia:** Decades of research into pain management indicate that pain is best managed early and aggressively while it is hard to combat pain once it is well established. In current veterinary practice, administration of multiple analgesics in combination with acting through different mechanisms, “multimodal analgesia”, is often advocated to maximize analgesic effect. The multimodal approach for analgesia also provides a great benefit of concomitant reduction of adverse effects with additive or synergistic analgesic effect produced by lower doses of each analgesic. In addition, an administration of analgesics before the patient receives painful stimuli, “preventive analgesia”, is advocated to reduce the requirement of anesthetics during surgery and to minimize post-operative pain in animals. In surgical patients, the preventive and multimodal approach for analgesia is successfully achieved by the premedication using multiple analgesics, such as a combination of a non-steroidal anti-inflammatory drug (NSAID) and an opioid. An effective perioperative pain management regimen would normally incorporate several different classes of such as opioids (e.g. morphine, fentanyl, remifentanil, butorphanol, buprenorphine, tramadol), NSAIDs (e.g. carprofen, meloxicam, robenacoxib), α2-adrenoceptor agonists (α2-agonists; e.g. xylazine, medetomidine), local anesthetics (e.g. lidocaine, bupivacaine, mepivacaine, ropivacaine), and N-methyl-D-aspartate (NMDA) antagonists (e.g. ketamine). These drugs not only reduce the severity of acute postoperative pain, but in some cases also reduce the incidence of chronic postoperative pain.

**Drug choice:** The choice of drug(s) used to treat pain will depend on the underlying cause, severity, and duration of pain. Knowledge of the pharmacology of analgesics in each species is required to optimize...
drug choice. Factors including age, breed and physical status may influence drug pharmacology and consequently the efficacy and dosing regimen of analgesic drugs. For example, when compared to ‘adults’, drugs in very young animals (puppies and kittens less than 12 weeks of age) and geriatric animals (>75% life expectancy) often have a different pharmacokinetic profile that may alter the effective dose and dosing interval. It is unwise to extrapolate pharmacokinetic data from one species to another; this is particularly true between the dog and the cat. For the management of acute postoperative pain, in particular severe pain, drugs should be titrated to effect, and a multimodal approach used. Dosing intervals are influenced by the severity of pain, patient factors and the combination of drugs used, and should be modified according to patient response.

**Loco-regional techniques:** Potent analgesia is provided by loco-regional techniques such as Infiltration anesthesia (e.g. intratesticular block, ring block, intraperitoneal anesthesia, wound soaker catheters), limb nerve blocks, facial nerve blocks, intravenous regional anesthesia, intraarticular blocks, and neuraxial blocks. For all loco-regional anesthetic techniques, it is imperative to maintain sterile injection techniques (clipping and sterile preparation of the injection site). The techniques are performed on the anaesthetized or deeply sedated (with analgesia as these are painul to perform) animal. While many landmarks and nerves themselves can be palpated transcervaneously, use of neurostimulator or ultrasound localization techniques can reduce the risk of incomplete blocks and damage to the nervous, vascular and other structures.

**Tender loving care:** Surgical technique can have an important impact on perioperative pain. Gentle tissue handling and techniques that minimize trauma (e.g. small incisions, arthroscopy, laparoscopy) should be employed whenever possible. The site of surgery also impacts on postoperative pain, for example, movements that place tension on the incision (e.g. deep breathing and coughing) increase the intensity of pain after thoracic/abdominal surgery. The most important step in managing perioperative pain is to actively assess the animal for signs of pain on a regular basis and use the outcomes of these assessments along with knowledge of the disease/surgical status and history of the animal to make a judgment on the pain state. It is recommended to adopt a specific protocol and approach in each animal and to assess their pain in a consistent manner. High-quality nursing care (tender loving care) should be applied to animals as an adjunct to pain management to create an environment where the animal is emotionally and physically comfortable.

**Keywords:** Cats, Dogs, Multimodal Analgesia, Perioperative Pain Management, Preventive Analgesia

**References**
Anesthetic Protocol for Dogs and Cats with Diseases

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Summary
No longer is a successful anesthetic procedure defined as one that the patient simply recovers from unconsciousness. The goal of current anesthetic techniques should be to have the patient recover from anesthesia with no lasting physiologic or psychologically detrimental effects from the anesthetic procedure itself. To this end, knowledge concerning veterinary anesthesia and analgesia is greatly expanding and continually developing, as the breadth and depth of our profession are evolving with the emergence of species-specific and disease-specific research. Accordingly, changes in cases become more challenging and our patient populations are growing older with more complex disease states. In the current fields of veterinary anesthesia and analgesia. The emphasis is given to the impact of various diseases on anesthetic management in dogs and cats [1]. In this lecture, an outline of planning anesthetic protocol based on the current concepts will be presented for dogs and cats with cardiovascular diseases, respiratory diseases, hematological disorders, neurologic diseases, hepatic diseases, and renal diseases.

Oxygen is essential for production of adenosine triphosphate (the "molecular unit of currency" of intracellular energy transfer) in the mitochondria. Lack of oxygen delivery (DO₂) can trigger the cascade leading to temporary, permanent, or irreversible cell death. The mathematical definition of DO₂ is the product of oxygen content (CaO₂) and cardiac output (CO). CaO₂ is composed of both dissolved and hemoglobin (Hb)-bound oxygen in the blood takes into account the arterial partial pressure of oxygen (PaO₂), and saturation of arterial Hb with oxygen (SaO₂). Blood flow through a tissue capillary bed is held constantly by autoregulatory mechanism between 60 and 160mmHg of mean arterial blood pressure (ABP). The ABP is the product of CO and systemic vascular resistance (SVR). The CO is the product of heart rate (HR) and stroke volume (SV). The SV is determined by preload, diastolic function and contractility of ventricle muscle, and afterload. These relations are described as follows: DO₂ (ml/min) = CaO₂ (ml/dL) x CO (L/min); CaO₂ (ml/dL) = (1.34ml/g O₂ x Hb x SaO₂) + (0.003ml O₂/dL/mmHg x PaO₂); ABP (mmHg) = CO (L/min) x SVR (dynes sec/cm⁵); CO (L/min) = HR (beats/min) x SV (ml/beat); SV = preload x diastolic function x contractility / afterload.

Almost anesthetics can compromise cardiovascular function and DO₂ in patients with cardiovascular, respiratory, and hematologic disorders. The brain, liver, and kidney receive a high proportion of CO and the anesthetics can deteriorate their functions in patients with neurologic, hepatic, and renal diseases. The anesthetist must minimize these detrimental effects and maximize the resemblance to patient homeostasis. Special considerations for planning anesthetic protocol are the preanesthetic physical status of the patient and the nature of the procedures to be performed (magnitudes of surgical invasion and postoperative pain, site, duration). When there is any doubt about the patient’s physical status, procedures are best delayed until it is improved. The anesthetic protocol should be incorporated the concepts of "preventive" and "multimodal" analgesia (balanced anesthesia). The placements of intravenous catheter and endotracheal tube are mandatory and mechanical ventilation must always be available. Sophisticated anesthesia monitoring including indirect ABP and SaO₂ (SpO₂), end-tidal CO₂ pressure (PETCO₂), electrocardiography (ECG),
and core body temperature is recommended. Furthermore, the most reliable cardiopulmonary monitoring (e.g. arterial blood gas analysis, direct ABP, central venous pressure) should be applied in serious cases.

**Cardiovascular disease**
As anesthetics are associated with cardiovascular depressions, a large step toward cardiovascular stability can be provided with good sedation with appropriate premedication. Preoxygenation can be critical to preventing hypoxia before induction to anesthesia. Patients with non-compensated cardiovascular disease are at risk for heart failure due to poor cardiac function or cascade of neurohormonal mechanisms that lead to an increase in circulating blood volume, activations of the sympathetic nerve system and renin-aldosterone-angiotensin system, and increased secretion of antidiuretic hormone. Patients with a history of heart failure or chronic volume overload (e.g. mitral, tricuspid, and aortic valve insufficiency, left to right shunt) may be less likely to tolerate high fluid infusion rates during surgery, and lower infusion rates should be used.

**Premedication:** Opioids are a mainstay of anesthesia as they have minimal cardiovascular effects. Bradycardia induced by opioid can be controlled with anticholinergics. Benzodiazepines are good choices for sedation because of their minimal cardiovascular effects although they are inconsistent sedatives. Alpha₂-adrenergic receptor agonists (α₂-agonists) are contraindicated because they increase SVR.

**Induction to anesthesia:** Propofol, etomidate, and high dose fentanyl with benzodiazepine are excellent choices. Dissociative anesthetics increase in myocardial work and oxygen demand that may worsen cardiac function and arrhythmias. Thus, ketamine and tiletamine are contraindicated in hypertrophic cardiomyopathy and should be avoided in any patient with other cardiomyopathy and valvular cardiac disease.

**Anesthetic maintenance:** Inhaled anesthetics are commonly chosen but depress cardiovascular function dose-dependently. These side effects are managed either by minimizing their requirement with intraoperative analgesia and/or by counteracting with cardiovascular supports (e.g. dobutamine, dopamine).

**Respiratory disease**
Anesthetic management of patients with respiratory diseases should be provided to secure adequate oxygenation and CO₂ elimination. Proper premedication with analgesics and sedatives, rapid patient’s airway control with endotracheal tube, ventilatory support, and PETCO₂ monitoring are essential. Alveolar ventilation is primarily driven by the arterial partial pressure of CO₂ (PaCO₂) and measured as "minute ventilation (MV)". The MV is composed of tidal volume (VT) and respiratory rate (RR) as follows: MV = RR x VT.

**Premedication:** Opioids reduce the ventilatory response to hypoxia and hypercapnia and depress MV. Careful monitoring of ventilation and oxygenation is highly recommended when opioids are administered. Acepromazine and benzodiazepines are associated with minimal effects on the respiratory system.

**Induction and anesthetic maintenance:** Most anesthetics depress MV significantly in dose-dependent manners, while ketamine appears less respiratory depression. Low-dose ketamine infusion (e.g. 0.6mg/kg/hr following 0.5mg/kg) improves respiratory and cardiovascular depression associated with inhaled anesthetics.

**Hematologic disorders**
Anemia and dyshemoglobinemias have a great impact on oxygen delivery to tissue because approximately 97% of CaO₂ is bound to Hb. Hemostatic disorders can be generally characterized as those that predispose the animal to be hemorrhagic (hypocoagulability) or to thrombosis (hypercoagulability).

**Anesthetic protocol for anemic patients:** Anesthetic management of anemic patients should include preoxygenation, careful quantification of hemorrhage, minimization of hemodilution, and anesthetic protocols that have a minimum influence on circulation of red blood cell (RBC). Anesthetics may significantly decrease circulation erythrocyte mass by causing RBC sequestration in the spleen. Acepromazine and thiopental are relatively contraindicated in anemic patients. Propofol should be used sparingly because it may promote vasodilation and splenic enlargement. Etomidate and diazepam should be avoided because they known to cause hemolysis. Application of a multimodal approach can minimize the negative side effects of each drug.
Anesthetic protocol for patients with hemostatic disorders: Patients should be gently handled to avoid iatrogenic trauma and hemorrhage. The IM injection should be minimized or avoided. Jugular venipuncture, femoral arterial puncture, and regional nerve blocks are contraindicated. The drugs that impair platelet function (e.g. acepromazine, non-steroidal anti-inflammatory drugs [NSAIDs]) are contraindicated. Synthetic colloids (e.g. hetastarch) may also impair platelet function and should be used judiciously.

Neurologic disease
Anesthetic management should be purposed to optimize cerebral blood flow (CBF) and prevent increases in intracranial pressure (ICP). Cerebral perfusion pressure (CPP) has a large effect on CBF. CPP is defined as follow: CPP = mean ABP - ICP. Hyperventilation is the most effective method for rapidly reducing ICP. A common recommendation is to maintain the PaCO₂ 30-35mmHg using mechanical ventilation. Hyperosmotic therapy (e.g. mannitol, hypertonic saline) may be used to reduce or prevent increase in ICP.

Premedication: A recent study has indicated that acepromazine can be used without the risk of increasing the likelihood of seizure. Acepromazine should be used with caution because of vasodilation and subsequent hypotension. Both benzodiazepines and α₂-agonists appear to be rational choice since they decrease in CBF. Opioids are an acceptable choice as they have minimal direct effect on CBF and ICP although it can indirectly increase ICP by hyperventilation and emesis.

Induction and anesthetic maintenance: The most injectable anesthetics decrease CBF and ICP and contribute to the decrease in the cerebral metabolic requirement of oxygen (CMRO₂). However, ketamine is controversial because this agent does not reduce CBF, ICP, or CMRO₂. In general, inhaled anesthetics increase CBF and decrease CMRO₂. Most volatile anesthetics used at concentrations below 1.0 minimum alveolar concentration (MAC) minimally affect CBF, although CBF and ICP will increase above 1.0 MAC. Thus, it is important to use a balanced anesthesia approach due to reduce the inhalant requirement.

Hepatobiliary disease
The liver serves a number of vital functions including drug metabolism and protein synthesis such as albumin (critical in providing plasma oncotic pressure) and coagulation factor. It is recommended to use the drug that its main route of excretion is not via liver (e.g. inhaled anesthetics, propofol, etomidate, remifentanil) or an antagonist is available (e.g. α₂-agonists, benzodiazepines, opioids). In any patient with liver dysfunction presenting for invasive procedures, it is important to check the coagulation status to rule out coagulopathy.

Premedication: The α₂-agonists offer excellent sedative property at the cost of significant cardiovascular depression. The administration of benzodiazepine for premedication of most patients with liver dysfunction is an excellent choice, however, care must be taken in patients with hepatic encephalopathy. Neurons from animals with hepatic encephalopathy demonstrate increased sensitivity to benzodiazepines due to the presence of endogenous benzodiazepines and the administration of exogenous benzodiazepines can aggravate hepatic encephalopathy. In general, opioids are considered safe for use in patients with liver diseases because a nonselective opioid receptor antagonist (e.g. naloxone) may be given when if drug effect is prolonged or profound. There is no clinical evidence to suggest that morphine should be avoided in patients with hepatobiliary disease, although morphine causes contraction of the sphincter of Oddi and increase in biliary pressure in several species. Most opioids primarily undergo hepatic biotransformation but remifentanil is entirely extrahepatic via ester hydrolysis. Therefore, remifentanil is advantageous as duration of effect should be unchanged in the presence of liver dysfunction. Although not contraindicated, caution should be used when administering NSAIDs owing to the potential for idiosyncratic hepatotoxicity and adverse effects on platelet aggregation. It is prudent to avoid NSAIDs in cases of hepatic dysfunction with coagulopathy.

Induction to anesthesia: Propofol and etomidate are excellent choices for anesthetic induction in patients with liver dysfunction because of their extrahepatic metabolism. Ketamine is considered safe but propofol and etomidate offer smooth induction and more rapid recovery than ketamine.

Anesthetic maintenance: Isoflurane, sevoflurane, and desflurane are minimally metabolized, not directly associated with hepatotoxicity, and recovery is unaltered by hepatic dysfunction. However, they produced a
number of adverse physiologic effects (e.g. decrease in hepatic blood flow) in dose-dependent manner. It is important to use a balanced or multimodal approach to anesthesia to reduce the requirement of inhalants.

**Renal disease**
The maintenance of normotension, isovolemia, and adequate CO sufficient to maintain renal perfusion has the greatest effect on the prevention of initial or ongoing renal insult. Careful ABP monitoring is advised in patients with existing renal disease. Historically, the administration of a low dose infusion of dopamine (1-3μg/kg/min) was commonly used in the management of patients with acute kidney injury (AKI) and chronic kidney disease (CKD). However, a number of studies have found this regimen to be ineffective. Higher doses of dopamine (5-10μg/kg/min) may be indicated for inotropic support.

**Premedication:** Although use of benzodiazepines is generally recommended, caution must be taken because of paradoxical excitement on administration in juvenile patients. Coadministration a benzodiazepine with an opioid or other sedative is advised to improve sedation. The effect and duration of opioids are largely unaffected by renal failure. However, active metabolites of morphine and meperidine relay on renal excretion and significant renal disease may result in prolonged sedation, respiratory depression, or neuroexcitation. The NSAIDs have the potential to cause detrimental effects in animals with renal disease.

**Induction to anesthesia:** Metabolic acidosis accompanied by renal dysfunction may potentiate thiopental effect owing to an increase in the nonionized fraction. Thiopental dosage should be decreased in patients with significant renal impairment. Propofol and etomidate are excellent choices. Renal disease should have little effect on metabolism of moderate doses of ketamine. However, elimination of ketamine relies extensively on renal excretions in cats. Therefore, ketamine should be avoided in cats with renal disease.

**Anesthetic maintenance:** Isoflurane, sevoflurane, and desflurane are appropriate choice. High fresh gas flows and avoidance of CO₂ absorbents containing potassium hydroxide and sodium hydroxide and CO₂ absorbent desiccation will greatly decrease formation of potential nephrotoxic degradation products (e.g. inorganic fluoride ions, compund A), particularly in sevoflurane anesthesia.

**Keywords:** Anesthetic Protocol, Cats, Disease, Dogs

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Injectable Anesthetic Drug Delivery: Repeat Administration, CRI and TCI Using Alfaxan as an Example Drug

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Typically the modus operandi for anesthetizing veterinary patients is to premedicate with single or multiple drugs, perform induction with a registered injectable drug(s) and then maintain anesthesia in the patient with an inhalant anesthetic. Another less frequent but certainly viable means of performing induction and maintenance of anesthesia is to replace the inhalant anesthetic with an injectable anesthetic which equates to total intravenous anesthesia (TIVA). The intravenous anesthetic provides hypnosis and muscle relaxation. Analgesia can be provided by the administration of an opioid, alpha-2 adrenergic agonist, ketamine, lidocaine or a combination thereof. Anesthesia can be maintained by intermittent boluses but continuous rate infusion (CRI) produces a more stable plane of anesthesia with less variation in hemodynamic, respiratory and central effects and thus is safer for the animal. CRI can also be administered with manual adjustment (i.e. stepped or variable rate infusion [VRI]) with or without a loading dose. The loading dose (LD) is calculated as LD = target plasma concentration of injectable anesthetic (Cp) x volume of distribution of the central compartment (Vc). The maintenance dose for the CRI = Cp x total body clearance (Clb). Clb is calculated from a good quality pharmacokinetic study(ies) of the injectable anesthetic; ideally using compartmental modelling. Compartmental modelling will also yield the micro constants (V1, k10 ± k12 & k21 ± k31), necessary to perform Target Controlled Infusion (TCI) calculations.

The concept of TCI was first described by Krüger-Thiemer in 19681. The first theoretical model of TCI was by Schwilden et al2 and called the computer-assisted total intravenous anesthesia system (CATIA). Since that time a number of different computer programs have been developed for TCI including the STANPUMP (Stanford University), the STELPUMP (University of Stellenbosch), RUGLOOP from Ghent University and the Diprifusor System (first commercial system for propofol)3. The distinct advantage of TCI over CRI is that with TCI the “effect site concentration” is reached quickly while slowly increasing drug in the peripheral compartment(s) until the concentration of anesthetic in all compartments equals the targeted effect site concentration. Figure 1 shows the conceptual difference between rate and plasma drug concentration for CRI and TCI.

Calculation of the effect site concentration EC (EC) for an injectable anesthetic is roughly performed in the same manner as a minimum alveolar concentration (MAC) study using a calibrated and validated noxious stimulus except the TCI infusion pump replaces the vaporizer. Both studies are designed to calculate the lowest drug concentration preventing movement in response to a maximal noxious stimulus in a population. In a recent study in cats we calculated the alfaxalone EC 99% to be 7.6 (5.5-9.7) mg L⁻¹ 4. Alfaxalone, like propofol, is a drug that has attributes necessary for TIVA. It theoretically has a short context sensitive half-life (unpublished data) and does not appear to pharmacokinetically or pharmacodynamically accumulate during infusion at clinically recommended dose rates. Alfaxalone in 2-hydroxypropyl-β-cyclodextrin has been successfully used for TIVA or partial intravenous anesthesia (PIVA) in dogs5-15, cats16-24, horses25-27, ponies28-29, pigs30, sheep31-33, goats34-36, monkeys37, rabbits38 and rats39-41.
Keywords: Alfaxan, Repeat, Infusion

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Alfaxan in Other Species and by Different Routes of Administration

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In the 1940s the Hungarian born endocrinologist Hans Selye showed that reversible unconsciousness could be produced in rats administered intraperitoneal injections of large quantities of steroid hormones\(^1\). Of the steroids injected desoxytococosterone acetate, a pregnanedione, was the most potent and devoid of hormonal activity\(^2\). In 1955 P’An and colleagues reported that a close structural analogue of pregnanedione, hydroxydione, was more potent and safer than the thiobarbiturate thiopenitone\(^3\). However, hydroxydione was not the ideal anaesthetic induction agent in that it produced a delayed anaesthetic induction of up to 3 minutes and it had to be solubilised in an alkaline pH causing venous thrombosis. Further structure activity relationship on this neurosteroid showed that manipulation of the 3 and 21 carbon positions altered anaesthetic potency\(^4,5\). Eventually, the active molecule 3α-hydroxy-5α-pregnane-11,20-dione (alfaxalone) was discovered by the Glaxo UK Pharmacology Department. Similar to the barbiturates, benzodiazepines, propofol and isoflurane it is thought that the main mechanism of action is through the gamma-aminobutyric acid type A (GABA\(_A\)) receptor found in neurons and other excitable cells.

Alfaxalone was later combined with alfadolone (21-acetoxy-3α-hydroxy-5α-pregnane-11,20-dione), Cremophor\(^®\) EL (B.A.S.F.) and sodium chloride to yield formulation CT1341. The alfadolone was placed in the formulation to improve the solubility of the alfaxalone\(^6\). Child et al performed a battery of pharmacological tests on CT1341 in laboratory animals and found it offered significant advantages over the other injectable anaesthetics in that it had a higher margin of safety, it was non-irritant to tissues including veins, it was compatible with the adjuvant and pre-anaesthetic drugs, it did not accumulate and it produced a pleasant anaesthetic experience for the patient\(^7\). In the early 1970s, CT1341 was introduced as an intravenous (IV) anaesthetic induction agent for humans (Althesin\(^®\)) and as an IV and intramuscular anaesthetic (Saffan\(^®\)) for cats and monkeys. Saffan\(^®\) was not licensed in dogs because in dogs polyoxethylated emulsifying agents like Cremophor\(^®\) EL cause histamine release and potential anaphylaxis\(^8\). In an effort to remove the side effects observed with drug carriers such as Cremophor\(^®\) EL, groups like Brewster et al found that alfaxalone and other drugs could be solubilised in safe carriers like 2-hydroxypropyl cyclodextrins (2-HPCD)\(^9,10\).

Since 2000, alfaxalone in 2-HPCD (Alfaxan\(^®\)) has been commercially available as an anaesthetic for intravenous injection. The product is registered and labelled for the induction and maintenance of anaesthesia in dogs and cats in multiple countries. However, the formulation has been administered in many other species by different routes of administration to produce sedation or anaesthesia. By definition the actual use or intended use of a drug in an animal in a manner that is not in accordance with the approved labeling is extralabel drug use.

An internal corporate search and an external search of Alfaxan\(^®\) administration to species other than dogs and cats yielded numerous studies. Routes of administration included IV, IM, IP, SC and topical routes of administration. Some studies were corporate sponsored internal studies, drug in kind or collaborations\(^11-30\), however, most of these studies were independent studies cited in refereed journals\(^31-93\).
Keywords: Alfaxan, Species, Route

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Historical Perspective

Corporate Internal Studies, Drug in Kind or Collaborations


**Independent Studies**


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Total Intravenous Anesthesia in Horses

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Summary

Intravenous (IV) anesthetic drugs and techniques are primary means for producing general anesthesia in equine practice. Induction and maintenance of general anesthesia with intravenous drugs, total intravenous anesthesia (TIVA), has many potential advantages over inhalation anesthesia. The use of TIVA reduces the cost of equipment and eliminates any possible hazards associated with exposure to volatile and gaseous anesthetic drugs. TIVA in horses provides better cardiovascular function and suppression of the endocrine stress response during anesthesia than inhalation anesthesia. In addition, TIVA reduces the production of pro-inflammatory and anti-inflammatory cytokines compared with inhalation anesthesia. Therefore, TIVA produces favorable effects on endocrine and inflammatory responses triggered by anesthesia and surgery. A major concern regarding the use of TIVA in horses is that the long-acting and cumulative effects of many IV anesthetic drugs may prolong or result in a poor quality of recovery. In halation anesthesia is more controllable than conventional TIVA and preferred for prolonged periods of anesthesia (>60 to 90 min). Currently developed TIVA protocols using propofol or alfaxalone may overcome these concerns.

The ideal drugs for TIVA have pharmacokinetic properties that they and their metabolites are non-cumulative after prolonged infusion. However, many IV anesthetics are long acting and cumulative, therefore, extending the length of anesthesia with an IV drug for a longer period may result in a prolonged and poor recovery. Current TIVA techniques can be grouped into three categories: (1) those that are suitable for short-term procedures such as castration (<30 min) and that result in a very rapid recovery, (2) those suitable for intermediate-duration procedures (<90 min), and (3) those that could be extended indefinitely (>120 min).

Total intravenous anesthesia for short-duration procedures (<30 min)

Anesthesia for procedures lasting 10-15 min can be produced with boluses of IV drugs. The most popular IV drugs for short-term anesthesia are combinations of α2-adrenoceptor agonists (α2-agonists; e.g. xylazine, detomidine, medetomidine, romifidine) with dissociative anesthetics (e.g. ketamine, tiletamine) or barbiturates (e.g. thiopental, thiamylal). Centrally acting muscle relaxants (e.g. guaifenesin, diazepam, midazolam) are administered to ensure smooth induction to anesthesia. Butorphanol, an opioid agonist-antagonist, is coadministered with α2-agonists to enhance analgesia. Small IV doses of thiopental (0.5-1mg/kg) or ketamine (0.5-1mg/kg) can be administered to extend anesthesia.

**Xylazine-Ketamine**: Sedation with xylazine (1.1mg/kg IV) followed in 3-5 min by ketamine (2.2mg/kg IV) produces quite and uneventful induction to anesthesia in most horses. Respiration is depressed transiently (arterial partial pressure of carbon dioxide [PaCO₂] 40-50mmHg) and arterial partial pressure of oxygen (PaO₂) decreases to 60mmHg in spontaneously air-breathing horses. Hemodynamic variables remain normal. Recovery is generally uneventful and horses stand within 15-25 min after ketamine administration. Repeated reduced doses of ketamine and xylazine can be administered to prolong the duration of anesthesia.
**Xylazine-Butorphanol-Ketamine:** Sedation with xylazine (1.1mg/kg IV) and butorphanol (0.04mg/kg IV) followed by ketamine (2.2mg/kg IV) produces rapid and smooth induction. Respiratory rate decreases briefly at early phase of anesthesia. The PaCO₂ is maintained at 40-50mmHg but PaO₂ decreases to 60 mmHg in spontaneously air-breathing horses. Hemodynamic variables remain normal. Recovery is good to excellent and horses stand within 30 min.

**Xylazine-Diazepam-Ketamine:** Sedation with xylazine (1.1mg/kg IV) followed by administration of ketamine (2.2mg/kg IV) and diazepam (0.04mg/kg IV) enhances muscle relaxation resulting in quiet and uneventful induction. Hemodynamic variables remain normal. The PaO₂ decreases to 60mmHg in spontaneously air-breathing horses. Most horses stand with mild ataxia within 35 min.

**Xylazine-Guaifenesin-Ketamine:** Following sedation with xylazine (1.1mg/kg IV), a 5% or 10% solution of guaifenesin (35-50mg/kg) is infused until the horse demonstrates marked ataxia. Then, a bolus IV dose of ketamine (2.2mg/kg) is administered to produce quiet and uneventful induction. Cardiorespiratory depression is minimal during anesthesia. Recovery is quiet and uneventful and horses stand within 40-50 min.

**Xylazine-Thiopental/Xylazine-Thiamylal:** Sedation with xylazine (1.1mg/kg IV) followed by thiopental (5-8mg/kg IV) or thiamylal (4-6mg/kg IV) produces rapid and smooth induction. Temporal apnea and hypoventilation are common. Anesthesia is short (5-10 min) and not associated with significant analgesia. Recovery is usually rapid but may be uncoordinated. Horses may require assistance to stand using head and tail ropes. Repeated administration of thiopental/thiamylal to maintain anesthesia can lead to drug accumulation and result in prolonged and uncoordinated recoveries.

**Xylazine-Guaifenesin-Thiopental:** Following sedation with xylazine (1.1mg/kg IV), a 5% or 10% solution of guaifenesin is infused until the horse shows marked ataxia. Then, a bolus thiopental (5mg/kg IV) is administered to produce rapid and uneventful induction. Alternatively, Induction can be achieved by infusion of a mixture (adding 1g of thiopental to 500mL of a 5% guaifenesin solution) to effect (recumbent). A small bolus thiopental (0.5-1mg/kg IV) may be required to hasten induction in the horses showing panic because of inadequate sedation and muscle relaxation. Recovery occurs within 30-40 min and residual muscle weakness is common if high doses of guaifenesin are used.

**Detomidine-Ketamine:** Sedation with detomidine (20μg/kg IV) followed by ketamine (2.2mg/kg IV) produces rapid and smooth induction. However, some horses occasionally require the administration of thiopental because of unsatisfactory induction. Respiratory depression is similar to xylazine-ketamine. However, some horses demonstrate incoordination or excitement during recovery. The higher arterial blood pressure and poor recovery caused by lingering effects of detomidine (vasoconstriction, sedation).

**Xylazine-Propofol/Detomidine-Propofol/Xylazine-midazolam-Propofol:** The administration of propofol (4mg/kg IV) alone produces a short-term anesthesia and very rapid and smooth recovery. However, transition to recumbent is slow and the horse commonly shows leg paddling after recumbent and tachycardia (60-80bpm) during anesthesia. The premedication with xylazine (1.1mg/kg IV) or detomidine (15μg/kg IV) prevents tachycardia and enhances the quality of induction but fails to prevent the other undesirable characteristics of propofol (hypoventilation, apnea). The premedication with xylazine (1.1mg/kg IV) and midazolam (0.04mg/kg IV) minimizes paddling but excessive muscle tremors still remain.

**Xylazine-Guaifenesin-Alfaxalone [2]:** The premedication with xylazine (0.5mg/kg IV) and guaifenesin (35mg/kg IV) followed by alfaxalone (1mg/kg IV) produces rapid and smooth induction. However, horses exhibit a higher incidence of tremor/shaking on induction. The PaO₂ decreases to 60mmHg and the PaCO₂ is maintained at 40-50mmHg in spontaneously air-breathing horses. Hemodynamic variables remain with normal limits. Recovery is satisfactorily and horses stand within 30 min.

**Medetomidine-Midazolam-Alfaxalone [3]:** The premedication with medetomidine (6μg/kg IV) and midazolam (0.02mg/kg IV) followed by alfaxalone (1mg/kg IV) produces rapid and smooth induction. The PaO₂ decreases to 70mmHg and the PaCO₂ is maintained at 50mmHg in spontaneously air-breathing horses. Hemodynamic variables remain with normal limits. Recovery is good and horses stand within 45 min.

Total intravenous anesthesia for intermediate-duration procedures (30 to 90 min)
Surgical procedures requiring anesthesia for more than 30 min can achieved with simultaneous infusions of α2-agonists, dissociative anesthetics, and centrally acting muscle relaxants. The combination of guaifenesin, ketamine, and xylazine (“Triple Drip”) has been popularized since 1986. This technique and its modifications using different α2-agonists or water-soluble benzodiazepines can be safely administered to horses for 90 min.

**Guaifenesin-Ketamine-Xylazine Infusion (Triple Drip):** A mixture of guaifenesin (50mg/mL), ketamine (1mg/mL), and xylazine (0.5mg/mL) is administered to horses to effect (recumbent) and anesthesia is maintained by an infusion of the mixture (1-1.5mL/kg/hr). Infusion rate is increased or decreased as needed to maintain anesthesia. Alternatively, horses are anesthetized with ketamine (2.2mg/kg IV) after the premedication with xylazine (1.1mg/kg IV) and then the mixture is infused at a rate of 2.75mL/kg/hr. Hemodynamic variables remain within normal limits. Hypoxia and hypoventilation do not occur in spontaneously 100% oxygen-breathing horses. Recovery from prolonged infusion (>60 min) of triple drip may take more than 1 hr.

**Midazolam-Ketamine-Medetomidine Infusion:** Surgical anesthesia can be maintained with an infusion of a mixture of midazolam (0.8mg/mL), ketamine (40mg/mL), and medetomidine (100μg/mL) using an infusion rate of 0.1mL/kg/hr in horses anesthetized with ketamine (2mg/kg IV) after the premedication with medetomidine (5μg/kg IV) and midazolam (0.04mg/kg IV). Hemodynamic valuables remain with normal limits. The PaCO₂ is maintained at 50mmHg but PaO₂ decreases to 60mmHg in spontaneously air-breathing horses. Recovery is good to acceptable and the horses stand in 59 min after 60 min of anesthesia.

**Alfaxalone-Medetomidine [4]:** Surgical anesthesia can be maintained with infusions of alfaxalone (2mg/kg/hr) and medetomidine (5μg/kg/hr) in horses anesthetized with alfaxalone (1mg/kg IV) after premedication with acepromazine (0.03mg/kg IV), medetomidine (7μg/kg IV) and guaifenesin (35mg/kg IV). The PaO₂ maintains at 117-172mmHg and PaCO₂ is maintained at 50-56mmHg in spontaneously 100% oxygen-breathing horses. Hemodynamic valuables remain with normal limits. Recovery is good and horses stand within 37 min after 45 min of anesthesia.

**Alfaxalone-Medetomidine-Butorphanol [5]:** Surgical anesthesia can be maintained with infusions of alfaxalone (2mg/kg/hr), medetomidine (5μg/kg/hr), and butorphanol (50μg/kg/hr) in horses anesthetized with alfaxalone (1mg/kg IV) after premedication with medetomidine (7μg/kg IV) and butorphanol (25μg/kg IV). The PaO₂ decreases to 50mmHg and PaCO₂ is maintained at 50mmHg in spontaneously air-breathing horses. Hemodynamic valuables remain with normal limits. Recovery is good and horses stand within 70 min after 60 min of anesthesia.

**Total intravenous anesthesia for prolonged procedures (>120 min)**

At present, propofol is the only IV anesthetic that has been evaluated for prolonged TIVA in horses. However, propofol is unsatisfactory as the sole agent for horses because of its poor analgesic, large volumes required, unpredictable induction, and significant respiratory depression. Minimum infusion rate (MIR) is the median effective dose (ED₅₀) of an IV anesthetic required to prevent movement in response to a noxious stimuli. The MIR of propofol in horses is 0.20mg/kg/min [6]. The dose of propofol to maintain surgical anesthesia may be greater than 0.28mg/kg/min. Several studies have suggested that the propofol MIR in horses can be reduced to 0.10-0.18mg/kg/min by premedication with xylazine (1mg/kg IV) or detomidine (15μg/kg IV).

**Ketamine-Propofol Infusion:** Surgical anesthesia can be maintained with infusions of ketamine (3mg/kg/hr) and propofol (0.16mg/kg/min) in horses anesthetized with propofol (3mg/kg IV) after premedication with medetomidine (5μg/kg IV) and butorphanol (20μg/kg IV). Cardiovascular function is adequate, however, artificial ventilation is required to maintain respiratory function. Recovery is good to fair and the horses stand within 70 min after 120 min anesthesia.

**Medetomidine-Propofol Infusion:** Surgical anesthesia can be maintained with medetomidine (3.5μg/kg/hr) and propofol (0.10-0.11mg/kg/min) infusions in horses anesthetized with ketamine (2mg/kg IV) after premedication with medetomidine (7μg/kg IV). Cardiovascular function is acceptable but artificial ventilation is required. Recovery is uneventful and horses stand within 42 min after 112 min anesthesia.
**Ketamine-Medetomidine-Propofol Infusion** [7]: Surgical anesthesia can be maintained with infusions of ketamine (1mg/kg/hr), medetomidine (1.25μg/kg/hr), and propofol (0.13-0.17mg/kg/min) in horses anesthetized with ketamine (2.5mg/kg IV) and midazolam (0.04mg/kg IV) after premedication with medetomidine (5μg/kg IV). Cardiovascular function is acceptable, however, artificial ventilation is required. Recovery is good and the horses stood in about 74 min after 175 min anesthesia.

**Medetomidine-Lidocaine-Butorphanol-Propofol Infusion** [8]: Surgical anesthesia can be maintained with infusions of medetomidine (3.5μg/kg/hr), lidocaine (3mg/kg/hr), butorphanol (24μg/kg/hr), and propofol (0.1mg/kg/min) in horses anesthetized with lidocaine (1mg/kg IV) and propofol (3mg/kg IV) after premedication with medetomidine (5μg/kg IV) and butorphanol (0.02mg/kg IV). Recovery is good and the horses stood in about 46 min after 129 min of anesthesia. Cardiovascular function is acceptable, however, respiratory rate decreases, PaCO₂ increases to 70mmHg and PaO₂ is maintained at 250mmHg in spontaneously 100% oxygen-breathing horses. Artificial ventilation is recommended in prolonged anesthesia.

**Keywords:** Alfaxalone, Ketamine, Horses, Midazolam, Medetomidine, Propofol, Total Intravenous Anesthesia

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Cardiopulmonary Resuscitation in Dogs and Cats

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Summary

Cardiopulmonary resuscitation (CPR) is the attempt to return spontaneous circulation (ROSC) in patients with cardiopulmonary arrest (CPA). In previous reports on CPR in veterinary patients from 1992 to 2009, the prognosis was grave for dogs with CPA and the rates of ROSC and survival discharge from the hospital for dogs with CPA had been reported to be 13-35% and 3-6% in those reports [1-3]. In June 2012, the first consensus CPR guidelines for dogs and cats were formulated on the basis of a large-scale, systematic literature survey under the title “Reassessment of Campaign on Veterinary Resuscitation (RECOVER)” and evidence-based consensus clinical CPR guidelines (RECOVER guidelines) was introduced in veterinary practice [4]. It is strongly expected that outcome of veterinary CPR may be improved by incorporating the RECOVER guidelines into veterinary practice. In this lecture, an outline of veterinary CPR based on the RECOVER guidelines will be presented.

The RECOVER guidelines presented CPR-related topics in 5 domains: Preparedness and Prevention, Basic Life Support (BLS), Advanced Life Support (ALS), Monitoring, and Post-Cardiac Arrest Care. The main elements of CPR and their temporal sequence with the clinical guidelines most relevant to the CPA patients have been summarized in a CPR algorithm chart (Figure 1a). The box surrounded by the dashed line in the Figure 1a contains the initial BLS and ALS actions for a patient diagnosed with CPA: (1) chest compressions, (2) ventilation support, (3) initiation of electrocardiogram (ECG) and end-tidal carbon dioxide (EtCO₂) monitoring, (4) obtaining vascular access for drug administration, and (5) administration of reversal agents if any anesthetic/sedative agents have been administered. Then, the algorithm enters a loop of 2-min cycles of BLS with brief pauses to evaluate the patient for signs of ROSC and the ECG for a rhythm diagnosis. Patients in pulse-less electrical activity (PEA) or asystole should be treated with vasopressors and anticholinergic drugs. Patients in ventricle fibrillation (VF) or pulse-less ventricle tachycardia (VT) should be electrically defibrillated and another 2-min cycle of BLS should be started immediately. If BLS and ALS are performed promptly, initial ROSC rates may be as high as 50% in dogs and cats [5].

Preparedness and Prevention

Time-sensitive and coordinated actions necessary to maximize survival from CPA have the potential to lead to improved outcomes. It is recommended that the location, storage, and content of resuscitation equipment should be standardized and regularly audited. In addition, the presence of cognitive aids (e.g. checklists, algorithm charts, dosing charts) has been shown to improve compliance with CPR guidelines. Formal training of personnel in the use of these cognitive aids is also crucial to effective utilization during CPR. Availability and clear visibility of these charts in areas in which CPA may occur (e.g. procedure areas, anesthesia induction rooms, surgery suites) is recommended.

CPR training: Adherence to CPR guidelines can only be accomplished if personnel receive effective standardized training and regular opportunities to refresh skills. CPR training should include both didactic
components targeted at cognitive performance and opportunities to practice hands-on skills with quality feedback. CPR training at least every 6 months is recommended to reduce the risk of the decay of skills.

**Team dynamics:** Communication and team skills training can improve the effectiveness of CPR attempt. Specific leadership training is recommended for individuals who may need to lead in CPR attempts (e.g. veterinarians, technicians). Crucial roles of the team leader include distributing tasks to other team members and enforcing rules and procedures. Important leadership behaviors that can improve CPR team performance include intermittently summarizing the code to ensure a shared mental model among team members, actively soliciting input from team members to encourage situation awareness and identify issues and ideas from all members of the team, and assigning individual tasks to team members rather than performing them personally to allow better attention to the global status of the code rather than a specific task. Team performance can be enhanced with closed loop communication. Closed loop communication is accomplished by a clear, directed order being given to one team member by another, after which the receiving team member repeats the order back to the requestor to verify the accuracy of the receiver’s perception. This simple technique drastically reduces medical errors, especially in an emergency situation, due to misunderstanding of orders and prevents the possibility of an order not being carried out because the receiver did not hear the request.

**Basic Life Support (BLS)**

High-quality chest compressions should be delivered in uninterrupted cycles of 2 min with most patients in lateral recumbency, at a compression rate of 100–120/min and a compression depth of 1/3–1/2 the width of the chest while allowing for full elastic recoil of the chest between individual compressions. Early intubation and ventilation is highly valuable, with a ventilation rate of approximately 10 breaths/min, a tidal volume of 10 mL/kg, and an inspiratory time of 1 sec delivered simultaneously with compressions. If intubation supplies are not available, mouth-to-snout ventilation is an acceptable alternative, and should be delivered in repeated rounds of 30 chest compressions followed by 2 rapid breaths. After each 2-min cycle of BLS, the compressor should be rotated to prevent fatigue, which may decrease the quality of chest compressions. Every effort should be made to minimize the duration of chest compression interruptions between cycles.

**Advanced Life Support (ALS)**

ALS encompasses the components of CPR performed after BLS has been initiated and until ROSC is achieved. The ECG is evaluated for a rhythm diagnosis at a brief pause after the first 2-min cycle of BLS. Because only 25–30% of a normal cardiac output is achieved with even high-quality external chest compressions, generation of adequate coronary and cerebral perfusion pressures during CPR requires high peripheral vascular resistance. Therefore, vasopressors are an essential component of ALS drug therapy. Patients in PEA/asystole should be treated with vasopressors (e.g. epinephrine, vasopressin) and, potentially, anticholinergic drugs (e.g. atropine). Patients in VF/VT should be electrically defibrillated and another 2-min cycle of BLS should be started immediately.

**Epinephrine:** Epinephrine, a catecholamine that acts as a nonspecific adrenergic agonist, has been widely used for its vasopressor (α<sub>1</sub>) activity during CPR for decades. It also has β1-adrenergic activity, the inotropic and chronotropic effects of which are likely less crucial, and may be harmful when treating CPA due to increased myocardial oxygen demand, exacerbating myocardial ischemia, and predisposing to arrhythmias once ROSC is achieved. Low-dose (0.01 mg/kg IV) epinephrine every 3–5 min is recommended early in CPR, but high-dose (0.1 mg/kg IV) may be considered after prolonged (>10min) CPR.

**Vasopressin:** The vasopressor effects of vasopressin are mediated through the peripheral V<sub>1</sub>-receptor located on vascular smooth muscle. Unlike α<sub>1</sub>-receptors, V<sub>1</sub>-receptors remain responsive in an acidic pH, and vasopressin has no inotropic or chronotropic effects. Although evidence of the efficacy of vasopressin compared to epinephrine in dogs and cats during CPR is limited, the use of vasopressin (0.8 U/kg IV) with or without epinephrine every 3–5 min may be considered.
**Atropine:** Atropine is a parasympatholytic agent and most likely to be of use in dogs and cats with asystole/PEA associated with high vagal tone. Due to the lack of any clear detrimental effect, routine use of atropine (0.04 mg/kg IV) during CPR in dogs and cats may be considered.

**Defibrillation:** Electrical defibrillation is the most effective therapy for “shockable” rhythms (VF and pulse-less VT). Because VF/VT are the result of abnormal pacing of groups of ventricular myocardial cells by the myocardial cells themselves rather than the pacemakers, the goal of electrical defibrillation is to depolarize as many of these cells as possible, driving them into their refractory period, and stopping the random electrical and uncoordinated mechanical activity. If this is successful, the pacemakers may then begin establishing a sinus rhythm or the patient may develop asystole. Note that either of these outcomes is considered a successful defibrillation. The first dose of external electrical defibrillation is 4–6 J/kg with a monophasic defibrillator or 2–4 J/kg with a biphasic defibrillator. To maximize current through the ventricles, the paddles should be placed on opposite sides of the thorax approximately over the costochondral junction directly over the heart. To facilitate this, the patient will likely have to be placed in dorsal recumbency. If the first shock is unsuccessful, defibrillation energy escalation (e.g., 50% dose increase) is reasonable. In the absence of an electrical defibrillator, mechanical defibrillation may be accomplished with a precordial thump, but the efficacy of this intervention is likely poor.

**Monitoring during CPR**

Strong evidence supports the use of ECG and EtCO₂ monitoring in dogs and cats with CPA.

**ECG:** Although it is susceptible to artifact during chest compressions, evaluation of the ECG during inter-cycle pauses is recommended to obtain an accurate rhythm diagnosis and to guide ALS therapy. However, the ECG must evaluate rapidly followed by immediate resumption of chest compressions.

**EtCO₂:** Strong evidence supports the use of EtCO₂ monitoring during CPR as an early indicator of ROSC and as a measure of efficacy of CPR, potentially allowing rescuers to adjust their treatment to maximize perfusion during CPR. Because EtCO₂ is affected by both pulmonary perfusion and minute ventilation, rescuers should be cautious to maintain constant minute ventilation when using EtCO₂ measurement for these purposes. Sudden increases in EtCO₂ occur with ROSC due to an increase in pulmonary blood flow. There is limited data in dogs and cats suggesting that higher EtCO₂ values during CPR (>15 mm Hg in dogs) may be associated with an increased rate of ROSC.

**Post-Cardiac Arrest Care (PCA care)**

Many animals will ultimately die despite initial successful resuscitation, leading to the conclusion that ROSC is only an intermediate endpoint in CPR. Survival to discharge rates range from 2 to 10% for dogs and cats, despite initial ROSC in 35 to 45% of the animals [1,2]. Optimizing care after ROSC positively impact outcome of CPR. This may suggest that a superior intensive care unit providing advanced post-PCA care could have benefits to veterinary CPR patients. The RECOVER PCA care algorithm (Figure 1b) suggests a bundle of care including respiratory optimization, hemodynamic optimization, and neuroprotective interventions [4]. This chart summarizes a comprehensive treatment protocol for PCA care that includes components of controlled ventilation and oxygenation, goal-directed hemodynamic optimization, and neuroprotective strategies. The sequence shown reflects the order in which each component should be assessed and treatment initiated. Assessment and initiation of treatment for the subsequent component will likely commence before the endpoints of the previous component have been completely met. Thus respiratory, hemodynamic, and neuroprotective treatment strategies will be initiated in parallel in most cases.
Keywords: Cardiopulmonary Resuscitation, Cats, Dogs

References

Figure 1 CPR and PCA care algorithm charts in dogs and cats [4].
The Emerging Concern of Chagas' Disease in Working Dogs

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Summary

Chagas’ disease (American trypanosomiasis) is a zoonotic disease caused by the protozoan parasite Trypanosoma cruzi. Approximately 8-12 million people have contracted Chagas’ disease worldwide, with the majority located in South America, Central America, and Mexico, where it is the most common cause of infectious cardiomyopathy (1). Primarily due to migration, approximately 300,000 people with Chagas’ disease reside in the United States, and smaller populations of infected immigrants live in Europe, Asia, and Oceania (2). The disease can be fatal to about one-third of all people affected, often remains undiagnosed due to a long latent period, and is difficult to treat, with treatment failure common in patients suffering from the chronic form of the disease (1). Chagas’ disease is considered one of the major neglected tropical diseases by the World Health Organization (3-4).

In Latin America, dogs are considered an important reservoir host for T. cruzi due to their close relationship with people in rural areas and the feeding habits of insects that harbor T. cruzi (5-7). In the United States, infected dogs most commonly suffer from cardiac disease with cases of sudden death, myocarditis, arrhythmias, dilated cardiomyopathy, congestive heart failure, lymphadenopathy, and weight loss commonly reported from both naturally occurring and experimentally induced infections (8-18). Military working dogs (MWDs) stationed in Texas have become infected with Chagas’ disease since at least 2006, when the reported positive seroprevalence rate in San Antonio, TX was 8% in 180 MWDs tested (19). Although many infected MWDs have remained healthy and effective working dogs, some died from myocarditis or developed dilated cardiomyopathy, congestive heart failure, or arrhythmias resulting in their retirement from service or euthanasia. Because of the potential to negatively impact the health of MWDs stationed in Texas, where the insect vectors for transmission of T. cruzi to dogs are native, Chagas’ disease has emerged as a pathogen of concern for the United States Army (15, 19-20). Considering that most MWDs reside in Texas for their initial training, there is increased potential for exposure and development of canine Chagas’ disease in MWDs. This seminar will review Chagas’ disease in the dog, to include a brief summary of the parasite and its life cycle, mechanisms of transmission, clinical presentations, diagnosis, and treatment options.

Clinical Presentation

In dogs, there are acute, indeterminate, and chronic symptomatic forms of Chagas’ disease. The acute phase of the disease may go largely unnoticed due to vague, mild clinical signs similar to what occurs in humans. Privately-owned dogs in Texas have shown lethargy, anorexia, and lymphadenopathy, severe enough in some cases to produce draining tracts from which the T. cruzi parasite can be identified cytologically (Roy Madigan, personal communication). Dogs severely affected by the acute phase are more likely to be puppies less than 6 months old; older dogs may not show any clinical signs (21). There have been 2 cases of sudden death in young MWDs in Texas, including a 16 week-old puppy, that were attributed to acute myocarditis due to T. cruzi infection.
Most dogs survive the acute phase and enter an indeterminate phase similar to humans. Infected dogs may never develop any clinical signs and live a normal, healthy life. It is unknown the percentage of chronically infected dogs that develop clinical signs of Chagas’ disease. However, most MWDs and other dogs develop cardiac disease. Experimental inoculation of dogs with T. cruzi demonstrated that in 5 out of 8 dogs, ventricular premature contractions (VPCs) and ventricular tachycardia developed during the chronic phase between 60-170 days post-inoculation, and chronic myocarditis with cardiac dilatation over 8-36 months (21). Reported clinical signs in naturally infected dogs include lethargy, inappetance, exercise intolerance, ascites, tachycardia, tachypnea, dyspnea, weight loss, heart murmur, bradycardia, coughing, and lateral recumbency. Electrocardiographic and echocardiographic changes include right bundle branch block, VPCs, ventricular tachycardia, third degree atrioventricular block, enlargement of the right ventricle and right atrium, tricuspid regurgitation, decreased fractional shortening, and occasionally pericardial effusion (21). MWDs stationed in San Antonio have suffered from dilated cardiomyopathy, acute myocarditis, and congestive heart failure due to chronic T. cruzi infection.

Diagnosis and Treatment
A positive serology test from the indirect fluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), or radioimmunoprecipitation assay (RIA) is the gold standard for diagnosing Chagas’ disease in dogs. A positive serology test in conjunction with clinical signs and electrocardiographic, radiographic, or echocardiographic changes associated with cardiac disease is a convincing means of diagnosing Chagasic cardiomyopathy or myocarditis in dogs and humans (21). Antibody titers can remain high for many years, sometimes for the entire lifetime of the patient, regardless of whether the patient is symptomatic or healthy. During the acute phase, examination of blood smears or buffy coat smears may reveal the motile trypanastigote form of T. cruzi. Trypomastigotes may also be found cytologically from infected tissues such as lymph nodes or effusions (21). The spherical amastigotes may also be found from histopathological examination of any tissues, particularly myocardial or smooth muscle tissue. Polymerase chain reaction (PCR) has emerged as a means to detect low levels of circulating parasites during chronic infection, but this test lacks appropriate sensitivity to assure its use as a sole modality for parasite detection or measuring efficacy of drug therapy (22-24).

In humans, treatment for Chagas’ disease has primarily focused on the use of two drugs: benznidazole and nifurtimox. Benznidazole may cause dermatitis and photosensitization in 25-50% of patients, peripheral neuropathy in 30% of patients, or other side effects such as vomiting, anorexia, weight loss, paresthesia, or bone marrow suppression. Up to 13% of all patients prescribed benznidazole cannot complete therapy. Nifurtimox causes nausea, vomiting anorexia, weight loss, and abdominal pain in up to 50-75% of patients. Neurotoxicity is a common finding with nifurtimox, with up to 50% of patients suffering from a myriad of neurologic signs. Up to 40% of patients prescribed nifurtimox do not complete therapy (1).

Amiodarone and itraconazole have shown promise as an alternative therapeutic treatment option when prescribed together. Historically, amiodarone has been used to treat arrhythmias secondary to Chagasic cardiomyopathy in people (25). It disrupts calcium homeostasis in T. cruzi in vitro, and has a synergistic effect on the survival of trypanosomes when coupled with the ergosterol inhibitor posaconazole (25-26). Itraconazole, a more widely available ergosterol inhibitor than posaconazole, was recently paired with amiodarone in the successful treatment of a Venezuelan man in critical condition due to his Chagasic cardiomyopathy (27).

Treatment of Chagas’ disease in the dog is poorly documented. Drug availability may have contributed to this: neither nifurtimox or benznidazole is readily available in the United States. The main documented side effect of benznidazole in dogs is vomiting (21). One recent experimental study demonstrated a 62.5% parasitological cure for acute infection in 22 dogs, and a 38.7% parasitological cure for 10 chronically infected dogs with Chagas’ disease (28).

Recently, The US Army Veterinary Corps embarked on a clinical trial evaluating amiodarone and itraconazole as a treatment modality for chronically infected MWDs. To date, 12 MWDs have been enrolled in the treatment
group, which received 7.5 mg/kg of amiodarone and 10 mg/kg of itraconazole once daily. In general, the medications were well tolerated, with transient liver enzyme elevations and vomiting being the most common side effects. Results of the study are pending publication (Madigan, personal communication).

**Keywords:** Military Working Dog, Chagas' Disease, Cardiomyopathy

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Critical Care Anesthesia - A Case-Based Review of What's New and Different

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Summary

The critically-ill patient requires careful assessment, planning, monitoring, and support in the event that sedation or anesthesia is required for surgery, a minor procedure, or long-term mechanical ventilation. Critically-ill small animals have altered physiology and may lack the ability to metabolize anesthetic drugs efficiently, putting them at greater risk to exhibit the deleterious side effects of these drugs, particularly hypotension, hypothermia, hypoxemia, and hypoventilation. These patients may develop coagulation abnormalities, cardiac arrhythmias, and multiple organ dysfunction due to their systemic inflammatory response (1, 2). It is important that veterinary teams have the proper training, equipment, medications and support in place prior to hospitalizing critically-ill patients that may require sedation or anesthesia. The acronym LIFESAVER may help guide veterinarians to properly plan for management of critically-ill sedation and anesthesia cases. This lecture will explain the meaning of this acronym and use case examples to reinforce key points.

(Learn equipment function and limitations)

Equipment readiness is a key step required to ensure the veterinary team is ready and capable of managing critically-ill anesthesia cases prior to patient hospitalization. Basic anesthesia and monitoring equipment, to include the anesthesia machine, oxygen source, and vital signs monitors, must be operable and regularly maintained. Sterilized surgery packs, electrocautery, suction equipment, fluid and syringe pumps, defibrillator and a crash cart stocked with appropriate resuscitation drugs should be placed in the operating room and routinely checked for readiness. Anesthesia ventilators should be ready for use in cases that require its use, such as thoracotomies or head trauma. The emergency room should have trochars, stomach tubes, a tracheostomy pack, and various supplies for performing emergent life-saving procedures such as pericardiocentesis, gastric decompression, or thoracostomy tube placement.

(Identify Illness and make specific plan)

Planning anesthesia for critically-ill patients requires a basic understanding of the impact each specific illness has on physiology and drug metabolism. For instance, septic patients are at increased risk for hypotension due to the effects of systemic inflammation and changes in the structural integrity of the endothelium that may occur secondary to this condition. Patients with hepatic or renal dysfunction may have altered metabolism and clearance of anesthetic drugs. In general, however, critically-ill patients may already have exhibited a robust systemic inflammatory response prior to anesthesia, resulting in an increased risk for hyper- or hypocoagulation, acidosis, endothelial dysfunction, hypotension, hypoxemia, hypothermia, and cardiac arrhythmias prior to sedation or anesthetic induction. Clinicians should evaluate their patients carefully and tailor the types and doses of anesthetic drugs based on patient status.
(F)luid support- crystalloids, colloids, or blood products?
Patient condition dictates the use of crystalloids, colloids, or blood products during anesthesia. Crystalloids, such as lactated ringer’s solution or isotonic saline, are most commonly used but a conservative approach should be exercised for patients with trauma or coagulopathy because of the risk of exacerbating hemorrhage and worsening coagulation abnormalities. Typically, continuous rates of infusion are maintained at 5-10 ml/kg/hr with bolus therapy executed in 5-10 ml/kg increments for treatment of hypotension. Colloids such as hetastarch, voluven, and dextrans are used with caution and recommended for only short term, bolus therapy (5-10 ml/kg) for treatment of hypotension during anesthesia. Evidence in human medicine indicates that these colloids may result in a higher risk for acute kidney injury and bleeding, can worsen patient outcome in sepsis cases, and may not provide an improvement in outcome compared to crystalloids in critical illness (2, 3). Blood products, such as fresh whole blood, packed red blood cells, and fresh frozen plasma are commonly used in critically-ill cases and should be quickly accessible for patients that require them. In all cases, use of fluids should be driven by goal-directed resuscitation of certain physiological endpoints such as blood pressure or lactate.

(E)valuate your patient frequently
Patient status can change rapidly during critical illness. For example, it is not uncommon for trauma patients to decompensate due to worsening hemorrhage once they are hospitalized and fluid support has been initiated. Serial physical, and if necessary, neurological exams should be performed at regular intervals prior to, during, and after anesthesia to ensure adequate patient support. Use of cageside ultrasound exams such as the AFAST, TFAST, or VETBLUE are extremely useful to evaluate changes in effusions that may occur in trauma, pericardial disease, or congestive heart failure and can help immediately guide therapy (2, 4).

(S)tabilize your patient prior to surgery
Veterinary teams should work carefully and efficiently to stabilize patients using goal-directed physiological endpoints before anesthetic induction and surgery. Most cases that are properly stabilized prior to surgery have fewer peri-operative anesthetic complications and may be easier to manage. Examples for patient stabilization prior to surgery include packed red blood cell transfusions for traumatic and non-traumatic hemoabdomen cases, intravenous broad spectrum antibiotics and vasopressor support for patients with sepsis and hypotension refractory to fluid support, and gastric decompression and anti-arrhythmic therapy for GDV cases with sustained ventricular tachycardia.

(A)djust your drug doses and regimens
For critically-ill patients, dosages for certain anesthetic drugs may have to be reduced to account for their decreased metabolism and clearance. In general, anesthetic drugs should be chosen that are reversible, rapidly metabolized, and have minimal hypotensive effects. Adequate analgesia must be planned into the anesthetic plan. A multimodal approach, combining an opioid with a benzodiazepine, is a common example of a drug protocol used in critical illness that provides strong analgesic effects, sedation, and can be reversed. Local anesthetics are useful not only to provide analgesia, but to dose-reduce the amount of intravenous opioids and volatile gas anesthesia used during surgery. Several different anesthetic drug protocols will be discussed during the lecture.

(V)ascular access from two or more sites or ports
Management of critically-ill patients during the perioperative period and recovery time in the hospital is going to be significantly easier if multiple vascular access sites are maintained. Critically-ill patients benefit from the placement of a central line with multiple ports (double or triple lumen jugular venous catheters) because of the frequent blood sampling and multiple intravenous drugs that may be needed to support them. Arterial catheters should be placed in patients where hypotension is a significant risk factor, or in patients where
measurement of arterial blood gases would be useful. Dedicated peripheral or central venous catheters are necessary for blood transfusions and parenteral nutrition.

**E**fficiently perform the most appropriate surgery
Surgery on truly critically-ill patients should be performed by the surgeon most experienced with the necessary surgical procedure. In patients where extended surgical and anesthesia time may increase morbidity or mortality, surgeries can be staged to be done on multiple days. For example, damage control surgery for trauma cases may result in the surgeon packing (balloting) a fractured liver to control hemorrhage and immediately closing the abdomen, only to return 12-24 hours later to perform a definitive repair.

**R**e-evaluate your patient frequently after surgery
In most truly critically-ill surgical patients, intensive care is required for the 48-72 hours after surgery. Immediately following surgery, critically-ill patients may exhibit prolonged recovery times requiring use of reversal agents, coagulopathies, arrhythmias, or organ dysfunction secondary to the effects of systemic inflammation. Careful patient evaluation during this time period will allow the veterinary team to appropriately adjust monitoring and treatment protocols and hopefully ensure a successful patient outcome.

**Keywords:** Critical Care, Anesthesia, Small Animal

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Intracranial Pressure Elevation
- Why Dangerous? What to Do?

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Summary
Elevation of intracranial pressure (ICP) is often speculated or encountered in veterinary practice. While ICP elevation can occur due to various underlying intracranial conditions, two common subsequent events associated with ICP elevation are cerebral ischemia and brain herniation. Prevention of these two is the main focus in managing ICP elevation. The goal of this lecture is to understand the basic physiology of ICP and basic principles in managing patients with elevated ICP.

Physiology of the intracranial pressure
Intracranial pressure is the pressure inside the intracranial cavity. Physiologic range of the ICP is between 7-15 mmHg. Intracranial pressure in physiologic status is not constant. It rather is dynamic and pulsatile. Transient increase ICP can be seen associated with coughing, sneezing, and straining. Sustained increase ICP > 20 mm Hg is pathologic and lowering ICP therapy is generally indicated, as the tissues within the cranial vault are no longer able to compensate.

Change in the intracranial pressure is caused by the change in the total volume of the intracranial contents. Monro-Kellie doctrine is an application of some of the principles of physics to the intracranial contents, hypothesized first by Alexander Monro with the experimental support by George Kellie. This doctrine dictates the sum of the total volume in the rigid skull cavity is constant. Among three intracranial components, brain parenchyma (80%), cerebrospinal fluid (10%), and blood component (10%), an increase in one should cause a decrease in one or both of the remaining two.

Although intracranial pressure can be altered by many factors, the following factors are commonly associated with the ICP elevation; 1) increase in CSF, 2) increase in the cerebral blood volume (CBV), 3) increase in the brain parenchyma due to several pathologies such as granuloma, neoplasia, or foreign bodies, or 4) vasogenic brain edema. Among these, elevation of ICP is most commonly caused by 1), 3), 4), or combination of these in small animal practice. According to the Monro-Kellie doctrine, increase in the brain parenchyma causes decrease in other two components, blood and/or CSF, as the compensatory system. This can result in cerebral ischemia associated with the decrement in blood component within the intracranial space.

The following formula is often used to consider the cause and the consequence/associated events of ICP elevation.

\[ CPP = MAP - ICP; \quad CBF = CPP/CVR; \quad CVR = \frac{Lh}{pr^4} \]

(L is vessel length, h is viscosity and r is vessel radius)

CPP: cerebral perfusion pressure
MAP: mean arterial pressure
ICP: intracranial pressure
CBF: cerebral blood flow
CVR: cerebral vascular resistance

When ICP elevation occurs, MAP needs to be increased to maintain CPP. CPP is a primary determinant of CBF. CBF refers to the volume of blood in the cerebral vasculature per unit time. While autoregulatory systems exist for constant CBF in face with physiologic changes in CPP, CBF usually decreases when CPP decreases. CBF is maintained constant despite physiologic changes in CPP as originally hypothesized by Alexander Monro, attributed to the pressure autoregulatory system of the intracranial vascular resistance. Within the physiologic range of CPP (50-150 mmHg), CBF is maintained constant. Intravascular viscosity also contributes to this pressure autoregulation of the intracranial vasculature. Other mechanisms attributing to the constant CBF are as follows; 1) Flow-metabolism coupling, and 2) Chemical autoregulation system. 1) flow-metabolism coupling system changes the intracranial vascular diameter in response to H+ and lactate, which can be considered as byproducts of anaerobic metabolism. 2) Chemical autoregulation system refers to the responsiveness of the cerebral vasculature to the PaCO₂ and PaO₂ if <60mmHg, which can be considered as indicator of total metabolic rate. With these three systems, CBF is maintained constant in order to meet the various metabolic demand in the particular area of the brain. Our goal in maintaining CBF is to control the above three systems; i.e. maintenance of CPP through lowering ICP, restoring or maintaining the aerobic metabolism, and maintenance of normal PaCO₂.

Cushing reflex can be seen in some patients with acute ICP elevation. With ICP elevation, vasomotor center-mediated elevation of the mean arterial blood pressure (MAP) and intracranial vascular dilation occurs aiming to maintain CPP. In response to the elevation of MAP, baroreceptor-mediated reflex causes decrease in the heart rate. The classic findings in Cushing reflex; systemic hypertension, bradycardia, mental disturbance, and change in respiratory pattern, are typically recognized in patients with acute elevation of ICP. In contrast, chronic conditions such as intracranial neoplasia tend to show the characteristic findings of classic Cushing’s reflex less frequently. Systemic hypertension along with other findings such as loss of appetite, intermittent tetra/para-paresis, lethargy, personality change, can be often seen in this subpopulation.

Brain edema and ICP
Brain edema can cause elevation of ICP, and elevation of ICP can lead to the progression of brain edema. Once ICP elevation occurs, cerebral vascular dilation occurs to maintain CBF. This increase in CBF can lead to the development or progression of the vasogenic brain edema. As the vasogenic brain edema results in increase in the brain parenchyma volume, it causes ICP elevation. To minimize this vicious cycle, vasogenic edema should be addressed promptly.

Clinical manifestation
Clinical manifestation of ICP elevation greatly varies, and the clinical signs are generally not sensitive indicator of ICP elevation. Common clinical findings with ICP elevation are as follows; mental disturbance, behavior change, lethargy, anorexia, intermittent/episodic collapse or tetra-/para-paresis, systemic hypertension with or without bradycardia, pupillary changes, cervical hyperesthesia, nausea, and cranial nerve dysfunction. It appears that the degree but also the speed of elevation greatly influence the clinical manifestation.

ICP reduction therapy
Prevention of cerebral ischemia and brain herniation are two main goals with ICP reduction therapy. Although it is ideal to confirm ICP elevation with the aid of ICP monitor or some objective findings such as MRI prior to commence ICP reduction treatment, it is not uncommon ICP elevation to be suspected or speculated based on the clinical manifestation. For the treatment, along with the treatment for the underlying etiology,
corticosteroid for vasogenic edema and/or osmotic diuresis such as Mannitol (0.25-1.0g/kg CRI over >20 min) or hypertonic saline solution (3% - 3-5mL/kg, 7% - 2-4mL/kg CRI >20 min) for acutely deteriorating patients, head elevation by 20-30 degrees to promote venous return, IV fluid for maintenance of normotension are commonly used. While rare in veterinary medicine, emergency CSF shunting procedure or skull bur hall procedure for extra-axial intracranial hematoma evacuation have been described in human medicine.

Keywords: Intracranial Pressure, Cushing's Reflex, Brain Herniation
Traumatic Brain Injury

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Summary

Traumatic brain injury (TBI) can be a life-threatening condition. Although general principles in trauma management can be applied, additional consideration as for the intracranial component can dramatically change the course of action. Modified Glasgow Coma Scale (MGCS) can be utilized to aid the objective assessment of the patients' neurologic status over time. According to the guidelines for the management of severe TBI published by the Brain Trauma Foundation, osmotic diuretics and intravenous fluid to maintain normotension are listed as the recommended treatments.

Primary Injury

Mechanical disruption of brain tissue at the time of impact. Frequent cause of traumatic brain injury in small animals are motor vehicle accident, animal fight, penetrating injuries, falls, or abuse. Types of primary injury occurring at the moment of impact include lacerations, skull fractures, cortical contusions, hemorrhage, and diffuse axonal injury. Coup contusion occurs at the site of impact in the absence of a fracture. A countercoup contusion occurs in the brain opposite the point of impact. Skull fractures may or may not have significant consequences. While rare, contaminated and severe depressed fractures need immediate surgical intervention.

Diffuse axonal injury (DAI) results from accelerative-decelerative forces associated with high-energy trauma, most commonly with the motor vehicle accident or shaken baby syndrome in humans. The patients with DAI are commonly presented with coma. Axonal fibers are diffusely disrupted, most commonly recognized in the gray matter-white matter junctions with microbleeding. While DAI can be suggested with cross-sectional imaging as specific pattern of microbleeding, it can also occur without significant abnormal findings in MRI/CT.

Hemorrhage

Hemorrhage may be severe if there is laceration of a venous sinus. Intracranial hemorrhage is classified based on the location as below:

- Epidural – hemorrhage between the dura and the calvaria; usually caused by a skull fracture lacerating the middle meningeal artery. Commonly seen in young human patients without significant adhesion between the skull and dura; rare in animals
- Subdural – hemorrhage between the dura and the subarachnoid layer; considered rare
- Subarachnoid – hemorrhage into the subarachnoid space; usually caused by disruption of the vessels in the subarachnoid space; common in animals
- Intraparenchymal – hemorrhage into the brain parenchyma; caused by disruption of parenchymal vessels; common in animals

Secondary injury

Secondary injury follows the physiologic and metabolic abnormalities created by the primary injury. Among various mechanisms and metabolic cascades in the secondary biochemical injury, the most hazardous factors for neuroprotection are hypoxia and hypoperfusion. Ultimate consequence is apoptosis or cell death
of the neural tissue as the result of combination of these factors. Other major factors in secondary injury are excitotoxicity and reactive oxygen species. Our main therapeutic target in most of TBI patients is to minimize the secondary injury.

Intracranial pressure monitoring
Intracranial pressure (ICP) elevation is the leading cause of death in human patients with TBI. Although ICP can be measured and monitored with ICP monitors embedded within the cranial cavity, a recent study by Chesnut et al. in human patients with TBI demonstrated that measurement of ICP may or may not result in significantly improved outcome.

Medical management of TBI
General principles of trauma management should be applied – ABC (airway, breathing, circulation) should be secured first. Oxygen supplementation is routinely recommended as hyperoxegenation is considered beneficial. Use continuous pulse oximetry monitoring to ensure >95% SpO2.

Prevent systemic hypotension. Blood pressure measurement is routinely recommended as systemic hypotension is recognized as a negative prognostic factor in patients with TBI. The Brain Trauma Foundation recommends maintaining systolic arterial pressure > 90mmHg with the comment that recommended mean arterial pressure value should be investigated in the future. Isotonic crystalloid fluid CRI to maintain euvolemia in these patients improves hemodynamic stability without forcing water into the brain interstitial space with high static pressure. Other fluids or medications such as colloids, hypertonic saline, or vasopressor agents can also be used as necessary. As for vasopressors, controversy exists regarding which agent is superior to others.

Reduce ICP
Osmotic diuretics are extensively used in patients with TBI for this purpose. While mannitol and hypertonic saline are described elsewhere, it has not been concluded which is superior to the other.

1) Mannitol: exerts ICP reducing effect through multiple mechanisms; 1) reflexive vasoconstriction due to decreased intravascular viscosity – during and immediately after the infusion, 2) osmotic diuretic effect – 30-45 minutes following the infusion. The reflection coefficient of mannitol is 0.9, suggesting that repeated administration can result in the leak of mannitol molecules into the extravascular space, which can result in rebound elevation of ICP by promoting the water molecule shift from the intravascular space to the brain parenchyma. Plasma osmolality 320mmOsm/L is considered the upper limit of mannitol usage. Risk of renal injury increases if mannitol increases the plasma osmolality >330 Osm/L. Mannitol is contraindicated in patients with severe dehydration, severe hypovolemia, chronic anuric renal failure, pulmonary contusion, pulmonary hemorrhage, or pulmonary edema.

2) Hypertonic saline (HTS): exerts ICP reducing effect through similar mechanisms to those in mannitol. HTS has several advantages; exerts osmotic diuretic effect without reducing the intravascular volume, its reflection coefficient is 1.0, and requires relatively small volume. Plasma electrolytes should be monitored initially every 2 hours following HTS administration aiming plasma Na concentration of 150-155 mmol/L.

Corticosteroids for TBI?
Brain Trauma Foundation guideline states that there have been strong (Level 1) evidences not to recommend the usage of high dose methylprednisolone in patients with TBI. It is associated with increased mortality and is contraindicated in TBI. Although the exact mechanism leading to unfavorable outcome with high dose corticosteroid is unclear, the role of hyperglycemia which could be induced by corticosteroid may play some role. As the oxygenation and perfusion in the damaged brain tissue tend to be compromised, hyperglycemia with insufficient oxygen may result in lactic acidosis in the damaged microenvironment through anaerobic respiration.
Other treatment modalities such as insulin administration, induced hypothermia, pentobarbital coma, are still in some controversy.

**Keywords:** Traumatic Brain Injury, Head Trauma, Mannitol
The Acute Abdomen in Working Dogs - Differentials, Management, and Prevention Strategies

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Summary
Acute abdomen is a term used to describe the emergency presentation of an animal in which abdominal pain or discomfort is a primary clinical sign. There are a multitude of potential causes, such as trauma, neoplasia, sepsis, or other diseases affecting the hepatic, gastrointestinal, urinary, or reproductive systems. In the Military Working Dog (MWD), presentation with an acute abdomen is relatively uncommon. Most MWDs are German Shepherds or Belgian Malinois dogs, but Labrador Retrievers, German Short-haired Pointers, and other medium-to-large sized breeds comprise a very small minority of the MWD population. Because of the predominance of several highly energetic breeds within their population and the occupational hazards they encounter, MWDs who present with an acute abdomen may do so because of trauma, dietary indiscretion, gastric dilatation with volvulus (GDV), mesenteric volvulus, or neoplasia. However, other causes are possible and have been treated within the MWD population, such as biliary obstruction, pancreatitis, pyometra (within the small MWD breeding population), and splenic torsion. This lecture will review the general diagnostic strategy for acute abdomen and discuss several cases that highlight the more common acute abdomen presentations for MWDs. Finally, the impact of GDV and mesenteric volvulus on the MWD population will be presented.

Clinical presentation
The acute abdomen often presents with vague or non-specific clinical signs such as lethargy, anorexia, vomiting, diarrhea, weight loss, or abdominal distention. Abdominal pain is a key clinical sign in all cases and may be mild or severe in nature depending on the root cause or chronicity of the pain. The animal may present with varying degrees of dehydration or shock. GDV and mesenteric torsion cases in MWDs can present in severe shock with the animal in lateral recumbency or non-responsive. Unfortunately with mesenteric volvulus, MWDs may be found recumbent or even dead in their kennels because the condition can rapidly progress and deteriorate and dog handlers may miss early, subtle signs of pain. Note that referred abdominal pain from other conditions, such as discospondylitis or large volume ascites due to congestive heart failure, can confuse clinicians into initially diagnosing a primary abdominal cause for the acute abdomen (1).

Diagnosis
Besides a thorough physical exam, a complete history, abdominal imaging, and laboratory tests such as a complete blood count, serum biochemistry panel, and urinalysis comprise the minimum database for workup of an acute abdomen. Historical findings may help determine the chronicity of the condition and identify dietary indiscretion and trauma as potential root causes for the acute abdomen. Choosing the most helpful abdominal imaging modality for diagnosis can sometimes be challenging and is dependent upon availability of equipment and expertise with that equipment at the site of initial evaluation. In general, abdominal
radiography is very useful in diagnosing GDV, mesenteric volvulus, GI obstructions from foreign body ingestion, or uroabdomens (with use of contrast). Cageside ultrasound exams (AFAST or COAST) can easily identify peritoneal effusion, splenic or hepatic tumors, or biliary mucocele and should strongly be considered a standard test to perform early during patient assessment (3). If peritoneal effusion is identified, it must be aspirated via abdominocentesis and evaluated to determine its origin.

GDV
Historically, GDV comprised a significant mortality risk in MWDs, resulting in 9.1% of all German Shepherd and Belgian Malinois deaths from 1993-1996. In part due to this study, a comprehensive effort was made by the US Army Veterinary Corps to gastropexy all active duty MWDs. At present, MWDs are gastropexied before entering initial training by the veterinarians at the LTC Daniel E. Holland MWD Hospital on Lackland Air Force Base, TX. This effort has nearly completely eliminated the incidence of GDV in MWDs. Military veterinarians still do encounter GDV cases in deployed environments such as in Iraq or Afghanistan, but the working dogs who succumb to this condition are primarily non-gastropexied working dogs owned by private companies that enhance the Department of Defense’s war effort through government contracts (5).

Mesenteric volvulus
A perceived increase in the incidence of fatal mesenteric volvulus cases in MWDs in the early 2010s prompted several military veterinarians to do a comprehensive MWD record review to identify possible risk factors for this observed trend. 54 cases of MWD mesenteric volvulus were identified from 1990-2014. Comparing this population with 162 unaffected control MWDs, it was determined that both a prior prophylactic gastropexy, a prior abdominal surgery other than gastropexy, use of NSAIDs at the time of volvulus, and history of other gastrointestinal disease, were significant risk factors for the occurrence of mesenteric volvulus (4). At present it is unclear how or why these risk factors might increase the potential risk for mesenteric volvulus. Despite the risk identified in this study, MWDs are still routinely gastropexied to prevent the occurrence of GDV.

Keywords: Mesenteric Torsion, Acute Abdomen, Military Working Dog

References
**Ultrasound - A Vital Tool All Clinicians Should Master for Diagnosis and Management of Emergency and Critical Care Cases**

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**Summary**

The use of ultrasound as a rapid bedside modality for evaluating trauma in people developed into the standard of care during the 1990s (1). Veterinary medicine gradually adopted the use of ultrasound in emergency practice several years later, and validation of its use emerged in the literature during the 2000s by Boysen et al. (2) and Lisciandro et al (3-4). These studies prompted the Focused Assessment with Sonography for Trauma (FAST) scans for the thorax (TFAST) and abdomen (AFAST) to become an essential tool in the diagnosis and monitoring for small animal trauma patients. As ultrasound became more widely available in veterinary medicine, practitioners realized its value in evaluating emergency and critical care patients beyond those who are evaluated for trauma. The cageside ultrasound exam is a rapid point-of-care evaluation of the small animal patient that provides immediate and valuable diagnostic information for a variety of potentially critical conditions, including cardiac, respiratory, gastrointestinal, genitourinary, hepatic, renal, ocular, and musculoskeletal disorders (5). Furthermore, ultrasound is safe for the small animal patient and practitioner because there is no radiation exposure and it can be used with minimal restraint. As a result, veterinary medicine has seen the emergence of a variety of cageside ultrasound techniques such as VETBLUE (Veterinary Bedside Lung Ultrasound Exam), limited echocardiograph exams, and COAST® (Cageside Organ Assessments for Trauma, Triage, and Tracking) (6). These techniques not only have proven invaluable for initial triage and assessment of emergency patients, but are gradually becoming the standard of care to track progression of certain conditions and evaluate the patient’s response to therapy. This lecture will first briefly review the basic principles of ultrasound in order to enable the practitioner to understand how an image is formed and to recognize common artifacts. Probe choice and common settings for different types of focused exams will be discussed. Several of the most common types of exams used in emergency and critical care practice will be reviewed, to include AFAST, TFAST, VETBLUE, rapid echocardiograms, and several types of COAST® exams. Case examples will be provided for each type of exam along with a discussion of practice tips such as positioning, data collection, and interpretation of image findings. Intertwined with case discussion will be a review of the most recent veterinary literature that supports each type of focused ultrasound exam.

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**Keywords:** FAST Exam, VETBLUE, Ultrasound

**References**


Acute Spinal Cord Injury

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Summary
Acute spinal cord injury (ASCI) is commonly encountered in veterinary medicine, especially in small animal practice. ASCI generally is created by the primary injury followed by the subsequent secondary injury. The goal of this lecture is to understand the general pathophysiology of the ASCI, initial assessment, common differential lists, characteristic events accompanied with ASCI, consideration in imaging, and the medical management strategy of ASCI.

Primary Injury
Spinal cord injury can be created by either external or internal causes: external – automobile accident, falls, animal fight, natural disaster, and abuse; internal – intervertebral disc disease, pathologic fractures, and vascular damages. Mechanisms of primary spinal cord injury include compression, concussion, contusion, laceration, share, distraction, and bending. Along the spinal column, thoracolumbar spinal cord segment is the most commonly affected by the acute spinal cord injury. Vertebral fractures occur most commonly in the thoracolumbar and lumbosacral area, followed by the cranial cervical region. Fractures in the thoracolumbar regions are often associated with other systemic injuries: pulmonary contusions, pneumothorax, hepatic injuries, diaphragmatic hernia, or urogenital injuries. Fractures in the lumbosacral regions are often associated with pelvic fracture, femoral fracture, iliosacral luxation, or urogenital injuries.

Secondary Injury
Primary mechanical spinal cord injury is commonly followed by the secondary biochemical spinal cord injury. Acute spinal cord injury results in various systemic and local vascular events, including vascular endothelial damage, vascular spasm, hemorrhage, thrombosis, or ischemic events. These vascular compromising events can ultimately lead to the decreased perfusion or necrosis of the neural tissue. The primary spinal cord injury can also lead to excitotoxicity associated with abnormal release of excitatory neurotransmitters, activation of membrane phospholipases and production of free radicals. These various biomechanical events can sometimes be more detrimental to the neuronal tissue than the primary mechanical injury.

Initial Evaluation
If a spinal fracture-luxation is suspected, the patient should be secured and immobilized to a back board to prevent further injury. The patient should be evaluated in the position at the initial presentation with no or minimal additional movements or unnecessary handling. Chemical restraint can be added to this physical restraint if the animal struggles, ideally after concise neurologic evaluation. The basic principles in trauma patient management should be applied to the animals with spinal trauma by ensuring the stabilization of the physical status, including the assessment of the airway patency, breathing and the circulatory status. Particular attention should be paid to the respiratory status in the patients with cervical spinal cord injury. Quick assessment of the respiratory status as well as blood gas test and/or a set of chest radiographs should be performed accordingly.
Neurologic Assessment

The neurologic examination is used to determine neuroanatomic localization and severity of the spinal cord injury. It is important to perform the neurologic examination with care to prevent further injury and motion / displacement of the spine in patients with traumatic ASCI. Mental status, basic cranial nerves, spinal reflexes, paraspinal palpation for hyperesthesia, cutaneous trunci reflex, and assessment for deep pain perception can be performed in lateral recumbency. The most important prognostic indicator is presence or absence of deep pain perception. Patients without no deep pain for > 48 hours have a grave prognosis. Dogs with suspected intervertebral disc disease and absent deep nociception for < 24 hours have a 50-60% chance of regaining ambulatory status. In patients with traumatic ASCI and absent deep nociception, the prognosis for successful functional recovery is unfortunately hopeless.

Clinical characteristic findings in the respective neuroanatomical localization are as follows; a C1-C5 lesion results in tetraparesis/plegia or hemiparesis/plegia. Spinal reflexes are normal or exaggerated. Severe lesions can cause respiratory paresis along with paresis of the limbs. Patients with severe hypoventilation (PaCO$_2$ > 55mmHg or PvCO$_2$ >60mmHg) associated with cervical spinal cord disorders may require peri- and postoperative mechanical ventilatory support. A C6-T2 lesion results in tetraparesis/plegia or hemiparesis/plegia. Spinal reflexes in the thoracic limbs may be weak or absent with those in the pelvic limbs intact. A T3-L3 lesion causes varying degrees of paresis/paralysis in the pelvic limbs with intact spinal reflexes. A L4 through caudal vertebrae lesion can cause LMN weakness in the pelvic limbs and tail. Hindlimb and perineal reflexes may be weak or absent. Severe lesions result in loss of deep nociception to tail. Flaccid tail paralysis along with LMN urinary and/or fecal incontinence accompanied with relatively mild motor deficits in the hindlimbs can be sometimes seen in animals with acute injury to this sacral spinal cord segment or the lumbosacral nerve roots.

Medical management for ASCI

As the primary ASCI has been completed by the initial presentation, the main therapeutic target in the general medical management of ASCI has been focused on the neuroprotective approaches to attenuate the effects of secondary SCI. While methylprednisolone sodium succinate (MPSS) has been discussed for its potential role in neuroprotection, a recent study demonstrated that high dose (30mg/kg) of MPSS showed no significant beneficial effect on the functional outcome in dogs with severe thoracolumbar intervertebral disc disease, the most common underlying etiology of ASCI in dogs.

In patients with ASCI, maintenance of normal blood pressure to maintain normal perfusion pressure to the spinal cord and other organs, and adequate pain management are recommended as a part of general medical management strategy for patients with acute trauma. Hypoperfusion often follows acute CNS injury (ASCI or traumatic brain injury) due to the multiple factors; loss of sympathetic tone associated with the interruption to the first or second order neurons of the sympathetic nervous system, spinal shock causing loss of muscle tone and secondary hypovolemia from venous pooling, and blood loss from other organ injuries. To minimized hypoperfusion to the damaged spinal cord, adequate fluid therapy to maintain the normal blood pressure is generally recommended in ASCI patients.

Imaging study

The neurologic exam findings provide the most important and relevant information for the prognosis irrespective of the imaging findings in ASCI. Survey radiographs will provide baseline information regarding the vertebral column integrity, vertebral instability, or other bony abnormalities. A set of orthogonal views is always recommended to assess the vertebral integrity. Kinns et al. reported that sensitivity of the survey radiographs for the vertebral fracture/displacement was 72/77.5%, respectively, with CT findings being used as the gold standard. If a patient undergoes CT, the whole vertebral column scan is advised as multiple spinal fracture/displacement occurs in 10-15% of patients. In spinal trauma, the spinal instability and/or the significant spinal cord compression are two main factors for consideration of surgical intervention.

While other diagnostic imaging modalities have each own advantages and disadvantages, MRI is considered as the choice in ASCI, except for traumatic ASCI. CT is often suitable for those with obvious physical
evidence or clear history of traumatic ASCI. MRI then may follow as needed. While myelogram provides as great spatial resolution as survey radiographs, with the disadvantages such as inability to assess the spinal cord parenchyma, insufficient capability to provide the supplemental information regarding the pathophysiology of the extradural spinal compressive mass or the loss of the contrast column, careful case selection and the interpretation is mandatory with myelogram.

**Keywords:** Acute Spinal Cord Injury, Neuroanatomical Localization, Spinal Fracture
Generalized LMN Conditions

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Summary
Generalized Lower Motor Neuron (LMN) conditions can be challenging not only with diagnosis but also with management. These patients can present for various and sometimes confusing clinical manifestations. They are frequently localized to the cervical spinal cord or the intracranial lesion because of the clinical signs affecting all four limbs with occasional cranial nerve deficits, or could be confused with other systemic conditions. This lecture will provide the basic physiologic concept of lower motor neuron system as well as common differentials for acutely progressive generalized LMN conditions.

Paralysis
Paralysis generally refers to the loss of motor function, categorized into paresis and paralysis referring to the partial or complete loss of motor function in the affected area, respectively. Domestic animals have two different types of motor neuron systems; upper motor neuron system (UMN) and lower motor neuron (LMN) system. While the former is located within the central nervous system, the latter is originated within the CNS as the motor neuron cell bodies, then extends out of the CNS to form the peripheral motor nerve.

UMN system are generally excitatory to flexor muscles and inhibitory to extensor muscles. With UMN dysfunction, therefore, affected limbs tend to exhibit excessive extensor muscle tone while standing and walking. LMN system functions as the final common pathway of the motor system; i.e. contraction of the skeletal muscle. LMN dysfunction generally results in flaccid paralysis of the affected limbs. Generalized LMN conditions refers to those affecting the LMN system throughout the body. Although these conditions typically manifest as tetraparesis due to the trunk and all four limbs involvement, some conditions affect the cranial nerves as well. Most of the common generalized LMN conditions affect the peripheral portion of the LMN system, which makes the cross-sectional imaging modalities such as MRI or CT unsuitable for the diagnostic workup.

Neurolocalization
Neurolocalization is the essential component in diagnosing generalized LMN conditions. As the result of LMN dysfunction, the loss of skeletal muscle contraction occurs. Consequently, the following clinical findings are 3 hallmark signs of LMN dysfunction. 1) loss of skeletal muscle tone, 2) loss of spinal reflex, and 3) neurogenic muscle atrophy. The last finding can be seen only in the chronically denervated situation. If the patient exhibit all or some of these hallmark signs in all four limbs, generalized LMN condition is most likely. With pure generalized LMN conditions, patients should retain normal proprioception. The most reliable postural reaction is knuckling test with appropriate weight support. Unless extremely severely affected, patients retain the capacity to flip the knuckled toe back to the normal position as it requires minimum muscle strength and contraction.

Acute progressive generalized LMN conditions in dogs
The clinical course of generalized LMN conditions in dogs varies. Common differential conditions in dogs
with acute progressive conditions include acute idiopathic polyradiculoneuritis, fulminant myasthenia gravis, tick paralysis in selected regions, and botulism. Rabies should be included in the differential list if indicated. Chronic conditions include various etiologies such as infectious/inflammatory, metabolic, endocrine, paraneoplastic, genetic, or toxic etiology. Therefore, diagnostic workup may be extensive in some cases. These chronic conditions are beyond the scope of this lecture.

Hypoventilation
Hypoventilation refers to insufficient gas exchange in the pulmonary alveolar respiration. Severely affected lateral recumbent animals may be in insufficient ventilatory status. The clinician dealing with severely affected animals should check the ventilator status with arterial blood gas or venous blood gas combined with SPO2. Chest radiographs are also indicated in most patients. While the mechanical ventilator support may be considered in animals with severe hypoventilation with PaCO2 >55mmHg or PvCO2 >60mmHg, the prognosis is guarded. A recent study by Rutter et al. described that 6/14 dogs were successfully weaned from the ventilator with a median ventilator time of 49 hours, and 3/14 dogs survived to discharge.

Megaesophagus and aspiration pneumonia
Esophagus can be affected in generalized LMN conditions, especially in dogs as their esophageal musculature is predominantly composed of the skeletal muscle. Feline patients are less frequently affected as their esophagus is predominantly composed of the smooth muscle. Among several common generalized LMN conditions, myasthenia gravis in dogs commonly affect the esophagus. Animals with megaesophagus associated with generalized LMN conditions are at high risk of aspiration pneumonia, especially with non-ambulatory status.

Diagnostic workup
Patient's history and clinical signs generally provide the clinician some hint on differentiating common conditions in dogs (table 1). The etiology, diagnostic workup, and brief outline of the treatment for each condition will be discussed in the lecture.

Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cranial N signs</th>
<th>Autonomic signs</th>
<th>Spinal reflexes</th>
<th>Others</th>
<th>Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute idiopathic olyradiculoneuritis</td>
<td>Dysphonia Facial nerve</td>
<td>Rare</td>
<td>Decreased</td>
<td>Possible hyperesthesia</td>
<td>Ventral nerve roots +/- dorsal nerve root</td>
</tr>
<tr>
<td>Tick paralysis (US)</td>
<td>Possible</td>
<td>Rare</td>
<td>Decreased</td>
<td>Tick</td>
<td>Presynaptic terminal</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>Dysphonia Facial nerve Megaesophagus</td>
<td>Urinary retention</td>
<td>Generally normal</td>
<td>Fatigability</td>
<td>Postsynaptic (Ach-R)</td>
</tr>
<tr>
<td>Botulism</td>
<td>Common seen as tongue paralysis and pharyngeal paralysis</td>
<td>Common</td>
<td>Decreased</td>
<td></td>
<td>Presynaptic vesicle</td>
</tr>
</tbody>
</table>

*Ach-R: anti-acetylcholine recepeter

Keywords: Generalized LMN Dysfunction, Myasthenia Gravis, Tetraparesis
Advances in Hereditary Diseases and Genetic Predispositions in Dogs and Breed Characteristics

Urs GIGER

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Summary
Many of the characteristic breed traits and common and rare diseases seen in veterinary practice have a heritable basis. Recent exciting advances in our current knowledge of the completed dog genome sequence and the molecular genetic tests offer the opportunity to clinicians and clinical pathologists to use these emerging tools in clinical practice and have a positive impact on the health of dogs and in particular the diagnosis, management, and control of hereditary diseases. In this and subsequent sessions, practical aspects on breed structure and characteristics, practical diagnostic clinical, imaging, laboratory and genetic tools and management will be addressed and illustrated with many case examples.

Introduction
There are many unique traits of canine breeds and many hereditary disorders and genetic predispositions to disease have been identified. With the recent completion of the canine and feline genome sequences and molecular techniques these genetic (breed) traits and defects have been and are being characterized from the clinical signs to the molecular defect. Many specific breed traits such as size, chondrodysplasia, brachycephaly and many skin and coat color characteristics have recently been defined. While clinical and routine laboratory and imaging tests are helpful, specific biochemical and DNA tests have become available for >200 single gene defects through various laboratories in dogs. Moreover, with DNA tests it is now possible to determine the ancestry of mixed breed and purebred dogs, a first example of a complex trait. As it is difficult to keep track of all these hereditary diseases, tests, and treatments, a web-based database for available DNA tests on hereditary diseases in companion animals for clinicians is available (http://research.vet.upenn.edu/WSAVA- LabSearch).

Because of the increased awareness of breeders, pet owners, and veterinarians of genetic defects and the improved diagnostic abilities in clinical practice, the number of reported hereditary diseases in small animals is rapidly growing. At present, >900 hereditary diseases in dogs have been adequately documented. For the small animal practitioner, it can be a daunting, nearly impossible task to remember all these diseases and be aware of the many novel tests and their appropriate management and control.

Databases on hereditary diseases: It is difficult for a clinician to keep up with the rapidly accumulating information on clinical genetics and the large spectrum of disorders and genetic predispositions. Thus, comprehensive update resources are needed. There are several web sites that provide some information on many different diseases in companion animals such as “Inherited Diseases in Dogs” (http://www.vet.cam.ac.uk/idid/); Mendelian Inheritance in Animals, http://www.angis.org.au/ Databases/BIRX/omia; Canine Inherited Disease Database http://www.upei.ca/~cidd/intro.htm; and the FAB list of feline hereditary disorders www.fabcats.org/breeders/inherited_disorders. The WSAVA Committee on Hereditary Diseases has set up a database on
genetic tests for hereditary diseases (http://research.vet.upenn.edu/WSAVA-LabSearch; www.wsava.org and www.VIN.com) with pertinent practical information on clinical features, genetic diagnostics, and management specifically for the clinician.

**Funding and Conflicts of Interest:** Author’s studies were supported in part by grants from the National Institutes of Health (OD010939) and the AKC Canine Health and other Foundations. The author is the director of the non-for-profit PennGen Laboratory offering genetic and hematological testing.

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**Keywords:** Hereditary Diseases, Genetic Predispositions, Dog

**References:** Specific references available upon request
Clinical Diagnostic Approach to Hereditary Diseases in Companion Animals

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Summary
While clinical and routine laboratory and imaging techniques are helpful, specific biochemical and DNA tests have become available for >200 disorders through various laboratories. This session will discuss various diagnostics for hereditary diseases and illustrate these tools with case examples. Simple test sample requirements and result interpretations are presented to use in clinical practice.

Beyond physical examination and imaging tools, genetic, metabolic, and other laboratory techniques are used to diagnose hereditary disorders in companion animals. Most genetic defects cause clinical signs early in life. The term congenital does only imply that the disease is present at birth, and does not necessarily mean it is inherited. A common presentation is failure-to-thrive compared to littermates. They are poor doers, often fade (hence the term fading puppy syndrome), and finally die. Failure-to-thrive should not be confused with growth retardation, dwarfism. In addition to these relatively unspecific clinical signs, some defects may cause specific clinical manifestations. Easy to recognize are malformations that involve any part of the skeleton and lead to disproportionate dwarfism, gait abnormalities, and/or facial dysmorphia. A large number of hereditary eye diseases have been described in dogs, some of which are not recognized until adulthood. Neuromuscular signs may vary from exercise intolerance to ataxia and seizures. Defects of many other internal organs are associated with unspecific clinical signs.

Diagnostic tests are generally required to further support a genetic disorder in a diseased animal. Radiology and other imaging techniques may reveal skeletal malformations or cardiac anomalies, and an ophthalmologic examination may further define an inherited eye disease, although some are not recognized until several years of age. Routine tests such as complete blood cell count, chemistry screen, and urinalysis may suggest some specific hematological or metabolic disorders or rule out many acquired disorders. Furthermore, clinical function studies may more clearly define a gastrointestinal, liver, kidney, or endocrine problem. Histopathology and/or electron microscopy of a tissue biopsy from an affected animal or from the necropsy of a littermate or relative may give the first clue to a genetic defect.

A few laboratories provide special diagnostic tests that allow a specific diagnosis of an inborn error of metabolism. Inborn errors of metabolism include all biochemical disorders due to a genetically determined, specific defect in the structure and/or function of a protein molecule. Disorders of intermediary metabolism typically produce a metabolic block in a biochemical pathway leading to product deficiency, accumulation of substrates, and production of substances via alternative pathways. The most useful specimen to detect biochemical derangements is urine because abnormal metabolites in the blood will be filtered through the glomeruli, but fail to be reabsorbed, as no specific renal transport system exist for most abnormal metabolites. The Metabolic Genetic Disease Laboratory at the University of Pennsylvania offers such tests.
http://research.vet.upenn.edu/pennngen. Similarly, Cornell’s Comparative Coagulation Laboratory offers functional testing for many bleeding disorders (http://ahdc.vet.cornell.edu) and the Comparative Neuromuscular Laboratory makes some functional and mostly histological analysis available for muscle and nerve disorders (http://vetneuromuscular.ucsd.edu). Once the failing system has been identified, the defect can be determined at the protein level. Homozygously affected animals have very low protein activity and/or quantities (0-10%). These tests may also be used to detect carriers (heterozygotes), who typically have intermediate quantities at the protein level (30-70%), but no clinical signs. Unfortunately, protein assays require submission of appropriate tissue or fluid under special conditions to specialized laboratories along with a control sample, and are labor intensive.

Many DNA screening tests have been developed. These tests are mutation or DNA marker specific and can, therefore, only be used in animals suspected to have the exact same gene defect. Small animals within the same or a closely related breed will likely have the same disease-causing mutation for a particular disease. However, dogs and cats as well as unrelated breeds of a species with the same disorder will likely have different mutations. On the other hand, a few mutations have been found in a few breeds or may be widespread within the canine population. For instances different mutations have been found to cause anemia due to pyruvate kinase deficiency in the different breeds, while a single mutation in the phosphofructokinase gene has been found to cause hemolytic anemia in English Springer Spaniels, Cocker Spaniels, Whippets, and mixed breed dogs. For many inherited disorders, the defective gene remains unknown; however, for a few, a polymorphic DNA marker that is linked to the mutant allele has been discovered. Some mutation and linkage tests have to be further defined such as renal dysplasia in a several terrier breeds. At present, mutation-specific and some linkage tests are available only for single gene defects in small animals; however, complex genetic traits may also soon be approached by these methods. Many predispositions such as inflammatory, immune-mediated, malignant disorders have a genetic basis. While many more single gene defects are being studied from clinical signs to the molecular defect, current investigations are shifting toward complex genetic traits. The many breed predispositions for various complex genetic traits are particularly attractive to further define their molecular bases.

DNA tests have several advantages over other biochemical tests. The test results are independent of the age of the animals, thus, the tests can be performed at birth or at least long before an animal is placed in a new home as well as before clinical signs become apparent. DNA is very stable and only the smallest quantities are needed; hence, there are no special shipping requirements as long as one follows the specific mailing instructions for biological products. DNA can be extracted from any nucleated cells, e.g., blood, buccal mucosa (using cheek swabs), hair follicle, semen, and even formalinized tissue. For instance, blood can be sent in an EDTA tube or a drop of blood can be applied to a special filter paper; buccal swabs can be obtained with special cytobrushes – the cheek cells and not the saliva is needed and swabs need to be completely dried. The DNA segment of interest, which is surrounding the mutation, is amplified with appropriate DNA primers utilizing the polymerase chain reaction (PCR). The mutant and/or normal alleles are identified by DNA fragment size or base pair differences. These tests are generally simple, robust, and accurate as long as appropriate techniques and controls are used. Furthermore, they can be used not only for the detection of affected animals, but also for carriers from birth on. All currently available DNA tests for hereditary diseases in dogs and cats and associated laboratories worldwide can be found at http://research.vet.upenn.edu/WSAVA-LabSearch. Furthermore panel screening for all reported mutations has been reported and may be most cost effective as long as one is assuring that the mutation found also causes disease in another breed and genetic counseling is provided.

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**Keywords:** Hereditary Diseases, Genetic Predispositions, Dog

**References:** Specific references available upon request
Therapy and Control of Hereditary Diseases

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Summary
While the therapeutic interventions for hereditary diseases are somewhat limited, some can be managed successfully with medical and/or surgical interventions. Cures for hereditary diseases are rare unless one considers mostly experimental transplantation and gene therapy. For several hereditary diseases, there are specific therapies available and practical. For others, supportive measures can make the animal more comfortable. Even more important is the control of these diseases in future generations by informed breeding of dogs to avoid the production of any affected animals and still to preserve the gene pool in each breed.

Surgical and Medical Therapy
At present, the therapeutic options in the treatment of hereditary diseases are limited and ethical principles need to be carefully considered. Many hereditary diseases are progressive with currently only palliative therapeutics available, and thus lead to the early demise of a diseased animal or euthanasia. Surgical interventions may correct some malformations including some orthopedic and eye problems as well as hepatic shunts, but such animals should be altered to prevent them from being used for breeding. Similarly, the newly discovered androgen-dependent cystinuria is cured by surgical or medical castration. In a few cases a deficient protein, cofactor, substrate, or metabolite can be supplemented to correct the defect. For instance, vitamin B12 (cobalamin) deficiency in cachectic and lethargic Giant schnauzers, Australian shepherds, beagles, and Border collies with an ileal receptor defect can be helped by bi-monthly cobalamin injections. Pancreatic enzyme supplementation and daily insulin injections are used to manage animals with exocrine or endocrine pancreatic insufficiencies, respectively. Another example of dietary management is copper hepatopathy in several breeds of dogs. Fresh frozen plasma is administered in the treatment of hereditary coagulopathies and von Willebrand disease, whenever animals excessively bleed. Other enzyme and protein replacements are also experimentally attempted. Many of these novel therapeutic options have been developed when the disease in animals was studied for humans.

Transplantation and Gene Therapy
Although kidney transplants have been established in clinical practice for chronic renal failure in dogs, they have not been applied in animals with hereditary (juvenile) renal disorders. Several hereditary disorders of hematopoietic cells have been experimentally corrected by bone marrow transplantation, e.g., pyruvate kinase and phosphofructokinase deficiency, cyclic hematopoiesis, and interleukin-2 (IL-2) receptor defects. Furthermore, bone marrow transplantation has been shown to deliver functional cells or active proteins (enzymes) to other tissues including liver, bone, and brain, e.g., for lysosomal storage diseases. Finally, gene therapy, the integration of a functional gene into the patient's own defective cells is clinically feasible within a decade. Experiments in rodent models have provided very encouraging results. However, effective gene therapy has proven more difficult in larger mammals, and the technology needs to be further improved.
to achieve persistent and regulated gene expression in larger mammals including humans, dogs and cats. The first and most promising canine gene therapy experiments have been the restoration of vison in severe retinal degenerations (Leber congenital amaurosis) with RPE65 incorporated into an adeno-associated virus vector and less bleeding in juvenile dogs with hemophilia A and B (FVIII or FIX in an adeno-associated virus) and mucopolysaccharidosis type VII in neonatal puppies with a retroviral vector carrying the beta-glucuronidase gene. Such treatments are being developed for humans, and once the technique is established, it may with ease also be applied in companion animals in the near future.

Control of Hereditary Diseases in Future Generations

Much more important than the treatment of hereditary disorders is the control of these traits in breeding programs for future health. Considering an autosomal-recessive transmission - the most common form of inheritance - breeding of two (asymptomatic) carriers results on average in 25% affecteds (homozygous for 2 mutant alleles), 50% carriers (heterozygote), and 25% normals (clear; 2 normal/wild-type alleles), or in other words, 75% show no clinical signs. However, as some diseases are mild or may not become clinical until a few years of life, unfortunately, even affecteds have been used for breeding. Our responsibility as veterinarians is to offer advice to breeders and prospective buyers who should become informed consumers.

Dominant traits are relative easy to control in a population as one of the parents would show clinical signs. However, some dominant traits are associated with incomplete penetrance, and, thus, their signs may be mild and missed. For autosomal recessive traits parents and offspring of affecteds are obligate carriers. They could even be affected, if it is a late onset [e.g. MPSIIIB in Schipperkes] or an intermittent disease or predisposition such as bleeding tendency. For X-chromosomal recessive traits the mother is typically the carrier passing the mutant or normal allele to the affected or healthy male offspring (hemizygote), respectively, while the female offspring are either again carriers or clear.

In order to reduce the frequency or eliminate altogether a recessive genetic defect, the further spread of the mutant gene (allele) has to be prevented in a family and eventually the entire breed. It is obvious that affected animals of any genetic disease should not be used for breeding. This approach is simple and effectively eliminates disorders with a dominant trait. For recessively inherited disorders, however, the elimination of affected animals is not sufficient and does not markedly reduce the prevalence of a defect within a breed or kennel. Although it may be safest not to breed any relatives of affected animals as they may be carriers or are obligate carriers, as requested by some kennel clubs, this practice may, because of inbreeding and narrow gene pools in some breeds, eliminate the most desirable traits and potentially all breeders in an entire kennel, and may severely reduce the genetic diversity of a breed. This may further result in the propagation of other defects in a breed. Thus, it will be pivotal to maintain genetic diversity and the desirable characteristic traits of a breed by detecting carriers (heterozygotes) and truly “clear” animals (homozygous normal) for simple recessively inherited disorders. Obligate carriers can be reliably identified for autosomal (both parents of affected and offspring of affecteds) and X-chromosomal recessive (mother of affected) disorders based upon the production of affected animals. For many diseases, reliable carrier detection tests are available and many breeders know about them and inform their veterinarian. For instance, carriers have approximately half normal (~50%) protein activity by functional assays, or have one normal and one mutant DNA sequence for the diseased gene by a DNA test.

Breeders should, therefore, be encouraged to screen their animals for known genetic diseases before breeding, whenever carrier tests are available. The availability of DNA genetic tests and nearest laboratories can be found at http://research.vet.upenn.edu/WSAVA-LabSearch. Unfortunately, many breeders mistrust these newer tests; either they were disappointed by the inaccuracy of earlier tests, such as the radiographic examination for hip dysplasia, or they fear that the results may become publicly known which could hurt their kennel and thus business. If carriers can be identified, they need to be bred to clear and again any
offspring intended for breeding should be screened. Breeders need to be educated by well-informed veterinarians; clinical genetic counseling is labor intense and not necessarily lucrative, but has the potential to affect the health of numerous animals far beyond the kennel involved and thereby also improve the future health of the breed.

These advancements have far-reaching benefits for promoting canine health permitting the elimination of deleterious gene defects, while preserving desirable traits in a breed. Several genetic disease registries have been established. While breeders and veterinarians are encouraged to participate in them, these registries are often biased just like frequency reports from testing laboratories. Often only clear animals are registered and more animals related to known affecteds and carriers are tested than in the random population. Thus, the true frequency of deleterious trait is likely overestimated by a screening laboratory and under-reported in a registry. Canine Health Information Center or CHIC (www.caninehealthinfo.org/) is a database which includes test results obtained from DNA testing but also OFA hip dysplasia and elbow scores, degree of patella luxation, and serum thyroid hormone values. Breed clubs are determining the tests included in CHIC and as more tests are included there will be fewer unaffected animals. Various examples on how to improve a breed or a kennel will be discussed.

In conclusion, it is most exciting to learn about many recent advances for the management of many hereditary disorders and genetic predispositions in small animal practice, be it for the diagnostic approach to a hereditary disease, the understanding of its pathophysiology, or its control. In addition to the clinician’s responsibility to suspect a genetic disease and to appropriately diagnose it with modern specific techniques, clinicians must become involved in the control of these disorders with breeders. Clinicians and clinical pathologists can thereby make an important contribution toward controlling the further spread of mutant genes and reducing future suffering of animals.

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**Keywords:** Hereditary Diseases, Genetic Predispositions, Dog

**References:** Specific references are available upon request
Lysosomal Storage Diseases: Clinical and Molecular Characteristics

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Summary

Lysosomal storage diseases are a group of rare genetic disorders of cellular catabolism, which are composed of more than 50 disorders. Most of them are inherited as autosomal recessive traits and results from mutations in the coding sequence of one of the acid hydrolases or their activating factors located in the lysosome. The genetic abnormality results in the reduction or elimination of the catalytic activity of the particular enzyme, and the reduction in enzyme activity results in the accumulation of the substrate of that enzyme within the lysosome. The accumulation of unprocessed or partly processed substrates in lysosomes causes progressive damage of affected tissues, resulting in progressive cellular and organ damages that affects appearance, physical abilities, organ and system functioning, and, in most cases, neuronal development. Based on the catabolism pathways, lysosomal storage diseases are categorized into a few subgroups: sphingolipidosis, mucopolysaccharidosis, mucolipidosis, glycoproteinosis, and other uncategorized diseases including neuronal ceroid lipofuscinosis. In this symposium, a number of companion cases with lysosomal storage diseases especially in sphingolipidosis, mucopolysaccharidosis, and neuronal ceroid lipofuscinosis are introduced along with their clinico-pathological and imaging findings that are helpful for the diagnosis.

Backgrounds: Lysosomal storage diseases are a group of rare genetic disorders of cellular catabolism, which are composed of more than 50 disorders and categorized into a few subgroups such as sphingolipidosis, mucopolysaccharidosis (MPS), mucolipidosis, glycoproteinosis, etc (Fig. 1 and Table 1)\(^1\)\(^2\). Sphingolipidosis includes GM1 and GM2 gangliosidosis. MPS is categorized into at least 6 types: I, II, III, VI, VI, and VII. Neuronal ceroid lipofuscinosis (NCL) include at least 16 different diseases, therefore, a number of cases with NCL have been identified. The animal with lysosomal storage disease is also an excellent model for human diseases.\(^3\)

Gangliosidosis: GM1 and GM2 gangliosidoses are progressive neurodegenerative lysosomal storage diseases resulting mainly from the excessive accumulation of GM1 and GM2 gangliosidosis in the lysosomes, respectively. These diseases result in the premature death due to brain damage with progressive neurological signs. In GM1 gangliosidosis, the accumulation of GM1 ganglioside is caused by an inherited deficiency of the lysosomal acid β-galactosidase encoded by the GLB1 gene. In GM2 gangliosidosis, the accumulation of GM2 ganglioside is caused by an inherited deficiency of the lysosomal acid β-hexosaminidase A or GM2 activator protein in GM2 gangliosidosis, and the disease is accordingly categorized into three variants: Tay-Sachs disease (B variant), Sandhoff disease (0 variant), and GM2 activator protein deficiency (AB variant), which are caused by the molecular defects of the HEXA, HEXB, and GM2A genes, respectively. GM1 gangliosidosis is identified in Shiba inus and domestic shorthair cats. Sandhoff disease is identified in the Golden retriever, Toy Poodle, mixed-breed dog, and domestic shorthair cat.
**Mucopolysaccharidosis**: MPS is a group of lysosomal storage disorders caused by a deficiency in glycosaminoglycan (GAG) degrading enzymes which is also characterized by the accumulation of GAGs due to the impaired functions of lysosomal enzymes. The resulting accumulation of unprocessed or partly processed GAGs: dermatan sulfate, heparan sulfate, keratan sulfate, and chondroitin sulfate in lysosomes causes progressive damage of affected tissues, including heart, respiratory system, bones, joints, and central nervous system. This accumulation causes permanent, progressive cellular damage that affects appearance, physical abilities, organ and system functioning, and, in some cases, neuronal development. MPS is categorized into at least 6 types: I, II, III, VI, VI, and VII, which are caused by 12 different genes. Several types of MPS have been diagnosed, and a number of causative mutations have been identified in canine and feline MPS.

**Neuronal ceroid lipofuscinosis**: NCL is a rare group of inherited, neurodegenerative lysosomal storage diseases characterized histopathologically by the abnormal accumulation of ceroid- or lipofuscin-like autofluorescent lipopigments in neurons, retinal cells, and other visceral cells throughout the body. NCL shares certain clinical features in both human beings and animals, including behavioral abnormalities, such as personality changes and aggressiveness, mental retardation and/or dementia; motor disturbances, such as ataxia and incoordination; visual problems leading to central and/or retinal blindness; premature death, but these differ in degree based on the causative gene, of which there are currently at least 16, almost all recessively inherited. A few types of NCL in dogs such as Border Collies and Chihuahuas, and mixed-breed cats have been identified in Japan.

**Diagnostic methods**: Clinical pathology or laboratory medicine is a powerful tool for the diagnosis of genetic diseases because many of genetic diseases have definite or subtle abnormal findings in clinico-pathological and laboratory tests such as blood films and urinalysis. Therefore, veterinary practitioners should take note of such results that provide a clue for the diagnosis of genetic diseases.

**Figure 1** Classification of lysosomal storage diseases
Table 1 Deficient proteins, genes, and accumulated compounds in lysosomal storage diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Deficient enzyme or protein</th>
<th>Gene</th>
<th>Main accumulated compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphingolipidosis</td>
<td>β-glucocerebrosidase</td>
<td>GLC3</td>
<td>GM1 ganglioside</td>
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<td>GM1 gangliosidosis</td>
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<td>GM1 ganglioside</td>
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<tr>
<td>GM2 gangliosidosis</td>
<td></td>
<td></td>
<td>GM2 ganglioside</td>
</tr>
<tr>
<td>Tay-Sachs disease</td>
<td>α-glucosidase A</td>
<td>HEXA</td>
<td>GM1 ganglioside</td>
</tr>
<tr>
<td>Sandhoff disease</td>
<td>α-glucosidase A &amp; B</td>
<td>HEXB</td>
<td>GM1 ganglioside, glucosidase</td>
</tr>
<tr>
<td>Farber disease</td>
<td>acid lipase</td>
<td>LIPA</td>
<td>cholesteryl ester, highdensity</td>
</tr>
<tr>
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<td>cholesteryl ester, highdensity</td>
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Keywords: Lysosomal Storage Disease, Gangliosidosis, Mucopolysaccharidosis

References
Congenital Metabolic Disorders in Purebred Companion Animals

Osamu YAMATO

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Summary

Inherited diseases such as inborn errors in metabolism are numerous in not only humans but also animals. It is estimated that many domestic animals have been afflicted with inherited diseases, and have died or have been euthanized without any proper diagnosis. Underlying inherited diseases must cause abnormalities within individual animals. In addition, not a few inherited diseases lead to increased mortality and decreased productivity in herds of animals in case the prevalence is high enough. We routinely experience several inherited diseases with high prevalence in dogs and cats, which include degenerative myelopathy in multiple canine breeds, trapped neutrophil syndrome in Border Collies, etc. Furthermore, we routinely experience several kinds of inherited eye diseases such as Collie eye anomaly and progressive retinal degeneration (e.g. cone-rod dystrophy in Dachshunds and progressive rod-cone degeneration in multiple canine breeds), which are also problems because there are many animals with visual abnormality in certain pure breeds of dogs and cats. In this lecture, the clinical, molecular, and epidemiological characteristics such diseases are introduced.

Backgrounds: Not a few inherited diseases lead to increased mortality and decreased productivity in herds of animals in case the prevalence is high enough. We routinely experience several inherited diseases with high prevalence in dogs and cats, which include degenerative myelopathy (DM) in multiple canine breeds, trapped neutrophil syndrome (TNS) in Border Collies. Furthermore, we routinely experience several kinds of inherited eye diseases such as Collie eye anomaly (CEA) and progressive retinal degeneration including cone-rod dystrophy (CRD) in Dachshunds and progressive rod-cone degeneration (PRCD) in multiple canine breeds, which are also problems because there are many animals with visual abnormality in certain pure breeds of dogs and cats.

Degenerative myelopathy: Canine DM is an adult-onset, progressive neurodegenerative disease that occurs in multiple dog breeds, including Pembroke Welsh Corgis (PWCs), German Shepherd Dogs, Boxers, Bernese Mountain Dogs (BMDs), and Collies. Most of the affected dogs are at least 8 years of age at the onset of clinical signs, which may include progressive, asymmetric upper motor neuron paraparesis, pelvic limb to generalized proprioceptive ataxia and lack of paraspinal hyperesthesia; these signs ultimately leading to paraplegia and dyspnea that may necessitate euthanasia. In addition to identification of these typical clinical signs, diagnosis of DM is made by post-mortem histopathological analysis of the spinal cord for markers of disease, which typically include demyelination, axonal loss or degeneration and astrogliosis. Cytoplasmic accumulation and aggregate formation of a mutant form of the superoxide dismutase 1 (SOD1) protein in the spinal cord of DM-affected dogs, which can be detected by anti-SOD1 antibodies, are closely associated with the pathogenesis of DM. To date, two DM-associated mutations have been identified in the canine SOD1 gene: c.118G>A (p.E40K) and c.52A>T (p.S18T) that is present in only BMDs. These mutations are likely to cause formation of misfolded proteins that accumulate into insoluble aggregates. The molecular epidemiological survey demonstrated the frequencies of the G/G wild-type, G/A heterozygote,
and A/A homozygote to be 9.0, 42.6, and 48.4%, respectively, indicating that the prevalence of the mutant A allele (0.697) in Pembroke Welsh Corgis is extremely high in Japan. In addition, the prevalence of the mutant A allele is 0.276 in Collies in Japan.

Trapped neutrophil syndrome: TNS is an autosomal recessive inherited neutropenia that was first identified in Border collies in Australia and New Zealand in the 1990s and has also been identified in Japan and other countries. TNS is characterized by a marked reduction in the number of neutrophils in the peripheral blood and hyperplasia of myeloid cells in the bone marrow. Affected dogs are susceptible to life-threatening infections due to severe neutropenia, resulting in death at a young age. Chronic and recurrent infections often mask the original leukopenia by inducing increased numbers of monocytes and eosinophils. In 2011, the causative mutation of TNS in Border collies was demonstrated to be a 4-base pair deletion in exon 19 of the canine VPS13B gene. The carrier frequency was 11.1% in Japan, suggesting that the mutant allele frequency is high enough to warrant measures to control and prevent the disease. Treatment with prednisolone and antibiotics is effective to make affected dogs survived to adulthood with a good short-to medium-term outcome.

Inherited eye diseases: CEA is a congenital inherited canine ocular disorder affecting the posterior segment of the eye in Collie-related breeds including Australian Shepherd, Border Collie, Lancashire Heeler, Rough Collie, Shetland Sheepdog, and Smooth Collie. CEA is a pleomorphic syndrome, with variability in manifestation and severity of clinical and ophthalmologic lesions, which is associated with an intronic 7.8-kilo base deletion in the canine NHEJ1 gene. The two main ophthalmoscopic changes are regional choroidal hypoplasia and coloboma of the optic disk or adjacent areas, which may be bilateral and are often symmetrical with equal severity. Recently, Hokkaido Inu, a traditional Japanese breed, has the CEA-associated mutation with high prevalence (mutant allele frequency 0.667).

Progressive retinal atrophy or degeneration is a hereditary retinal disorder that includes a group of conditions with similar clinical presentation, although the age of onset and rate of progression vary considerably by breed. PRCD is one of forms in canine inherited eye diseases, which is a middle-to late-onset, autosomal recessive retinal disorder causing photoreceptor degeneration. It has been reported that a point mutation c.5G>A (p.Cys2Tyr) in the canine PRCD gene is associated with PRCD in 29 or more breeds. The mutant allele frequency was 0.088 in Toy Poodles in Japan. CRD is an autosomal recessive, inherited retinal disorder caused by a 44-bp insertion in exon 2 of the canine RGRIP1 gene. It was first identified in Miniature Dachshund dogs. Dogs with CRD experience early-to-late onset and show individual differences in progression to blindness. The mutant allele frequency was approximately 0.3 in Miniature and Kaninchen Dachshunds.

Figure 1 Spinal cords stained with H&E and LFB from normal and DM dogs
Figure 2 Wright-Giemsa-stained blood film from a Border Collie with TNS

Keywords: Degenerative Myelopathy, Trapped Neutrophil Syndrome, Inherited Eye Disease

References
Recommendation and Counseling for a Purebred Dogs with Multiple Genetic Disorders

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Summary
Breeders and veterinarians should prevent deleterious genetic disorders in purebred dogs through reproductive management. Since canine whole genome sequencing was completed 14 years ago, mutations causing or associated with approximately 225 disorders have been identified in dogs. Specific information about these mutations can be obtained from Internet based databases, such as Online Mendelian Inheritance in Animals (OMIA). These advances in canine genetics have contributed to the diagnosis and prevention of genetic disorders in purebred dogs. Genotyping or screening prior to breeding can avoid any breeding of affected animals and breeding between carriers and thereby prevent production of any affected dogs in the future while preserving the gene pool in each breed. For example, we succeeded in preventing neuronal ceroid lipofuscinosis in Border Collies in Japan because the population of this breed is not so large in Japan. In contrast, we have not completed the prevention of GM1 gangliosidosis in Shiba Inus because the population of Shiba Inus is very large in Japan, and it is likely that this disease has been diffused to other countries. Furthermore, comprehensive investigation aimed at preventing multiple underlying genetic disorders in a single breed has not been performed thoroughly. We examined mutant allele frequencies associated with multiple genetic disorders in Border Collies, in order to develop recommendations for prevention of these disorders, and to promote broader prevention of canine genetic diseases. In this lecture, several preventive strategies will be introduced based on the data of genetic diseases in Shiba Inus and Border Collies.

Backgrounds: Breeders and veterinarians should prevent deleterious genetic disorders in purebred dogs through reproductive management. Since canine whole genome sequencing was completed 14 years ago, mutations causing or associated with approximately 225 disorders have been identified in dogs. Specific information about these mutations can be obtained from Internet based databases, such as Online Mendelian Inheritance in Animals (OMIA). These advances in canine genetics have contributed to the diagnosis and prevention of genetic disorders in purebred dogs. Genotyping or screening prior to breeding can avoid any breeding of affected animals and breeding between carriers and thereby prevent production of any affected dogs in the future while preserving the gene pool in each breed.

GM1 gangliosidosis in Shiba Inus: GM1 gangliosidosis, a lysosomal storage disease that affects the brain and multiple systemic organs, is caused by an autosomal recessively inherited deficiency in acid β-galactosidase, which is encoded by the GLB1 gene. GM1 gangliosidosis in Shiba Inus was first reported in 2000. The causative mutation has been identified as a deletion of the cytosine in exon 15 at nucleotide position 1647 in the putative coding region (c.1647delC) of the canine GLB1 gene, thereby enabling molecular diagnosis and/or genotyping with polymerase chain reaction (PCR)-based DNA tests. Affected dogs manifest neurological signs of progressive motor dysfunction from 5–6 months of age and die at 12–18 months after...
a clearly defined clinical course, which is associated with progressive accumulation of GM1 ganglioside and the subsequent neuronal damage in the central nervous system. The previous molecular epidemiological survey showed that the carrier frequency for GM1 gangliosidosis is on the average 1.02% in Japan and rather high in the Kinki district, which may be related to the high prevalence observed in this region. In addition, it is likely that this disease has been diffused to other countries. Although the control and prevention of this disease is necessary, we have not completed the prevention of this disease because the population of Shiba Inus is very large in Japan.

**Neuronal ceroid lipofuscinosis in Border Collies:** Neuronal ceroid lipofuscinosis (NCL) is a rare group of inherited, neurodegenerative lysosomal storage diseases characterized histopathologically by the abnormal accumulation of ceroid- or lipofuscin-like autofluorescent lipopigments in neurons, retinal cells, and other visceral cells throughout the body. NCL shares certain clinical features in both human beings and animals, including behavioral abnormalities, such as personality changes and aggressiveness, mental retardation and/or dementia; motor disturbances, such as ataxia and incoordination; visual problems leading to central and/or retinal blindness; premature death, but these differ in degree based on the causative gene, of which there are currently at least 16, all recessively inherited. NCL in Border Collies was first identified in Australia in the 1980s, and a sporadic case with the disease was also reported in the USA in the 1990s. A diagnosis of the first case in Japan was made in a Border Collie that was born in 2000. The pathogenic mutation was reported in 2005 to be a nonsense mutation (c.619C>T) in exon 4 in the canine CLN5 gene, which enabled a DNA-based genotyping of affected dogs and carriers. Recently, several types of rapid genotyping assays for this mutation were developed, and the carrier frequency (8.1%) in Japan was determined by a genotyping survey using these assays, suggesting the mutant allele frequency of NCL in Border Collies is high enough in Japan that measures to control and prevent the disease would be warranted. For NCL control and prevention, we have been examining as many breeding dogs as possible, especially in kennels with a high prevalence, for 17 years. Such endeavors have successfully reduced NCL prevalence and may already be contributing to the recent decreasing trend in Japan.

**Multiple genetic disorders in Border Collies:** Previously we examined mutant allele frequencies associated with multiple genetic disorders (Table 1), using Border collies as a representative breed, and to make recommendations for prevention of the disorders. Genotyping of known mutations associated with seven recessive genetic disorders was performed using PCR assays. More than half (56%) of the Border collies had no mutant alleles associated with any of the seven disorders, suggesting that these disorders can be removed from the population over several generations. Since frequencies of each mutant allele differed among disorders, reproductive management should be performed after the establishment of prevention schemes that are appropriate for each disorder, the type and specificity of genetic test available, and the effective population size in each breeding colony.

**Table 1** Carrier rate and mutant allele frequency in multiple genetic disorders in Border Collies

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Dog number (%)</th>
<th>Mutant allele frequency</th>
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<tr>
<td></td>
<td>Carrier</td>
<td>Affected</td>
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<tr>
<td>CEA</td>
<td>123 (24.6%)</td>
<td>10 (2.0%)</td>
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<tr>
<td>DM</td>
<td>8 (1.6%)</td>
<td>0 (0%)</td>
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<td>IT</td>
<td>2 (0.4%)</td>
<td>0 (0%)</td>
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<tr>
<td>MER</td>
<td>16 (3.2%)</td>
<td>0 (0%)</td>
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<tr>
<td>NCL</td>
<td>35 (7.0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>SCM</td>
<td>15 (3.0%)</td>
<td>0 (0%)</td>
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<tr>
<td>TNS</td>
<td>59 (11.8%)</td>
<td>0 (0%)</td>
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![Figure 1 Distribution of Border Collies with known mutant alleles](image)

**Keywords:** Prevention, Genetic Disorder, Dog

**References**

Immune-Mediated and Other Hemolytic Anemias in Dogs

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Summary
This session will cover the different hemolytic anemias including immune-mediated, infectious, toxic and inherited causes. The diagnostics to establish evidence of hemolysis based upon hyperbilirubinuria, icterus, erythroid regeneration and occasionally Hemoglobinemia and -uria. Also, the specific diagnostics for each hemolytic anemia cause will be presented. The specific diagnostics for immune-mediated hemolytic anemia such as autoagglutination, spherocytosis and direct antiglobulin (Coombs’) test will be emphasized. A precise diagnosis of immune-mediated and other hemolytic anemias is crucial in determining an accurate prognosis and implement effective supportive and specific therapy.

Introduction
Immune-mediated hemolytic anemia (IMHA) is one of the most common and serious hemolytic anemias in dogs, but occurs rarely in other animal species. In IMHA an immune response, including anti-erythrocytic antibodies, complement and macrophages, targets directly or indirectly erythrocytes and a hemolytic anemia ensues. There are many triggers for IMHA such as infections, drugs and other agents, and cancer leading to secondary IMHA, but in many dogs no cause is identified (so-called idiopathic, autoimmune or primary IMHA) or a genetic predisposition has been proposed (Cocker spaniels). Furthermore, alloimmune hemolytic anemias, such as hemolytic transfusion reactions, both acute and delayed, and neonatal isoerythrolysis (only litters from transfused bitches), are caused by specific anti-erythrocytic alloantibodies. In contrast to other species, dogs with IMHA also develop an often overwhelming inflammatory response resulting in thrombosis and necrosis of various organs. And while the anemia can be corrected with transfusions, these complications in dogs are causing severe morbidity and mortality despite aggressive immunosuppression and antithrombotic interventions.

Immune Destruction of Erythrocytes
Regardless of the underlying cause, IMHA results from a breakdown in immune self-tolerance or from a deficit in the control mechanism that regulates B and T lymphocyte activity as well as macrophage reactivity. Immune destruction of erythrocytes is initiated by the binding of IgG or IgM antibodies to the surface of erythrocytes. Under most clinical circumstances, immune destruction is an extravascular process that depends on recognition of erythrocytes opsonized with IgG, IgM and/or complement by specific receptors on reticuloendothelial cells. Macrophages with engulfed erythrocytes may be noted on cytological examination of blood and tissue aspirates as erythrophagocytosis, but this is not definitive proof of an immune-mediated process. Antibody-coated erythrocytes may also be lysed by complement fixation and the membrane attack complex, which is clinically noted as intravascular hemolysis.

A diagnosis of IMHA must demonstrate accelerated immune destruction of erythrocytes. Evidence of a hemolytic anemia is suggested clinically by icterus and a regenerative anemia with hyperbilirubinuria, and
hemoglobinemia and hemoglobinuria refers to an intravascular process. However, the erythroid response in the bone marrow may be blunted by the immune and inflammatory process or the underlying disease thereby leading to non-regenerative anemias. Besides documenting a hemolytic anemia, one or more of the following three hallmarks must be present to support a diagnosis of immune-mediated hemolysis: persistent autoagglutination, marked spherocytosis and a positive direct Coombs’ test result. As in human medicine, the Coombs’ test should be considered the best test to definitively diagnose IMHA, although marked spherocytosis and persistent/true autoagglutination (after 3x washing of EDTA blood with saline) are other important parameters indicating immune-destruction of erythrocytes.

Autoagglutination
Anti-erythrocyclic IgM and in large quantities IgG antibodies may cause direct erythrocyte autoagglutination. The autoagglutination may be seen by naked eye in an EDTA tube or on a glass slide or may become apparent as small clumps of erythrocytes on blood smears. For yet unexplained reasons, canine erythrocytes have a tendency to unspecifically agglutinate in the presence of plasma and colder temperatures as well as possibly with excessive EDTA anticoagulant. Mixing blood with one drop of saline may break up rouleaux formation but not other forms of unspecific red cell agglutination. It is, therefore, important to determine whether the agglutination persists after “saline washing”, which has been coined persistent or true autoagglutination. This is accomplished by adding physiologic saline to the tube containing a small amount of EDTA-anticoagulated blood, mixing, centrifuging and removing the supernatant including the plasma and repeating this saline washing 3 times. True or persistent autoagglutination is indicative of an immune process, but precludes the performance of Coombs’ test or blood typing and crossmatching procedures which are based upon an agglutination reaction as result. Those based upon chromatographic techniques do not seem to be affected by autoagglutination as free red cells can move along the strip. If the agglutination breaks up after washing, the Coombs’ test is expected to be positive, if it is a case of IMHA. There is no evidence for washing away red cell bound antibodies in dogs.

Spherocytosis
If erythrocytes are only partially phagocytized or lysed by complement in circulation, erythrocytes with reduced surface area to volume ratio, known as spherocytes, are formed. They appear spherical and microcytic with no central pallor and are considered fragile. Note proper areas on the blood smear needs to be reviewed to find spherocytes in between single regular discoid red cells. Large numbers of spherocytes (>20/microscopic high power field) are nearly diagnostic for IMHA, whereas small numbers may be seen with other conditions including DIC, endotoxemia and zinc intoxication. In our experience all dogs with marked spherocytosis and suspected to have IMHA also had a positive Coombs’ test. However, only 60-80% of dogs with a positive Coombs’ test or clinically diagnosed with IMHA had marked spherocytosis. Hereditary spherocytosis due to genetic membrane defects has rarely been seen in dogs, but should be considered as a differential diagnosis in dogs with negative Coombs’ test results.

Because of the difficulties with the Coombs’ test (see below), Slappendale had proposed to use the erythrocytic osmotic fragility test at specific saline concentrations as a mean to diagnose IMHA and this test is currently used in various clinics in Europe. However, there are many other reasons for increased fragility of erythrocytes beside IMHA including hereditary red cell defects. This test is not used in human medicine and has not been shown to be superior to determination of marked spherocytosis and a positive Coombs’ test in dogs with IMHA. The osmotic fragility test is also a cumbersome and not well standardized technique.

Positive Direct Coombs’ Test Result
The direct Coombs’ test is also known as direct antiglobulin test (DAT) and is used to detect antibodies and complement on the surface of erythrocytes when the anti-erythrocyte antibody strength or concentration is too low to cause spontaneous agglutination (subagglutinating titer). Separate canine-specific IgG, IgM,
and C3b antibodies as well as polyvalent antiglobulin reagents are available. They are added at various concentrations after washing the patient's erythrocytes free of plasma (3x as shown above) and mixtures are generally incubated at room temperature or 37°C (cold agglutinins appear to be rarely of clinical importance and rarely cause hemolysis). The strength of the Coombs' reaction does not necessarily predict the severity of hemolysis, but reaction changes are useful in monitoring the disease.

Typically tube or microtiter methods have been used exclusively in the reference or teaching laboratory setting, but a flow cytometric method has also been introduced in a couple of places. A standardized, sensitive, and simple gel column method was available by DiaMed (Switzerland), but unfortunately the company was sold to another company which decided to not pursue the veterinary market. A novel standardized antiglobulin test method has just been developed by Alvedia (France) similar to the immunochromatographic strip technique for blood typing of dogs and cats (see updates on blood typing and crossmatching). Although many commercial laboratories offer Coombs' testing for dogs, clinicians have questioned the tests sensitivity and specificity and often forgo the test and/or use response to therapy as a diagnostic. However, negative Coombs' test results may be seen because of technical reasons, insufficient quantities of bound antibodies, the presence of weakly bound antibodies, or the disease in remission. The Coombs' test stays positive for days to months after initiating treatment. A few days of immunosuppressive therapy will likely not reverse the Coombs' test result, as unlikely a transfusion would cause a positive Coombs' test result. Thus, dogs with negative Coombs' test results should be reevaluated for other causes of hemolytic anemia.

In a recent prospective study of anemic and non-anemic dogs we compared various direct Coombs' test methods including microtiter plate assays, gel column, capillary, and immunochromatographic techniques using polyvalent antiglobulins in a laboratory setting and found excellent correlations between tests and with spherocytosis and without noticeable interference by immunosuppressive or transfusion therapy in anemic dogs.

**In conclusion**, a diagnosis of IMHA requires the documentation of red blood cell destruction and an immune process. While regenerative anemia, icterus, and hyperbilirubinuria are suggesting a hemolytic anemia, evidence of true autoagglutination, spherocytosis, and/or a positive direct Coombs' test are required to document immune destruction. The author also recommend monitoring IMHA patients for the disappearance of these immunological parameters to adjust and taper therapy.

**Funding and Conflicts of Interest**: Author's studies were supported in part by grants from the National Institutes of Health (OD010939) and the AKC Canine Health and other Foundations. Alvedia, Lyon, France provided the immunochromatographic strips for the studies. The author is the director of the non-for-profit PennGen Laboratory offering genetic and hematological testing.

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**Keywords**: Hemolysis, Immune-Mediated Hemolytic Anemia, Dog

**References**: Specific references available upon request
Canine Transfusion Medicine: Blood Types and Transfusion Reactions

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Summary
Veterinary clinicians, clinical pathologists, and veterinary technicians/nurses play a key role in providing safe and effective transfusion therapy. Blood typing is clinically important to ensure blood compatibility and therefore is recommended for any dog in need of a transfusion or considered to become a blood donor. Moreover, previously transfused dogs also should be crossmatched. Unless blood typing is performed regularly in practice, blood should be sent to a veterinary clinical pathology laboratory for typing and crossmatching. Different viewpoints exist regarding the extent and methods used for compatibility testing, and various techniques for laboratory and point-of-care use have been applied or are being developed. This presentation will present the current knowledge of the canine blood types and their clinical importance, review the different typing and crossmatching techniques, and illustrate this with case examples.

Canine Blood Types
Blood types are genetic markers on erythrocyte surfaces that are antigenic and species specific. A set of blood types of two or more alleles makes up a blood group system. Dogs have likely more than a dozen blood group systems mostly known as dog erythrocyte antigens (DEA). However, there is no DEA 2 blood group and some may be rather labeled high frequency or common red blood cell (RBC) antigens (e.g. DEA 4) and some have not yet received a DEA designation (e.g. Dal). Canine erythrocytes are either positive or negative for a blood type (e.g., DEA 4+ or DEA 4-), and these blood types are likely codominantly inherited. The DEA 1 system was thought to be an exception with DEA 1.1 (A1), DEA 1.2 (A2) and potentially DEA 1.3 (A3) being allelic. Thus, a dog could apparently be DEA 1.1+ or DEA 1.1-, and DEA 1.1- dogs can be DEA 1.2+ or DEA 1.2-. However, these studies were based upon weak polyclonal antibodies (DEA 1.1 and 1.2) requiring Coombs’ reagents. Recent studies with a monoclonal antibody showed that the DEA 1 blood group is a continuum from DEA 1- to weakly to strongly DEA 1+; hence DEA 1.2 typing is no longer offered. The degree of DEA 1 expression is constant and DEA 1+ appears to be dominantly inherited. A recent survey in North America indicates that most dogs are either DEA 1- or strongly DEA 1+ with fewer dogs being weakly to moderately DEA 1+. The biochemical structure of the DEA 1 remains still unknown, but a genome wide association study has identified a single DEA 1 locus.
Recent surveys revealed that the Dal- type is not restricted to Dalmatians but is also seen in Beagles, Doberman Pinschers, Shih Tzus, and Lhasa Apsos, and thus typing for this blood type is becoming more important particularly for those requiring multiple transfusions. In a related study dogs from North America were screened for two new blood types, called Kai 1 and Kai 2. Most dogs were Kai 1+ and only few dogs were Kai 2+ or Kai 1-/Kai2- but none were positive broth Kai types. The clinical importance is yet to be determined, albeit dogs can develop anti-Kai 1 alloantibodies. The PennGen Laboratory currently offers Dal and Kai 1 & 2 typing in the USA on a limited basis.
The clinically most important canine blood type is DEA 1, which elicits a strong alloantibody response after sensitization of a DEA 1- dog by a transfusion and thus can be responsible for a transfusion reaction in a DEA 1- dog previously transfused with DEA 1+ blood. It is currently unknown, if DEA 1- dogs are equally sensitized by weakly to strongly DEA 1+ blood, or if weakly DEA 1+ dogs are sensitized by strongly DEA 1+ blood. Furthermore, transfusion reactions against other blood types or common RBC antigens have rarely been observed and reported. They include reactions against the DEA 4, Dal, Kai 1 and common RBC antigens, and other clinically important blood types may be found in the future. No reagents currently are available against several antigens or are only available on a limited basis, and additional blood types continue to be recognized. Only limited surveys on the frequency of these blood types have been reported, which suggest possible geographic and at least for Dal major breed-associated differences. Strongly antigenic blood types are of great clinical importance because they can elicit a potent alloantibody response. These alloantibodies may be of the immunoglobulin G (IgG) or IgM class and may be hemagglutinins or hemolysins. Based upon experimental and clinical data, dogs can become sensitized after receiving a mismatched transfusion (i.e., a blood unit positive for one or more blood types not found on the recipient’s RBCs). There are no clinically important, naturally occurring alloantibodies (also known as isoantibodies) present before sensitization of a dog with a transfusion. Recent papers by Spada et al suggested naturally anti-DEA 7 antibodies but this has not been confirmed by others and is not experienced clinically. In fact no DEA 7 related transfusion reaction has been documented. Sensitizing dogs in experimental studies in the 1950s led to the documentation of some transfusion reactions caused by blood group incompatibilities and to the characterization of new blood types.

Clinically the most antigenic blood type in dogs is the DEA 1. Transfusion of DEA 1+ RBCs to a DEA 1- dog invariably elicits a strong alloantibody response. Following a first transfusion, anti-DEA 1 antibodies develop after more than 4 days and may cause a delayed transfusion reaction (rarely clinically documented). However, a previously sensitized DEA 1- dog develops an acute hemolytic reaction after a second transfusion of DEA 1+ blood. Transfusion reactions also may occur after a sensitized dog receives blood that is mismatched for a RBC antigen other than DEA 1 (e.g. DEA 4 and Dal). However, in most cases the incompatible blood type has not been determined. Because administration of a small (<1 ml) amount of incompatible blood can result in life-threatening reactions, the practice of giving small “test volumes” of donor blood to assess blood-type compatibilities is unacceptable. In contrast, pregnancy does not cause sensitization in dogs because of a complete placenta and does not induce alloantibody production; thus dogs with prior pregnancies can be used safely as blood donors.

**Canine Blood-Typing Procedures**

Because of the strong antigenticity of DEA 1, typing of donors for DEA 1 is recommended. Whenever possible, the recipient also should be typed to allow the use of DEA 1+ blood for DEA 1+ recipients. Canine blood typing is generally based on serologic identification by agglutination reactions but chromatographic strip methods are also offered. Originally sera from sensitized dogs have been used for typing, but such polyvalent alloantibodies vary from batch to batch, may require Coombs’ reagent to enhance agglutination reactions, and may not be always available and are therefore not optimal. Monoclonal antibodies against DEA 1 have been developed at Kansas State University and at the University of Lyon. The gel column technology, widely used in human blood banking, was found to be an excellent standardized laboratory method (DiaMed), but is unfortunately no longer commercially available. A blood typing card has been available with modifications since the mid-1990s as a simple in-practice kit to classify dogs as DEA 1- or DEA 1+ (degree of reaction can vary). A standardized simple immunochromatographic technique became available in the mid-2000s from Alvedia. Another cartridge with a similar strip technique was introduced by DMS/AgroLabo, but has not been evaluated. Moreover, a third cartridge method in which blood flows through the cartridge is also available (QickTest/Abaxis), but was producing some inconsistent results in one study.

Polyclonal reagents against other DEA types are currently only available on a limited bases for DEA 1, 3, 4 and 7 from Animal Blood Resource International (prior Michigan State University and Midwest Blood
Services). And only limited anti-Dal reagents from sensitized dogs are currently available in a couple of laboratories like University of Montreal and PennGen. Monoclonal anti-Kai 1 and anti-Kai 2 alloantibodies have been developed in South Korea and typing is available by PennGen Laboratory if incompatibilities are observed. DEA 1 typed and matched patients in need of a transfusion may be typed for DEA 4, Dal and Kai 1 & 2, which may then permit the localization of a type matched donor dog.

Caution should be exercised whenever the patient’s blood is autoagglutinating or has a low hematocrit (<10%). If autoagglutination is not too severe, it does not appear to affect the Alvedia chromatographic strip technique, because only free RBCs are moving up the strip. Clinicians and technicians should first check for autoagglutination of blood with buffer/saline on a slide or the card to avoid wasting a test kit. Autoagglutinating blood may be washed three times with ample physiological saline to overcome the apparent autoagglutination similar to what is done for the Coombs’ testing and crossmatching. However, if autoagglutination after three washes persists at more than 1+, it is considered to reflect true autoagglutination, which may preclude typing (as well as Coombs’ testing and crossmatching, because it always looks like DEA 1+ blood). In such circumstances, DEA 1- blood should be used, until the patient does not agglutinate anymore and can be retyped. DEA 1+ blood from severely anemic animals may not agglutinate when exposed to the anti-DEA 1 or other reagents because of a prozone effect. In these cases, some of the patient’s plasma may be discarded before applying a drop of blood onto the card. Finally, recently transfused dogs may display a mixed field reaction, with only the transfused or recipient cells agglutinating if they were DEA 1 mismatched.

**Blood Crossmatching Test**

Whereas blood typing tests reveal the blood group antigens on the red blood cell surface, blood crossmatching tests assess the serologic compatibility or incompatibility between donor and recipient. Thus, the crossmatch test checks for the presence or absence of naturally occurring and induced alloantibodies in serum (or plasma) without determining the blood type and does not replace blood typing. These antibodies may be hemagglutinins and/or hemolysins and can be directed against known blood groups or other RBC surface antigens. Many laboratories commonly use a standardized tube crossmatching procedure, but the interpretation of the agglutination reaction is highly variable. The crossmatching test requires some technical expertise, may be best accomplished through a veterinary laboratory along with blood typing, and is done with washed EDTA-anticoagulated blood from recipient and potential donor(s). The DiaMed neutral saline gel column technique and more recently the in-clinic DMS gel tube assay have been evaluated and were found to be simple, sensitive, and standardized methods to crossmatch dogs and cats. In addition, Alvedia introduced a simple strip crossmatch test with a Coombs’ phase which seems to readily identify incompatibilities in previously transfused dogs.

The major crossmatch tests search for alloantibodies in the recipient’s plasma against donor cells, whereas the minor crossmatch test looks for alloantibodies in the donor’s plasma against the recipient’s RBCs. Generally, tube segments from collection bags are used for this purpose in dogs. The presence of autoagglutination or severe hemolysis may preclude the crossmatch testing. A major crossmatch incompatibility is of greatest importance, because it predicts that the transfused donor cells will be attacked by the patient’s plasma, thereby causing a potentially life-threatening acute hemolytic transfusion reaction. Because fatal reactions may occur with less than 1 ml of incompatible blood, compatibility testing by administering a small amount of blood is not appropriate; this has been shown in experimental studies to potentially result in fatal reactions. A minor crossmatch incompatibility should not occur in dogs if canine donors have not been transfused previously and is of lesser concern because donor’s plasma volume is small, particularly with packed red cell products, and is diluted markedly in the patient. Do not use previously transfused dogs as donors. The initial blood crossmatch between two dogs that have never before received a transfusion should be compatible, because dogs do not have naturally occurring alloantibodies. Therefore, a crossmatch may be omitted before the first transfusion in clinical practice for dogs. Because the crossmatch does not determine the blood type of the patient and donor, a compatible crossmatch does not prevent sensitization of the
patient against donor cells within 1-2 weeks. Thus, previously transfused dogs should always be crossmatched, even when receiving again blood from the same donor. The time span between the initial transfusion and incompatibility reactions may be as short as 4 days and the induced alloantibody can last for many months to years (i.e., years after the last transfusion alloantibodies may be present). Again, a blood donor should never have received a blood transfusion to avoid sensitization and alloantibodies in donor plasma. The practice of transfusing patients with the least compatible unit does not have any scientific basis. Nevertheless, some minor agglutination results in crossmatching a patient may be unrelated to alloantibodies and unspecific (e.g., patient’s RBC damage by uremia and other illnesses, donor RBCs in tube segments and after extended storage of unit in the refrigerator). Of course, any patient with true/persistent autoagglutination may not be matched to any donor.

Although transfusion of blood and its components is usually a safe and temporarily effective form of therapy, there is always a risk for potential hazards. Adverse reactions usually occur during or shortly after the transfusion and can be due to any component of whole blood. Most transfusion reactions can be avoided by carefully selecting only healthy donors and extensive infectious disease screening; using appropriate collection, storage, and administration techniques; performing blood typing and crossmatching; and administering only the needed blood components.

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**Keywords:** Blood Types, Crossmatch, Transfusion Reactions

**References:** Specific references are available upon request
Body Cavity Effusions

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Summary
Effusions are the abnormal accumulation of fluid within a mesothelial-lined body cavity that result from many different disease processes. This lecture will review the mechanisms for normal and pathologic fluid accumulation plus discuss the classification of these effusions. General guidelines for the collection, handling, and processing of samples are reviewed for participants. The majority of the presentation will focus on the physical, cytologic and biochemical characteristics associated with specific diseases that will be demonstrated by case examples.

What is an effusion?
An effusion represents increased accumulations of fluid within a mesothelial cell-lined body cavity. A small amount usually less than 1ml is maintained between production and drainage.(1) The body cavities included are the abdominal or peritoneal, thoracic or pleural, and pericardial. The presence of an effusion is recognized when animals display clinical signs such as dyspnea, muffled heart sounds, ascites, or abdominal pain.

Mechanisms causing increased cavitary fluid:
- High hydrostatic pressure causing leakage from blood channels arising from flow obstruction such as volvulus
- Low oncotic pressure related to hypoalbuminemia causing poor water retention within vessels
- Increased vascular membrane permeability resulting from inflammation causing exudation of cells

Mechanisms for decreased drainage of excess fluid:
- Venous hypertension produces backup and poor removal of fluids in conditions like feline cardiomyopathy and heart failure
- Lymphatic vessel obstruction with pooling of lymph from leakage subsequent to compressive neoplasms such as lymphoma

How are effusions collected, preserved, and processed?
Peritoneal fluid: Collect fluid from the animal in left lateral recumbency or in a standing position. Following surgical preparation of the site between the umbilicus and urinary bladder, a 20-22 g needle is used to penetrate the abdomen slightly lateral to the midline. Fluid may drip into a tube or be suctioned using 6 or 12 ml syringes.

Pleural fluid: Collect fluid with the patient standing or sitting. A flexible (intravenous) catheter is placed in the 7th or 8th intercostal space following surgical preparation and infiltration with a local anesthetic. A 3-way stopcock may be used to remove large volumes of fluid and prevent air leakage.

Pericardial sac fluid: This fluid is removed with the patient sedated and in lateral recumbency. A venous catheter
is placed into the lower portion of the 4th intercostal space towards the heart until it penetrates the pericardium.

Handling and processing: For each of the sites, one ml of fluid should be placed into a red top tube for culture with the remainder used to make direct smears by coverslip or glass slide and then placed into purple EDTA tubes for cell counts and protein analysis. If direct smears cannot be made immediately the fluid can be placed into an EDTA tube and processed later. Refrigeration will help maintain cell integrity.

How are effusions evaluated?
- Color and degree of transparency is recorded along with any noticeable odor.
- Total solids or protein is measured by refractometer, especially after centrifugation in a microhematocrit tube if the fluid is not clear. Note that measurement of specific gravity using a refractometer has not been validated for use with body cavity fluids, only urine. Therefore values should be regarded as suggestive, not definitive.
- Cell counts may be made by automated counter, if clear. Alternatively fluids are counted by hemocytometer which is less accurate.
- Biochemical analysis of bilirubin, urea, lactate, triglycerides, and pancreatic enzymes may be helpful. (1)
- A smear (squash method) is performed between two coverslips or two glass slides for identification of cell types, presence of infectious agents or neoplasia.

Cell types often recognized in effusions
- Large mononuclear cells may be mesothelial cells or macrophages. These cells are difficult to distinguish and are therefore lumped into one category. These should be the predominant cells in most species, other than in the horse.
- Neutrophils are often present as the predominant cell type in horses but not in other species. Record the presence of degeneration if seen. Increased numbers signifies the presence of inflammation.
- Small mononuclear cells are lymphocytes which are present in low numbers normally.
- Eosinophils are occasionally present in effusions, but should be recorded separately, if present.

How are effusions classified?
Color and transparency are characteristics helpful in gaining the first impression as to the source or etiology of the fluid. (2) The red, white, or green-brown colors suggest hemorrhage, chyle, or bile as the sources, respectively. Immature cells unlike the normal or inflammatory cells suggest a neoplastic condition. When a specific diagnosis is possible, effusions may be described as hemorrhagic, chylous, bilious, or neoplastic. Fluids in which a specific etiology cannot be determined initially are then classified as transudate, modified transudate, or exudate.

Hemorrhagic effusion: Fluid is colored red, pink, or occasionally yellow. This category is used when the effusion is bloody due to acute or chronic hemorrhage without the presence of another abnormality. It should not be used to imply slight blood contamination. Commonly, this is associated with pericardial effusion. Acute hemorrhage is characterized by intact erythrocytes engulfed by macrophages or neutrophils. Peracute bleeding and blood contamination are associated with platelets which can remain intact for about one-half hour following collection. Chronic hemorrhage is characterized by hemosiderin-laden macrophages, cells containing coarse blue-black granules which are positive for iron using Prussian blue stain.

Chylous effusion: Fluid is white or pink-white lack of transparency; therefore is termed opaque. This is most often related to the presence of chyle from rupture of the thoracic duct lymphatics which may be caused by trauma, neoplasia, infection, or idiopathic reasons. Chyle consists of chylomicrons which are composed of triglycerides. The condition can be diagnosed by the presence of high triglyceride concentrations, often > 100 mg/dl in the effusion fluid. WBC counts are increased over normal but < 10,000/µl being the
predominant cells being small to medium lymphocytes. Acute chylous effusion is recognized by mainly small dense lymphocytes with few inflammatory cells. Following continued presence within the body cavity, chronic irritation of the mesothelial lining produces reactive or basophilic mesothelium, increased numbers of neutrophils and macrophages along with a mixed lymphoplasmacytic response. Macrophages may contain small clear vacuoles.

*Bilious effusion:* Fluid is formed from the rupture of the common bile duct or leakage from intrahepatic bile ducts. The color is dark yellow or green and generally opaque. Some bile is termed “white” since only amorphous gray mucus material from the gall bladder is present. Inflammation by a mixed cell population is common.

*Neoplastic effusion:* The presence of an abnormal cell population displaying features of malignancy is diagnostic. Common neoplasms to consider include lymphoma, carcinoma, or mesothelioma. Cell counts and protein may be mildly increased over normal or involve inflammation. Pericardial effusions may be distinguished by pH. Alkaline effusions (pH > 7.0) measured by a urine reagent dipstick are associated with neoplasia while effusions pH < 7.0 are associated with benign conditions or inflammation.

**Classification of Non-Colored or Non-Specific Effusions**

Fluids aside from those mentioned above, may be classified by features such as cell count, protein concentration, and predominance of cell types. Classification in this manner can suggest a pathophysiologic mechanism which assists in the diagnosis of an etiology.

*Transudate:* This fluid has low cellularity (<1000/µl for most animals, <1500/µl for horses) and low protein content (<2.5 g/dl). It is generally formed as a result of low oncotic pressure related to severe hypoalbuminemia (<1.0 g/dl) that can develop from nephrotic syndrome, for example. Low protein fluid may also develop from portal hypertension secondary to chronic liver disease or secondary to portal vein hypoplasia that result in leakage of low protein lymph from the intestinal vessels. Note that the small amounts of normal body cavity fluid resemble a transudate.

*Modified transudate:* This fluid has increased cellularity and/or protein content with predominance of mononuclear cells. It is often formed as a result of increased hydrostatic pressure or long-standing transudate fluids. Nonseptic intestinal disease in horses such as volvulus and right-sided heart failure are common causes.

*Exudate:* This fluid is synonymous with inflammation. Cell counts are increased (>5000/µl for most animals, >10,000/µl for horses). Protein is often increased (>3.0 g/dl). Due to the increased cell count, the character of the fluid is often cloudy. Septic exudate indicates a visible microorganism, usually bacteria or fungus, is present in the cells of the smear. Causes involve penetrating wounds, gut rupture, or neoplastic invasion. Nonseptic exudate indicates the lack of a visible microorganism within neutrophils or macrophages. Causes may include feline infectious peritonitis virus infection, bile leakage, uroperitoneum, tissue necrosis, and parasites such as cestodes.

**Keywords:** Hemorrhagic, Bilious, Chylous, Transudate, Exudate

**References**


Cytology of Canine and Feline Lymph Nodes

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Summary
Lymphadenomegaly is a common presenting sign in clinical practice. The ability to obtain diagnostic material and perform an accurate interpretation is emphasized in this presentation. Recognition of normal, hyperplastic, reactive, inflammatory, and neoplastic lymph nodes will be demonstrated through canine and feline case examples. As lymphoma may have variable appearances and biologic behaviors, cytologic evaluation of lymphoid tissue can be sometimes challenging. Differences between indolent and aggressive forms as well as immunophenotype will be discussed. Methods for improved diagnosis are presented including immunochemistry and clonality testing. The participant will gain confidence in lymph node interpretation from this presentation.

What are the indications for lymph node evaluation?
- **Lymphadenomegaly** or enlargement of one or multiple lymph nodes may be detected by palpation or by radiography and ultrasonography.
- **Evaluation of metastatic disease** involves evaluation of the lymph node(s) draining the primary lesion. (Table 1)
- **Classification of lymphoma** may be enhanced by the cytologic features stained with routine stains, or by cytochemical and immunocytochemical stains to distinguish B and T cell subtypes. The latter stains are performed at specialized laboratories. Histopathology is recommended for equivocal cases to demonstrate architectural changes. Material for clonality testing can be obtained as well.

Table 1 Selected peripheral lymph nodes for biopsy

<table>
<thead>
<tr>
<th>Lymph Node</th>
<th>Location</th>
<th>Drainage Features</th>
</tr>
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<tbody>
<tr>
<td>Mandibular (submandibular)</td>
<td>Group of 2 - 4 nodes located ventral to the angle of the jaw</td>
<td>Includes most of the head, including the rostral oral cavity</td>
</tr>
<tr>
<td>Superficial Cervical (prechapular)</td>
<td>Group of 2 - 3 nodes located in front of the supraspinatus muscle</td>
<td>Includes the caudal part of the head (pharynx, pinna), most of the thoracic limb, and part of the thoracic wall</td>
</tr>
<tr>
<td>Axillary</td>
<td>1-2 nodes located caudal and medial to the shoulder joint</td>
<td>Includes most of the thoracic wall, deep structures of the thoracic limb and neck, and the thoracic and cranial abdominal mammary glands</td>
</tr>
<tr>
<td>Superficial Inguinal</td>
<td>2 nodes located in the furrow between the abdominal wall and the medial thigh</td>
<td>Includes the caudal abdominal and inguinal mammary glands, ventral half of the abdominal wall, penis, prepuce, scrotal skin, tail, ventral pelvis, and medial part of the thigh and stifle</td>
</tr>
<tr>
<td>Popliteal</td>
<td>1 node located behind the stifle</td>
<td>Includes areas distal to the stifle</td>
</tr>
</tbody>
</table>
Popliteal and prescapular are the preferred biopsy sites for generalized lymphadenopathy. Submandibular lymph nodes are frequently enlarged and reactive related to constant exposure to antigens; therefore, these lymph nodes are a poor choice for biopsy in generalized lymphadenomegaly but useful when they are only involved.

**Cytologic biopsy procedure considerations**

- **Lymph node size**: The size of the lymph node should also be considered. Very large nodes may yield misleading information as it frequently contains necrotic or hemorrhagic tissue. The center of a very large lymph node should be avoided during aspiration.
- **Fine needle aspiration biopsy**: For aspirate smears use a 22 gauge needle alone or together with a 6 or 12 ml syringe. The skin over the lymph node is prepared as for a surgical procedure. The lymph node is immobilized between fingers and the needle is directed into the parenchyma in several directions. Alternatively, a syringe may be added to the needle or butterfly catheter to provide negative pressure. Use quick, sharp, and multiple withdraw motions of the plunger. Release the pressure on the plunger before removing the needle to avoid splattering the material within the syringe. Reattach an air-filled syringe and expel the needle contents onto the approximate center of a glass slide. The aspirate will appear creamy white, watery to viscous indicating a cellular sample. Gently squash the material with a second slide, sliding them apart horizontally. Dry smears rapidly with a hair dryer to avoid cell shrinkage.
- **Needle insertion biopsy**: The needle can be inserted multiple times into the lymph node to collect material without aspiration. This technique of “fine needle capillary sampling” is especially helpful in obtaining minimally blood contaminated specimens which is enhanced by aspiration.
- **Impression smears**: For impression smears from an excisional or incisional biopsy, it is important to blot excessive tissue fluids and gently touch the surface to a glass slide.
- **Formalin fumes**: When concurrent surgical biopsies are obtained, keep cytologic preparations away from formalin fumes to avoid poor staining. Cytologic and histologic samples are mailed separately.

**Cytodiagnostic groups for lymph node cytology**

Cytologic interpretation of lymph nodes is based on the presence of one or more cytodiagnostic groups. For example, inflammation may be accompanied by reactivity so they may not be mutually exclusive.(1)

- Normal
- Hyperplasia or reactivity
- Inflammation
- Lymphoma
- Metastatic neoplasia

**Normal lymph node**

- Small, well-differentiated lymphocytes which measure 1-1.5 times the diameter of a RBC, should compose more than 90% of the population. The chromatin of these cells is densely clumped with no visible nucleoli. Cytoplasm is scant. These cells are the darkest staining of all the lymphocytes.
- Medium (2-3 times RBC) and large (>3 times RBC) lymphocytes may be present in low numbers (<5-10%). Their nuclei have a fine, diffuse and light chromatin pattern. Nucleoli may be prominent. The cytoplasm is more abundant and often basophilic.
- Mature plasma cells represent a small portion of the cells found. Chromatin is densely clumped and often the nucleus is eccentrically placed within the abundant deeply basophilic cytoplasm. A pale zone or Golgi zone is seen paranuclear.
- Occasional macrophages (histiocytes) can be found as large mononuclear cells with abundant light cytoplasm, often containing debris. Nuclear chromatin is finely stippled and nucleoli may be found in activated macrophages.
Occasional mast cells and neutrophils also may be present.

**Reactive or hyperplastic lymph node**
- Small lymphocytes still predominate, but there is an increase in medium and/or large cell types, up to 15% of the total cell population.
- Plasma cells are mildly to markedly increased in number and may be shifted toward immaturity. Some highly activated plasma cells are termed Mott cells characterized by abundant cytoplasm filled with multiple large spherical pale vacuoles that represent immunoglobulin secretions.
- Macrophages increase in number with antigen stimulation along with cytoplasmic vacuolation.
- Neutrophils or eosinophils also may increase in number; however, these cells occur in lower numbers than expected for lymphadenitis.
- Reactive lymph nodes are associated with local or generalized conditions. Local conditions include neoplasia, infection, immune-mediated disease, and other inflammatory situations. Generalized conditions include infectious, immune-mediated, and an idiopathic enlargement in cats. In the latter condition, cats present with marked enlargements of lymph nodes that histologically resemble lymphoma. These cases generally spontaneously regress in one to 17 weeks.

**Lymphadenitis**

*Purulent lymphadenitis* composed of >5% neutrophils may be associated with bacterial, neoplastic, or immune-mediated conditions.

*Eosinophilic lymphadenitis* involves >3% eosinophils and is often related to allergic dermatitis, mast cell tumor, hypereosinophilic syndrome, feline fibrous eosinophilic gastroenteritis, and certain lymphomas.

*Histiocytic or mixed cell lymphadenitis* involves moderate to marked increases in macrophages without or with neutrophils, respectively. Conditions associated with this inflammatory response include systemic fungal infections (e.g., blastomycosis), other fungal infections, mycobacteriosis, leishmaniasis, salmon disease, and pythiosis. When epithelioid macrophages are noted along with neutrophils, “pyogranulomatous” may be used to characterize the lymphadenitis.

**Lymphoma**
- The predominant cell is usually an immature lymphocyte, since small well-differentiated lymphocytes are infrequently considered neoplastic in the dog or cat. The population is often homogenous. Medium or large sized lymphocytes account for 60-90% of the total cells.
- Mitotic figures may be frequent (2 or greater per five fields at 40x or 50x) and suggest a high turnover rate of the cells.
- Lymphoglandular bodies result from the rupture of lymphocytes and appear as small platelet-sized blue-staining cytoplasmic fragments within the background of the preparation. Although they may be seen in benign lymph node conditions, a higher frequency is expected in lymphoma due to the cell fragility.
- Prognosis or response to treatment for canine lymphoma has been associated with immunophenotype, clinical stage, and prior use of corticosteroids. B-cell types generally occur in 75% of the canine lymphoma population and T-cell types in the remaining 25%.
- Veterinary medicine has adopted use of the current 2008 human World Health Organization classification system for lymphoid neoplasms which separates these neoplasms into distinct disease entities. This system is based on immunophenotyping, morphology, clinical presentation, genotyping, and biologic behavior.
- Surgical removal and histologic examination of the lymph node is recommended in all questionable cases to make a definitive diagnosis. To further aid in prognosis and disease classification,
immunophenotyping should be performed. If morphology or immunophenotyping cannot distinguish neoplasia versus hyperplasia, PCR techniques for B and T cell receptor clonality should be considered.

**Metastatic lymph node**
- Metastasis is suggested by the presence of a cell population not normally expected in a lymph node e.g., epithelial cell clusters.
- These “foreign” cells often appear abnormal with several cytologic features of malignancy.
- The remaining lymphoid population may appear reactive with cell types as previously described.
- The metastatic neoplasm may replace the lymph node parenchyma completely and in so doing interfere with the cytologic identification of the tissue as lymph node.

**Keywords:** Lymphoma, Lymphadenitis, Immunochemistry, Lymphadenomegaly

**Reference**
Clinical Diagnosis of Lymphoma is Improved by Laboratory Diagnostics Including Genetic Markers

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Summary

Diagnosis of chronic enteritis and gastrointestinal (GI) lymphoma requires histopathological evaluation of the GI tract; however, these conditions are often still difficult to differentiate from each other. We recently developed a new multicolor GeneScan analytical system for detecting antigen receptor genes rearrangements in endoscopically biopsied intestinal specimens. Lymphocyte clonality was demonstrated not only in dogs with intestinal lymphoma but also in those with chronic enteritis. Moreover, the frequency was especially high with marked enteritis harboring a large number of intraepithelial lymphocytes. Based on the diagnostic criteria proposed by World Small Animal Veterinary Association Gastrointestinal Standardization Group, a number of dog patients could be categorized into small cell intestinal T-cell lymphoma. Moreover, clonal rearrangement of the antigen receptor genes was shown to be a negative prognostic factor in dogs with protein-losing enteropathy. Finally, the presence of serum IgA antibodies directed to gliadin and tissue transglutaminase in dogs with chronic enteritis and intestinal lymphoma suggests that repetitive inflammatory stimulation could be a dietary factor to develop chronic enteritis and subsequent intestinal lymphoma as shown in celiac disease in humans. In conclusion, genetic markers assessed by PARR can provide measures for understanding the pathogenesis of intestinal diseases in dogs.

Chronic enteropathy is one of the common clinical diagnoses in dogs, characterized by persistent or recurrent gastrointestinal (GI) symptoms such as diarrhea, vomiting, and weight loss. The common causes of chronic enteropathy include food-responsive enteropathy, antibiotic-responsive enteropathy, and inflammatory bowel disease (IBD). Although the pathogenesis of canine chronic enteropathy is not yet fully elucidated, interaction between the intestinal microenvironment (bacterial or dietary antigens), mucosal immune system dysfunction, and genetic factors are considered to be involved in its etiology. GI lymphoma should also be taken into consideration in dogs with chronic GI symptoms as shown in cats. Feline small cell GI lymphoma is characterized by uniform population of small T-lymphocytes with a large number of intraepithelial lymphocytes termed as nests and plaques (1).

Since its advent, polymerase chain reaction (PCR) for antigen receptor gene rearrangements (PARR) has been introduced into veterinary practice as a useful adjunctive for the diagnosis of lymphoma. This method is used to detect clonal lymphocytic expansion by amplifying the T-cell receptor gamma-chain (TCRγ) gene and the immunoglobulin heavy-chain (IgH) gene. There are several studies on the application of PARR in GI biopsy specimens. One of these reports suggested that PARR was a useful diagnostic tool for detecting latent GI lymphoma, which cannot be histopathologically diagnosed using endoscopic biopsy specimens.
(2). On the other hand, a more recent study reported that clonal lymphocytic infiltration was also detected in the GI tract of dogs with IBD, and reduced diversity of lymphocytic infiltrates significantly correlated with one-year survival rate (3).

We recently developed a new multicolor GeneScan analytical system for detecting antigen receptor genes rearrangements in dogs using multicolor-labeled multiple primers and capillary electrophoresis (4). In a series of our studies on chronic enteropathy, endoscopically biopsied samples were evaluated by histopathological examination and GeneScan analysis for PARR to understand the pathogenesis of a group of chronic enteropathy in dogs.

**Association between lymphocyte antigen receptor gene rearrangements and histopathological evaluation in canine chronic enteropathy**

Although definitive diagnosis of chronic enteritis and GI lymphoma requires histopathological evaluation of the GI tract, these conditions are often still difficult to differentiate from each other. Hiyoshi et al. (5) investigated the relationship between the results of PARR and histopathological diagnosis, degree of enteritis or lymphoma, and long-term prognosis in dogs, in order to evaluate the clinical significance of PARR. Endoscopic biopsy specimens obtained from 96 dogs with chronic enteritis (mild, n = 14; moderate, n = 20; marked, n = 62) and 21 dogs with GI lymphoma (large cell) were used. Clonality was observed in 51% of the animals with chronic enteritis; interestingly, it was also found in 29% of those with only mild enteritis. In dogs with marked enteritis, the rate of PARR was higher in those with lymphocyte epitheliotropism than in those without epitheliotropism. There was no significant prognostic difference between chronic enteritis with or without clonal rearrangements. In contrast, dogs histopathologically diagnosed with marked enteritis had a significantly shorter survival time than did those with mild or moderate enteritis. While the significance of PARR in the diagnosis of GI lymphoma remains uncertain, the pathological roles of clonally expanding lymphocytes in canine CE should be investigated further.

**Prognostic factors in dogs with protein-losing enteropathy**

Canine protein-losing enteropathy (PLE) is associated with severe gastrointestinal disorders and has a guarded to poor prognosis although little information is available regarding factors affecting prognosis. Nakashima et al. (6) performed a study on the prognostic factors for survival of dogs with PLE. Ninety-two dogs diagnosed with PLE were included in a retrospective cohort study. Variables recorded at the time of diagnosis were statistically analyzed for possible prognostic factors in a univariate and multivariate Cox proportional hazard model.

In the multivariate analysis, the predictors for mortality in dogs with PLE were more highly scored in terms of canine inflammatory bowel disease activity index (CIBDAI) \((P = 0.0003)\), clonal rearrangement of lymphocyte antigen receptor genes \((P = 0.003)\), and elevation of blood urea nitrogen (BUN) \((P = 0.03)\). Using histopathological diagnosis, both small- and large-cell lymphomas were associated with significantly shorter survival times than chronic enteritis and intestinal lymphangiectasia. Normalization of CIBDAI and plasma albumin concentration within 50 days of initial treatment was associated with a longer survival time. In conclusion, CIBDAI, clonal rearrangement of lymphocyte antigen receptor genes, histopathological diagnosis, and response to initial treatments would be valuable in separating the underlying causes and could be important in predicting prognosis in dogs with PLE.

**IgA antibodies against gliadin and tissue transglutaminase in dogs with chronic enteritis and intestinal T-cell lymphoma**

Molecular clonality analysis of TCR genes for diagnosing T-cell lymphoma is widely used in veterinary medicine. However, differentiating chronic enteritis from intestinal lymphoma is challenging because of the incompatibility between histopathological and clonality analyses results. On the basis of findings that canine intestinal T-cell lymphoma and celiac disease (CD) share some common features, Matsumoto et al. (7) conducted serological examinations in combination with histopathological and TCR clonality analyses in 48 dogs diagnosed with
either chronic enteritis or intestinal lymphoma (small cell and large cell). IgA and IgG antibodies against gliadin and tissue transglutaminase (tTG) were quantitatively measured using ELISA. Histopathological analysis showed that dogs with intestinal lymphoma were likely to have high levels of serum IgA antibodies against gliadin and tTG, and serum IgG antibodies against tTG. Interestingly, dogs with intestinal lymphoma had a higher serum IgA titer against gliadin and tTG than did dogs with chronic enteritis. These results suggest an association between repetitive inflammatory stimulation by gliadin peptides and subsequent intestinal lymphoma in dogs.

In a series of our studies on chronic enteropathy in dogs (5-7), existence of clonally expanded lymphocytes was demonstrated by GeneScan analysis for PARR in a certain number of dogs having lesions histopathologically diagnosed with chronic enteritis. Frequency of the clonality was especially high in the lesions containing a large number of intraepithelial lymphocytes. Based on the diagnostic criteria proposed by World Small Animal Veterinary Association Gastrointestinal Standardization Group (8), a number of dog patients could be categorized into small cell intestinal T-cell lymphoma supported by the histopathological features and lymphocyte clonality demonstrated by PARR.

Considering the fact that a clonal population of lymphocytes was frequently present not only in intestinal lymphoma but also in chronic enteritis, it may be more reasonable to consider these two diseases as a continuum. Future studies may eventually provide supportive information to characterize subcategory of chronic enteritis cases at risk of developing intestinal T-cell lymphoma.

**Keywords:** Chronic Enteritis, Dog, Intestinal lymphoma, PARR, Small Cell Lymphoma

**References**


Prognosis and Management of Lymphoma are Improved by Laboratory Diagnostics and Genetic Markers

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Summary

Lymphoma is the most common hematopoietic malignancy in dogs. As a high proportion of dogs with lymphoma achieve remission soon after initiation of chemotherapy, an objective marker assessing treatment efficacy is required. Following clinical remission, the residual population of tumor cells can be referred to as the minimal residual disease (MRD). As an extension of the development of PCR for antigen receptor gene rearrangements (PARR) in dogs, there has been recent progress in the application of real-time quantitative PCR (RQ-PCR) to canine lymphoma patients. With the RQ-PCR system, a very high sensitivity (1 cell per 10,000 cells) has been achieved by preparing allele-specific oligonucleotide primers and probes designed from neoplastic clones of each patient. A series of MRD diagnostics studies employing the RQ-PCR system revealed its usefulness as a prognostic indicator, an objective marker of treatment efficacy, and a predictor of relapse for canine lymphoma patients receiving chemotherapy. Introduction of the MRD monitoring system will provide an innovative scientific tool in the development of superior treatments and monitoring strategies for canine lymphoma.

CHOP-based (cyclophosphamide [CPA], doxorubicin [DXR], vincristine [VCR], prednisone) protocols, were introduced for the treatment of canine lymphoma. The combination of CHOP-based protocols with L-asparaginase as adjunct therapy, is referred to as L-CHOP. In most cases such as in high-grade multicentric B-cell lymphoma, the disease is initially responsive to chemotherapy treatment. However, high rates of relapse and mortality are evident in nearly all cases as a result of disease progression. Overall response (OR) rate, progression-free survival (PFS), and overall survival (OS) in dogs with lymphoma treated with L-CHOP-based protocols were reported to be 70-90%, 7-12 months, and 10-17 months, respectively.

L-CHOP can rapidly induce complete remission (CR) in a large proportion of canine lymphoma patients. Median time from first day of L-CHOP to CR was reported to be 11 days (1). After achieving CR, the dogs continue to undertake chemotherapy for a certain period (e.g., up to 25 weeks in UW-25 protocol (2)). However, most dogs invariably experience relapse after termination of, or during, the protocol. This is indicative of a small but residual population of malignant tumor cells that persist within the body, referred to as the minimal residual disease (MRD), that may be implicated as the source of tumor relapses (3).

Prognostic significance of MRD in the early phase of chemotherapy in dogs with lymphoma

Although MRD levels after chemotherapy was found to have a prognostic significance in dogs with lymphoma (4), data at the end of chemotherapy cannot be obtained in nearly half of the sample population owing to the occurrence of progressive disease (PD) during the initial of remission induction therapy (5). In order
to apply the MRD monitoring system to the majority of dogs, Sato et al. (6) examined the prognostic significance of MRD levels in the early phases of a multidrug chemotherapy protocol in 36 dogs with multicentric high-grade B-cell lymphoma. The dogs were treated with a modified UW-25 protocol and evaluated for the MRD level at weeks 6 and 11 of UW-25. Of the 31 dogs that remained on the protocol by week 11, 14 were found to be MRD negative (less than 10 tumor cells/10^6 peripheral blood mononuclear cells [PBMCs]), whereas the other 17 were MRD positive. The PFS of the dogs with MRD-negative status at week 11 (median: 337 days) was significantly longer (P = 0.0002) than that of the MRD-positive dogs at the same time point (median: 196 days). These results highlight the clinical significance of MRD as a prognostic marker in the early phase of chemotherapy.

Increase in peripheral blood MRD before clinical relapse in dogs with lymphoma that achieved CR following chemotherapy

In order to identify the changes in MRD prior to clinical relapse in dogs with lymphoma that had achieved CR following chemotherapy, peripheral blood MRD was monitored by RQ-PCR in 20 dogs with multicentric high-grade B-cell lymphoma (7). During the follow-up period, 15 dogs relapsed in 28-320 days (median: 120 days) after completion of chemotherapy. An increase in MRD was detected 2 weeks or more prior to a relapse event in 14 of the 15 dogs. The time from increased MRD to clinical relapse was 0-63 days (median: 42 days). In contrast, no increase in MRD was detected in 5 dogs that did not experience clinical relapse. This study indicated that an increase in MRD can be detected before clinical relapse in dogs with lymphoma. This leads to the planning of early re-induction therapy based on an increase in MRD before clinical relapse (MRD-guided therapy) for the improvement of treatment outcome in canine lymphoma.

Evaluation of cytoreductive efficacy of VCR, CPA, and DXR in dogs with lymphoma as measured by RQ-PCR quantification of tumor cell number

As a high proportion of dogs with multicentric lymphoma respond well to the chemotherapeutic agents and achieve CR soon after initiation of chemotherapy, there is a difficulty in establishing drug efficacy from a standard clinical evaluation during treatment period. Cytoreductive efficacy of VCR, CPA, and DXR was evaluated in dogs with lymphoma that received the UW-25 protocol (2) without administration of L-asparaginase at week 1. The number of lymphoma cells in peripheral blood was measured from diagnosis to week 11 of the modified UW-25 in 29 dogs with high-grade B-cell multicentric lymphoma by using RQ-PCR (8). The number of lymphoma cells after the first administration of VCR, CPA, and DXR in weeks 1-4 was decreased in 100%, 52%, and 96% of the dogs, respectively. The cytoreductive efficacy of CPA was less than that of VCR and DXR. VCR, CPA, and DXR administered in weeks 6-9 were effective in 19%, 25%, and 74% of the dogs, respectively, indicating the sustained cytoreductive efficacy of DXR. CPA non-responders were heavier and exhibited a shorter first remission than CPA responders. The study provided several suggestions for the usage of these three antineoplastic agents in order to improve treatment efficacy. VCR use might be preferred in the early phase. CPA administration can be reconsidered, especially in large dogs (e.g., dose increase or substitution with other agents). DXR at the dosage used in the current protocol is highly effective, thus it can be recognized as a main drug in the combination protocol. Findings obtained in this study would be helpful to construct a new or modified chemotherapeutic protocol in order to obtain better treatment outcomes in dogs with lymphoma.

Quantitative MRD evaluation after or during antitumor therapy would provide an objective evaluation comparing the efficacy of different protocols, especially to indicate an advantage of newly introduced modalities such as high-dose therapy with autologous stem cell transplantation (9) and immunotherapy using monoclonal antibodies (10).

A series of works indicating the clinical usefulness of MRD monitoring (4,6,7,9) may potentially revolutionize the medical control of canine lymphoma. Measurement of MRD levels after a chemotherapy protocol can function as an objective parameter for the determination of treatment efficacy. This may potentially allow
clinicians to further recommend an additional treatment plan to a patient group harboring a relatively high MRD. MRD monitoring results obtained by a newly introduced protocol, in comparison to those obtained by conventional ones, could be used in assisting with the identification of the advantages and disadvantages of the new protocol. Consecutive monitoring of MRD during CR after completion of the protocols would be useful in predicting relapses, leading to the potential application of an early re-induction therapy prior to identifying clinical relapse.

Introduction of the MRD monitoring system will provide a tool to aid in the development of superior treatment strategies for canine lymphoma.

**Keywords:** Chemotherapy, Dog, Lymphoma, Minimal Residual Disease, Prognosis

**References**


Piezoelectric Bone Cutting Devices in Oro-Maxillofacial Surgery: Advantages and Clinical Use

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Summary
Piezoelectric bone surgery is a recent and innovative technology permitting a selective cut of mineralized tissue while sparing soft tissue. Similar to a dental scaler, a high frequency vibration, in the range of 25-35kHz, is transmitted to a metallic tip. However, the power of the piezosurgical instrument is 3 to 6 times higher than that of a dental scaler. The major advantages of this technology include high precision, a design that increases ease of curvilinear osteotomy, less trauma to soft tissue, preservation of neurological and vascular structures, reduced hemorrhage, minimal thermal damage to the bone as well as overall improvement of healing. The handpiece of the instrument is equipped with a sterile irrigation system and LED light, which improves visibility and overall safety. Piezoelectric surgery is particularly useful when performing delicate bone procedures such as periodontal or endodontic surgery. It is also indicated when performing more invasive bone surgery such as maxillectomy, mandibulectomy, and condylectomy, where preservation of neurovascular structures is important. Piezoelectric instruments are different from rotary instrumentation or oscillating saws, they require light pressure with constant motion of the tip. Training is required to master the technique.

Ultrasonic medical and surgical devices operate in the high-power (10–300W/cm²) low-frequency range (20–60 kHz) by creating vibratory energy for biological tissue cutting, ablation or fragmentation, and removal. This technology is used in many medical fields such as dentistry, phaco-emulsification in ophthalmology, tissue dissection in abdominal surgery, bone cutting in orthopaedic and maxillofacial surgery neurosurgery, selective fragmentation and removal of pathological (e.g. tumour) tissue, bone cement removal and lipoplasty (1). A recent review of its use in veterinary surgery has been published (2).

Technology
Ultrasonic bone cutting instruments have been first proposed by Vang in 1955 but did not gain popularity up until the 2000 when the need for precise bone cutting has been emphasized in maxillofacial surgery with the development of cortical bone graft and sinus surgery prior to dental implant placement (3). The technology is based on inverse piezoelectric activity: electricity applied to a piezoeactive ceramic generates high-frequency vibratory energy. Frequencies of 25–35 kHz (Hertz = vibrations per second) are specific for cutting mineralized tissue, reducing the risk of nerve and vessel damage. Soft tissue cutting requires frequency above 50 kHz (4) These vibrations results in a high-frequency almost linear reciprocal motion of the metallic tip of the instrument.

Advantages of piezoelectric bone surgery (table 1)
Piezoelectric bone surgery shows a selective cutting efficacy. At frequency in the range of 25-35 kHz only hard tissue are affected and soft tissue are spared. Soft tissue vibrates without fracture in contact with the osteotome tip. A study has shown that direct exposure of a peripheral nerve to a piezosurgery machine,
even in the worst-case scenarios, did not dissect the nerve but did induce some structural and functional damage. The perineurium of the nerve remained intact even after nerve contact at peak force, thus enhancing the potential for functional recovery. Importantly, the extent of damage was significantly higher with application of increased force on the nerve by the device, but not by activation of the ultrasonic vibration (5). The efficacy and safety of piezoelectric bone surgery has been challenged in neurosurgery where it has been shown that osteotomy of parietal bone of the cranial vault could be achieved without lesion of the dura mater (6).

The cutting instrument is made of a handle, not very different from the ultrasonic scaler handle, to which specific tips are attached. The tips can be made with different shapes and sizes and might be sharp, dentate (saw type) or diamond-coated. As for any ultrasonic device, the cutting efficiency resides in the device itself, the tip should be used with low pressure on the bone. With saw-type tips, it is recommended to first mark the bone with the teeth of the instrument, then to connect all the marks by drawing a line with the tip in a back and forth motion. Newest machine have a high power (up to 60 W), which enables them to cut dense cortical bone a lot faster than first generation machines. Though, it is generally accepted that a longer time is required for bone cutting with a piezoelectric scalpel compared to conventional saw or drills. In a study comparing osteotomy in sheep’s tibia using Er:YAG laser or Piezosurgery unit the mean cutting time of the tibial mid-shaft was 160-200 seconds for a 18x22 mm bone segment with the piezoelectric system and was longer with the Er:YAG laser (7). The amount of collateral soft tissue trauma and the risk of microfractures are reduced in these cutting techniques in comparison to oscillating saws or drills, as the application of high pressure is not necessary for cutting. The use of piezosurgery has been shown to be almost as fast as the use of rotary instruments in lower third molar teeth extractions in human patients (8). Use of piezosurgery resulted in less inflammation, less trismus and less discomfort with reduction in the need for pain medications (9).

The machine is equipped with a sterile irrigation system, which helps cleaning the operatory site, dissipating any heat due to friction (piezoelectric technology does not generate it itself) and favor hemostasis through cavitation effect (8). The handpiece is equipped with very high luminosity LEDs (100,000 Lux), which enable better visualization of the operatory site even in deep locations.

Table 1 Advantages of piezoelectric surgery (2)

<table>
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<td>Selective cutting of mineralized tissue</td>
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<td>Significant reduction of trauma to soft tissue.</td>
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<td>Reduced hemorrhage (cavitation effect)</td>
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<td>Excellent visibility within the surgical field,</td>
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<td>due in part to minimal bleeding, to high</td>
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<td>luminosity LED lights and effective irrigation.</td>
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<td>Precise cutting (limited vibration amplitude</td>
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<td>and specific design of osteotome tips).</td>
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<td>Curvilinear cutting.</td>
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<td>No thermal damage</td>
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<td>Sterile irrigation – Steam sterilization</td>
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Clinical uses
Due to the various and versatile tips that can be adapted on the handle of the instrument, and to the safety of the device in contact with soft tissue, there are numerous uses of piezoelectric bone surgery in dentistry and oromaxillofacial surgery (Figure 1). Though originally developed for implantology, it is nowadays used in many fields such as ENT surgery, neurosurgery and orthopaedic surgery in humans. Based on our clinical experience, the main fields where it can be used in veterinary surgery are: dentistry (periodontal
bone surgery, endodontic surgery), oral surgery (ostectomy-osteoplasty, surgical extractions, impacted teeth, root tips, harvesting cortical bone grafts), maxillofacial surgery (segmental osteotomy, rim excision, maxillectomy, orbitectomy, TMJ surgery) and ENT surgery (dorsal and ventral rhinotomy, tympanic bullae osteotomy and curettage).

![Figure 1 Piezotome2 LED® handpiece (Satelec, Mérignac, France) and various tips]

Compared to other bone cutting devices that we have been using in the past (manual bone chisels, rotary instrumentation and miniature oscillating saw) the biggest advantages of piezoelectric bone surgery reside in:

- the safety of the device when used in location where blood vessels and nerves are located and/or where the surgical access is limited (mandibular body, TMJ, vertical ramus of mandible, caudal maxillectomy, middle ear),
- the lack of thermal damage and bone necrosis
- the different cutting tips which can adapted to different bone density and different bone locations (saw-tip tips for dense cortical bone, angled-tip for limited accesses, diamond-tips for delicate cutting or grinding).
- the option for more conservative bone radical resection through the repositioning of the neurovascular bundle (infraorbital or mandibular).

**Limitations**

The progression from the first generation to the newest generation of piezoelectric handpiece for bone surgery with an expanded power has overcome most of the limits of this type of devices. It has to be acknowledged however that the use of the machine requires a training period. Other bone cutting devices such as hand chisels or rotative instrumentations might be faster in specific conditions, but lack the precision and safety of piezoelectric instruments. Beside specialists, veterinarians practicing oral-maxillofacial surgery but also orthopeadics and/or neurosurgery can make the best use of this instrument.

**Conclusion**

When facing with a difficult surgical access or the need for preservation of soft tissue is more important than speed, piezoelectric bone surgery offers a new exciting field in veterinary maxillofacial surgery.

**Keywords:** Piezoelectric, Ultrasonic, Maxillofacial, Surgery, Dog, Cats

**References**

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Periodontal Treatment: How Can Ultrasonic Devices Make Treatment Easier

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Summary
Subgingival treatment is the most important step of the initial periodontal treatment as it is aimed at removing plaque and plaque-associated calculus along the tooth root where it causes tissue destruction. In the past, this step has been performed with hand curettes. Hand instrumentation is very efficient but very difficult to teach and to master. It has been shown that specific ultrasonic subgingival tips used at a specific setting can be as efficient as hand curettes. Subgingival debridement with specific ultrasonic instruments is faster and easier to teach.

The goal of periodontal treatment is to suppress the plaque-induced inflammation in order to obtain healthy tissue. This is achieved through the removal of plaque and calculus both supragingivally and subgingivally (1). In most cases, this can be achieved by working blindly under the gum line with specific instruments. This primary and most important phase of the treatment is named the conservative periodontal treatment (also called hygienic etiological treatment or primary phase) by opposition to the surgical periodontal treatment.

Chemical reduction of the bacterial load and of the inflammation
The bacterial load can be reduced by flushing the oral cavity with a 0.12% or 0.2% chlorhexidine digluconate or with povidone iodine solution prior to periodontal treatment though the effect on bacteraemia is controversial (2). 97% reduction of salivary bacteria has been achieved in humans for up to 60 minutes during scaling and root planing following two 30-second rinses with 0.12% chlorhexidine (3). Good infection control is appropriate at all times, not just during the use of power-driven scalers because aerosols can be suspended in the air for up to 30 minutes. Adjunctive use of local antimicrobial after periodontal treatment has been studied with special focus on sustained release device or compounds. Though some statistically significant reduction of pocket depth and gain in attachment has been shown, there is no consensus on that use as these changes have not been shown to be of significant clinical benefit (4). Administration of systemic antibiotics for extended period of time cannot be used as a means of controlling dental plaque.

Mechanical scalers
Machines used are either ultrasonic scalers or sonic (pneumatic) scalers. Sonic scalers are air-turbine units that operate at low frequencies ranging between 1,500 – 16,000 cycles per second (Cps) or Herz (Hz), with a vibratory type tip movement that is primarily linear or elliptical in direction. They are fitted on one of the outlet of the air-driven unit. They are two different types of ultrasonic scalers: ferromagnetic (magnetostrictive) and piezoelectric (electrostrictive) ones. Magnetostrictive instruments operate between 20,000 and 45,000 Cps, using flat metal strips in a stack or a metal rod attached to a scaling tip. When an electrical current is supplied to a wire coil in the handpiece, a magnetic field is created around the stack or rod transducer causing it to constrict. An alternating current then produces an alternating magnetic field that causes the tip to vibrate. The tip movement of magnetostrictive units ranges from nearly linear,
to elliptical or circular, depending on the type of unit, and shape and length of the tip. Magnetostrictive tip movement allows for activation of all surfaces of the tip simultaneously, providing the option to use the side, back, or front of the tip for adaptation to the tooth surface. A piezoelectric unit operates in the 25,000 to 45,000 Cps range and is activated by dimensional changes in crystals housed within the handpiece as electricity is passed over the surface of the crystals. The resultant vibration produces tip movement that is primarily linear in direction, and generally allows only 2 sides of the tip to be active at any time (5).

Clinical effectiveness can be estimated from the displacement amplitude of the working scaling tip. The beneficial effects from the ultrasonic scaler is not only due to the mechanical oscillation of the tip but also to the cavitational activity and microstreaming forces of the water spray flowing over the oscillating tip. Acoustic microstreaming may play a role in disruption of subgingival biofilms.

Piezoelectric machines are characterized by a high efficiency (90%) with no loss of energy and no friction and subsequently almost no heat produced compared to magnetostrictive unit where 60% of the energy is converted to heat. Electric voltage applied to a piezoelectric ceramic generates ultrasonic energy, which is transmitted via a transducer to the handpiece. Modern machines allow vibration amplitudes of about 300 micrometers to be achieved within a frequency range of 27,000 – 32,000 Hz. Ultrasonic tips are screwed on the handpiece and the axial transmission generates vibration in one longitudinal plane whereas magnetostrictive scalers oscillate in an ovoid fashion.

Mechanical removal of plaque and associated calculus constitutes the single most important part of periodontal treatment. It comprises removal of supragingival debris but also, and most important, thorough removal of subgingival plaque and calculus attached to the tooth roots in the periodontal pockets. This latter step, though essential, is more complex to perform especially for the non-experimented practitioner.

**Subgingival debridment: a new concept for subgingival curettage and root planning**

The goal of periodontal treatment is the removal of plaque and calculus located in the pocket where it causes periodontitis. This is the single most important step. In the past, removal of subgingival plaque/calculus through subgingival scaling or curettage was completed by intense curettage of infected radicular cementum (root planing). The goal of root planing was to remove endotoxins (LPS) bound to root surface. It has been shown and it is today accepted that "radicular cleansing" is sufficient for achieving recovery of periodontal health as endotoxins are not deeply bound to cementum and, subsequently, aggressive root damage is unnecessary. Today, the term "subgingival debridment" is better used to describe the subgingival work (5). In this regard, the consensus report from the 1996 World Workshop in Periodontics states that intentional cementum removal should not be included in current periodontal debridement techniques for the purpose of removing toxic substances from the root surface (2).

The gold standard for subgingival debridement has been subgingival scaling using hand curettes. This is technique sensitive and may take a long time. Training is necessary before performing an efficient treatment. However, this step is fundamental as subgingival plaque/calculus is the cause of deep tissue inflammation and destruction. Various curettes exist in human dentistry. Gracey curettes with an offset blade and one cutting edge are convenient; when the terminal shank is parallel to the root surface, the cutting blade already forms a proper angle for subgingival scaling. Universal curettes have a right angle blade with two cutting edges. Curettes must be sharpened on regular basis (best is before each use) to keep a nice cutting edge. Attending a training session is highly recommended to learn how to use them properly.

In the past, periodontal debridement (scaling and root planing) was primarily performed with hand instrumentation since ultrasonic scalers were originally designed for gross supragingival calculus removal. Leon (1987) showed that power-driven instruments were as efficient as hand curettes. The superior ultrasonic effectiveness has been shown to be mainly in relation with its antibacterial effect (microacoustic streams)
New ultrasonic scalers have been modified to be used safely subgorgivally. Similar probing depth reduction and similar decrease in subgingival flora have been reported with both hand and power-driven scalers. Moreover, the lavage effect produced by the water coolant used with power-driven scalers provide a constant flushing activity during instrumentation that appears to have some therapeutic effects.

Table 1 Advantages of ultrasonic scalers

- High vibration amplitude (300 micrometers compared to 50 with magnetostrictive scalers).
- Large range of setting
- Several tips may be adapted on the same handpiece
- As effective as hand curettes for subgingival debridment but less time consuming.
- Less skill may be required to become competent with ultrasonic scalers than with manual scaling techniques.
- More effective in furcations than hand curettes.
- Better access to the bottom of the curette than hand curette.
- Ultrasonics used at a medium power setting cause less damage to root surface than hand or sonic scalers.

* Rootplaning: an old concept

The main difference between root planing and subgingival scaling was the depth of the cutting action. During root planing, infected, rough cementum covering the roots was removed with curettes. Technically, the difference is the angle and the pressure used during the treatment. As explained above, new development in periodontics have shown that complete removal of cementum to obtain a smooth surface is not necessary as infected cementum constitutes only a very superficial layer and better results may be obtained with a more conservative approach.

* Polishing

The concept of polishing is certainly better known than that of subgingival scaling because it is less difficult to perform and requires less training. The rational is to leave a surface as smooth as possible in order to delay plaque recolonization on tooth surface. The surfaces of the crown and the neck of the tooth are polished using a contrangle, rubber cups and prophy-paste. Performing a polishing after an ultrasonic scaling is useless if the subgingival work has not been properly performed. Root polishing with a specific device adapted to reach the pocket has not been shown to be more effective than ultrasonic subgingival debridment.

Treatment of periodontal disease must be followed by prevention of dental plaque regrowth (maintenance and dental home care) to avoid relapse of the disease

Keywords: Ultrasonic, Periodontal, Periodontitis, Dental Plaque, Calculus

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Contemporary Diagnostic Imaging in Dentistry and Maxillofacial Surgery

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Summary
Digital dental radiography remains the mainstay of imaging in dentistry. Systems based on photo-stimulated phosphor (PSP) technology, are preferred. The routine use of full-mouth radiographic series is the standard of care. Computed tomography is indicated for oral and maxillofacial trauma, tumors and soft tissue conditions. Cone-beam computed tomography is indicated for dental conditions and and Computed tomography with tridimensional (3D) imaging and 3D printing is very useful prior to advanced mandibular and maxillofacial surgery. Tridimensional imaging is especially useful for mandibular and maxillofacial fracture repair and palatal defect repair. Tridimensional printing is especially useful for surgical planning for mandibular reconstructions, complex oncologic excisions and correction of temporomandibular (TMJ) ankylosis.

Dental Radiography
Dental radiographs form an essential part of a comprehensive oral examination and radiological findings are a key element in dental decision-making. A commonly used approach is to radiograph those areas where one expects to find pathology, based on the visual oral examination and periodontal probing. The indications for dental radiography can be summarized as follows: (1) clinical signs of periodontal or endodontic disease; (2) prior to extraction, and post-extraction, if there is any suspicion of root fracture; (3) before, during and after endodontic procedures; (4) clinical staging of oral tumors; (5) dental trauma; and (6) diagnosis of missing teeth.

A "full-mouth survey" is defined as a series of radiographs depicting not only the teeth present, but also edentulous parts of the jaw bones that are normally tooth-bearing. It is common practice for a full-mouth radiographic survey to be obtained during a patient's first visit to a dentist. This is done for two main reasons: to determine the condition of the teeth and bones, and to establish a baseline for future changes. The routine use of full-mouth radiography is well established in human dentistry, although there is also some concern about the radiation safety aspects. However, for the adult, dentulous, new patient with clinical evidence of generalized dental disease, a full-mouth radiographic examination is considered appropriate.

Full-mouth radiography of small animal patients referred for dental treatment is not routinely done in practice, presumably because it is considered cost-prohibitive, and because it is not established practice to do so. With the increasing sophistication of veterinary dentistry and increased availability of suitable dental radiographic equipment, it is conceivable that the current standard of care should be upgraded to include full-mouth radiography when an animal is first presented for dental treatment.

A study of the clinical value of radiographs showed that the diagnostic yield of full-mouth radiography in dogs and cats is high.\(^1\,^2\) In 72.6 % of the dogs and 86.1 % of cats, radiography of teeth or areas with clinically evident dental disease provided additional, clinically useful, or essential information. Radiography of teeth or areas without clinically evident dental disease revealed incidental findings in 41.7 % of the dogs and 4.8 % of cats, and clinically important lesions in 27.8 % of dogs and 41.7 % of cats. Further inspection
indicated that older patients clearly derive more benefit from full-mouth radiography than do younger patients. The routine use of full-mouth radiography is therefore justifiable for new canine and feline dental patients. Direct digital imaging systems are becoming increasingly common in dental radiography. The majority of these systems are based on charge-coupled device (CCD) technology. The disadvantages of CCD systems include the restrictions associated with the bulky sensor, connecting wire, limited sensor size (no size #4), and high sensor cost. Systems based on photo-stimulated phosphor (PSP) technology, are preferred. Digital imaging based on PSP technology uses reusable imaging plates (including size #4) without cables or sensors, in combination with a conventional dental X-ray unit.

**Computed Tomography (CT)**
Computed tomography (CT) is becoming more readily available and affordable in veterinary medicine. It is indicated in dentistry and maxillofacial surgery in trauma cases to visualize the maxillofacial structures and temporomandibular joints. The use of CT with contrast medium is indicated for tumor cases and soft tissue conditions, such as masticatory muscle myositis. The CT scan should be acquired with a slice thickness as thin as possible.

**Cone-Beam Computer Tomography (CBCT)**
A recent advance in dental and maxillofacial imaging the cone-beam computed tomography (CBCT). With this imaging modality images are obtained with very high resolution. These can then be imported into special imaging software to evaluate the teeth and maxillofacial structures in great detail.

**Tridimensional Imaging and Printing**
Advanced mandibular and maxillofacial reconstruction surgery in veterinary medicine is becoming more common and receiving wider acceptance. However, these difficult cases require special preoperative planning due to the region’s complex anatomy. The use of tridimensional (3D) imaging and, more recently, 3D printing as surgical planning modalities for mandibular and maxillofacial surgery in dogs and cats were recently introduced. The use of 3D imaging following CT or CBCT is the standard of care at our institution and is performed by the attending surgeon. Several software programs are available for manipulation of DICOM files created by CT or CBCT for volume rendering and 3D imaging. This is routinely indicated for maxillofacial trauma cases\(^3\) as well as for oral tumor cases with bony involvement. It is also indicated for palatal defects, to compare the size and shape of the osseous defect with the soft tissue defect.\(^4\)

Having a 3D model provides the surgeon with the ability to perform precise preoperative planning and practice a virtual osteotomy and design a patient-specific implant preoperatively. The 3D printing of the affected skull overcomes this limitation and allows for a tangible understanding of the disorder and the precise surgical treatment. This may be further justified as precise presurgical planning may reduce the surgery time and allow for a reduction in overall surgical costs. Oral and maxillofacial tumors with bone involvement in difficult locations are indications for 3D printing. The 3D printed skulls allow for precise presurgical planning of the ostectomy sites. They are also excellent tools for client and student education.

Patients with complex mandibular and maxillofacial fractures may also benefit from 3D printing.\(^3\) The 3D printed skulls can be used for presurgical planning, plate selection and pre-bending of the plates, which saves on anesthetic time. For defect non-union mandibular fractures, the intact mandible can be mirrored for highly accurate pre-bending of the plate destined for the affected side.\(^5\)

We routinely use 3D printing of skulls prior to mandibulectomy and reconstruction.\(^6,7\) The 3D model of the intact mandibles, prior to mandibulectomy, can be used for ostectomy planning and for pre-bending the plate for the reconstruction.

Corrective ostectomies for ankylosis and pseudoankylosis of the temporomandibular joint can be very complex and not only involve the condylar process but also the coronoid process, zygomatic arch, and temporal bone. Precise preoperative planning and practicing a virtual osteotomy is possible with 3D printed models.\(^8\)
Keywords: Dental Radiography, Oral Radiography, Cone-Beam Computed Tomography, Computed Tomography, Tridimensional Imaging, Tridimensional Printing, Dogs

References
Principles of Oral and Maxillofacial Surgery

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Summary
The principles of oral and maxillofacial surgery, aspects of oral anatomy and histology, wound healing, hemostasis, instrumentation, and basic surgical and suturing techniques, will be discussed.

Overview
An overview will be given of aspects of oral anatomy and histology relevant to oral and maxillofacial surgery (OMFS). Wound healing of the oral soft tissues and maxillofacial bones will be discussed. The essential and some unique instruments used in OMFS will be illustrated. The importance of atraumatic tissue handling and hemostasis will be emphasized. The commonly used tissue flaps, and suturing techniques and suture materials will be discussed.

Keywords: Oral Surgery, Maxillofacial Surgery, Oral Wound Healing, Dogs, Cats.

References
Disorders Affecting Jaw Opening/Closing:
Diagnosis and Treatment

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Summary
there are many oral conditions that may interfere with mouth opening/closing. They may directly affect
TMJ and motion or may indirectly prevent voluntary motion because of pain, tissue swelling and
space-occupying effect. Based on history and clinical presentation, diagnosis may require blood tests,
tissue biopsy, electro-diagnostic tests and diagnostic imaging (radiographs, ultrasounds, CT-scan or MRI).
Specific treatment depends on the identification of the cause of the disorder.

Inability to fully open or close the oral cavity is a disorder that can be easily clinically identified but which
may be due to various causes interfering with the biomechanics of the jaws.

Anatomy and biomechanics
The mandibles and maxillae articulate by a synovial condylar joint, the temporomandibular joint (TMJ),
composed of the head of the mandible and of the mandibular fossa, which is part of the squamous part
of the temporal bone. Because of the shape and angulation of the joint, mandibular movement is mainly
in vertical dimensions (hinge motion) with very little lateral displacement contrary to humans. Jaw closing
is due to temporal, masseter and pterygoid muscles, which are innervated by the trigeminal nerve. Jaw
opening is due to gravity and the action of the digasticus, which is innervated in its caudal part by facial
nerve and in its rostral part by the trigeminal nerve. During opening of the mouth, the mandibles rotates
around the axis of the TMJ and the ramus and coronoid process are rotating rostrally.

Clinical presentations
Disorders affecting mouth opening/closing may arise from dental, osteo-articular and soft tissue structures
causes. Disease processes affecting mouth opening are more frequent than these affecting mouth closing.
Clinical examination helps differentiate between both. Trismus (restricted mouth opening) is most of the
time due to a painful local condition resulting in a constriction of masticatory muscles. In specific cases,
a systemic cause may also be identified. Clinical examination under general anaesthesia with analgesic
drugs is mandatory to help determining whether the restricted opening is transitory (linked to pain) or permanent.
Permanent restricted mouth opening is most likely due to a chronic disease process affecting osteo-articular
structures or surrounding soft tissue or and cannot be improved during physical examination. Etiological
diagnosis may require blood tests, tissue biopsy, electro-diagnostic tests and diagnostic imaging (radiographs,
CT-scan or MRI).

Osteo-articular diseases
Cranio-mandibular osteopathy (CMO): this condition affecting mainly West Highland White Terrier has an
autosomal recessive basis (1). It occurs in young dogs (3-8 months) as a painful ventral mandibular bony
swelling, which can extend on the TMJ and tympanic bulla. Inability to open the mouth is due to the pain and to ankylosis in case of extension to the TMJ. The condition is self-limiting and resolves in most dogs at the age of 1 year. Steroids are used to limit swelling and associated pain and to facilitate intake of food during the diseased period.

**TMJ diseases (2):** any TMJ trauma (luxation/fracture) may lead mouth opening or mouth closing disorders. Trauma, and less commonly infection or tumours, may also further lead to TMJ ankylosis. Diagnostic is mainly based on history, clinical and diagnostic imaging (CT scan being mostly useful). Treatment of ankylosis is gap condylectomy.

Jaw fractures: pain, displacement of bony parts and subsequent malocclusion may prevent normal mouth opening/closing.

**Coronoid process displacement:** this condition mostly occurs in Basset Hound and Setters. Due to muscular dysfunction and abnormal TMJ anatomy, the coronoid process (tip of the ramus) is displaced laterally during opening and closure of the jaw. At time of closure, the coronoid process may hit the ventral border of the zygomatic arch or move lateral to it, thus preventing full closure of the mouth, which appears locked. This condition is painful and easily diagnosed based on history and clinical examination. Treatment consists in ventral zygomatic arch osteotomy and/or coronoid process osteotomy.

**Soft tissue conditions**

**Muscular diseases (3,4):** masticatory muscles are particular as myosin fibres are of a different type than other muscles, type-2M fibres. A specific disease named masticatory muscle myositis (MMM) is specifically affecting this group of muscles and progressively results in the closure of the mouth and impossibility to open it, even with anaesthesia. In the acute phase, swelling and pain is present. In the chronic phase, muscle atrophy and fibrosis is present. Diagnosis is based on history, clinical examination, muscle biopsy and eventually dosage of anti-2M antibodies (see internet address below). This disease is considered immune mediated and treatment is based on corticosteroids and, sometimes, immunosuppressive drugs.

**Trigeminal paralysis:** clinical presentation is a dog with inability to close his mouth. It appears as a dropped mandible. The mandible is flaccid and can be moved but the dog is not able to hold it. Usually no other neurological signs is present. The exact aetiology is unknown though trauma to trigeminal nerve is likely. The condition resolves without treatment within 3-6 weeks. Dietary support is needed during this period.

**Infections (5,6,7):** infection in oral cavity, especially in the caudal area, but also in the pharyngeal, orbital and retro-orbital area may induce pain, swelling and restriction in mouth opening due to the space occupying lesion. Orbital cellulitis of dental origin and wood stick penetration are some of the recognized conditions. Diagnosis may require ultrasound or MRI to thoroughly evaluate the location and extent of the lesion.

**Tumours:** tumours of the mucosa or connective tissue may restrict jaw mobility because of pain, fibrosis or space-occupying lesion. As for inflammatory soft tissue conditions, MRI may be superior to CT scan in evaluating the extent of the lesion.

**Dental diseases**

Pain and soft tissue swelling associated with infections as well as tooth displacement associated with trauma or mobile teeth, secondary to periodontitis and bone loss, or may prevent normal mouth opening/closing.

**Systemic causes**

Tetanus is the most common systemic cause that can interfere with mouth opening. As there is no specific
test, diagnosis is based on the specific clinical presentation and on the exclusion of other neuro-muscular causes. Early antibiotic treatment with fluid and dietary supports are key-factors for recovery. In a recent paper, survival rate was only 50%.

**Keywords:** Trismus, Oral Disease, TMJ, Myositis, Jaws, Mandible

**References**

Rethinking Jaw Fracture Management: Non Surgical and Surgical Approaches

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Summary
Jaw fractures, particularly mandibular fractures, have been traditionally treated with open reduction and placement of metallic implants (surgical approach). Because these bones have specific anatomical and biomechanical characteristics (presence of dental structures, masticatory function) it cannot be merely considered as a long bone. Placement of metallic implants may lead to severe complications (infections, malocclusion). Closed reduction with placement of a tooth-born intra oral device can be used to stabilize the fracture (non-surgical approach) in specific conditions. Interdental fixation can be used for fracture in the dentate area rostral to the carnassial tooth and Intermaxillary fixation can be used for caudal fracture. Very good clinical results have been achieved. Cases where a non-surgical treatment cannot be used constitute good indications for surgical treatments. Titanium maxillofacial locking plates and screws are the best choice for osteosynthesis of the mandible.

Jaw fractures are more frequent in young adult dogs and are mostly due to trauma by cars and dog's fights. Mandibular fractures are more prevalent than maxillary fractures. The body of the mandible appears to be particularly involved, and the molar region accounts for 41 to 47.1% of mandibular fractures (1,2). In addition, a high incidence (70.6%) of mandibular fractures was observed in small breed dogs (2). In cats, 75% of the lesions are located in the rostral part of the mouth (symphysis and para-symphyseal area) (3). The bone of the jaws differs from other bones as it contains specific hard structures, the teeth. The mandibular body contrary to the ramus is not protected by thick muscular tissue. As a consequence, 75% of the mandibular fractures in dogs are open fractures. Almost half (43%) of the fractures are complex fractures and almost all open fractures (94%) are located in a dentate area and involve the alveolar socket (4). Jaw fractures may be managed by invasive treatments involving external or internal fixation devices (plate and screws, external fixators, intramedullary pins and wires) and non-invasive treatments relying on tooth-born devices. Reported complications of conservative (tape muzzle) and invasive treatments are malocclusions (35%), osteomyelitis (27%), non-union (17%) and delayed bone healing (11%) (4).

Biological considerations
Restoration of dental occlusion and preservation of periosteal attachment and vascularization at the fracture site while minimizing dental trauma are key-elements in the management of mandibular and maxillary fractures (5,6). The mandibular alveolar bone has been shown to have a 30% lower blood flow compared to the maxillary alveolar bone in dogs, which suggests there is less potential for the mandible to heal (7). Small breed dogs have comparably large first mandibular molar teeth with roots that occupy most of the jaw bone height (8), which provides only limited safe space for the placement of intraosseous metallic implants without risking damage to dental structures and the associated neurovascular bundle in that region. Biomechanical studies have shown that placement of a fixation device on the tension surface of the mandible
provides better neutralization of mastication forces (9). Champy’s technique developed in humans uses this principle and proposed a stabilization of the mandibular fracture by a titanium mini maxillo-facial plate placed on the tension side and screwed to the bone with monocortical screws to prevent root damage. Such a technique is not fully applicable to dogs and cats due to their specific anatomical characteristics. Nevertheless, an interdental splints applied on the tension surface of the mandible (alveolar margin) offers such a mechanical advantage. To proper restore occlusion while making an interdental splint of placing a plate and screws, pharyngostomy intubation is recommended.

**Non-invasive treatments**

*Maxillo-mandibular fixation:* maxillo-mandibular fixation (MMF) can be made by binding maxillary and mandibular canine teeth in occlusion in a semi-closed fashion with dental composite resin. Because of the size and specific occlusion of canine teeth in dogs and cats with normal occlusion, both jaws can still be locked leaving a 1 to 2 cm opening between maxillae and mandibles. This allows the animal spontaneous feeding with a liquid or soft diet. This technique can be used for different type of fractures pending the four canine teeth are stable. Good clinical results have been reported in dogs and cats (10, 11). In emergency cases, for a transitory period, or for non-displaced stable fractures, a tape-muzzle can be used to prevent opening and closing of the jaw. However, restoration of the occlusion and stabilization of the fracture is inferior to that obtain with a fixed MMF.

*Wire-Reinforced Interdental Splinting:* an Interdental splint can be used for various types of fracture pending there is a stable and sound large tooth rostral to the fracture line and another one caudal to the fracture line. In most typical cases, whether it is a maxillary or mandibular fracture, the two anchoring teeth are the canine tooth, rostrally, and the carnassial tooth, caudally. Fracture at the level of or caudal to the distal root of mandibular carnassial tooth can seldom be treated with this technique. Clinical healing occurs in about 2 months with few complications. There is a relationship between the extent of lesion on the tooth at the line of fracture and the clinical healing time and the rate of complications. Subsequently, thorough assessment of the tooth in the line of fracture is mandatory. Intraoral radiographs are preferred over standard extraoral radiographic technique as they provide better assessment of the fracture line and tooth in the fracture line.

*Interdental wiring:* the role of the interdental wire is to provide mechanical retention of the splint on the teeth and to increase the biomechanical properties of the device compared to acrylic splints alone. Various patterns of interdental wiring have been described. No biomechanical differences were found in a recent in vitro study that compared two commonly used interdental wiring techniques, including the Stout loop and crossover techniques.

*Splint: prior* to placing the splint, the tooth surface has to be scaled and the mouth rinsed and dried. Oral wounds needs to be sutured. Soft tissue can be protected with K.Y. gel. Intraoral composite resin material manufactured for temporary bridges and crown is preferred as the polymerisation of the material is not associated with an exothermic reaction, and teeth scaled, rinsed and dried. The interdental wire provides macro-mechanical retention for the splint. In addition, acid-etching of enamel can be used to increase micro-mechanical retention of the resin material on the teeth and/or to place a bonding agent (liquid organic resin material), which will increase adhesion of the composite resin on the enamel surface. Once the resin material is placed, the mouth is closed in occlusion and maintained closed until full polymerisation. Excess material is removed, if possible, prior to curing or with a dental bur after polymerisation. When making a mandibular splint limited amount of resin material must be placed on the vestibular surface of the carnassial teeth to avoid interference with the occlusion. On the opposite, when making a maxillary splint, no or little material must placed on the lingual side.
Invasive treatments
Titanium maxillofacial locking plate (2.0 or 2.4 mm) constitutes our first choice for mandibular plating. The plate can be 3-D contoured to adapt the specific anatomy of the mandible. Once screwed, a standard plate is held by friction on the bone. With locking plates, the threaded head of the screws locks in the holes of the plate. The plate and screws form a mechanical couple ensuring stability without compression on the bone. The load transfer of this construction occurs via the plate, not by preload and friction, and provides relative stability. This is the same principle as that of an external fixator but at the level of the bone surface.

Fate of the tooth in the line of fracture
Teeth are important because they may serve as anchor points when using tooth-born fixation techniques such as interdental splints but also because of their bone-maintaining function. On the opposite, an infected root in the line of fracture will contribute to bone resorption and will prevent healing. It is accepted in humans that teeth in the line of fracture should be saved if not infected and if they allow reduction of the occlusion and stabilisation of the fracture. A recent meta-analysis on teeth in the line of mandibular fracture in humans reported that intact teeth in the fracture line should be left in situ if they show no evidence of severe loosening or inflammatory change but should be extracted if they prevent the proper reduction of fractures, in the presence of fractured roots or extensive periapical lesion or periodontal damage, with broken alveolar walls, resulting in the formation of a deep pocket. In specific cases, when only one root of the mandibular carnassial tooth is severely affected, the other root can be retained and temporary or definitive endodontic treatment performed. Any injured tooth and any tooth located in the vicinity of the fracture line must be evaluated along time. There is no consensus in veterinary medicine for the optimal timing of the radiographic follow-up. We routinely recommend a first radiographic control at 4 weeks in dogs and cats treated with dental splints and at 2 months for the ones treated with an internal fixation device. Subsequent radiographic follow-up examinations are based on the aspect of the healing at the first examination but are most of the time done at 6-8 weeks for dental splints (at time of removal) and at 4 to 6 months for internal fixation. Depending on potential remaining problems associated with the teeth, other recheck examinations may be recommended as deemed necessary. The decision whether to perform treatment, or not, of the tooth in the fracture line at the time of jaw fracture repair is based on the clinician’s decision and experience. When the complication is associated with the tooth in the fracture line, an antibiotic treatment cannot solve the problem without undertaking a dental treatment. When bone healing has occurred, the tooth in the line of fracture, which was initially saved, may be definitively treated or extracted if required.

**Keywords:** Mandible, Maxilla, Fracture, Trauma, Interdental Splint, Dogs, Cats

**References**
Recent Advances in Maxillofacial Fracture Management

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Summary
Internal fixation of maxillofacial fractures allows quick return to normal function and excellent cosmesis. Non-locking plates and screws system are easier to contour to the complex maxillofacial anatomy. Appropriate multidisciplinary preoperative management and diagnostic imaging are essential. Titanium miniplates and screws are typically not removed, unless complications ensue, which is rare.

Introduction
The maxillofacial complex in the dog is the most prominent part of the skull rendering it vulnerable to severe injuries.1 The term 'maxillofacial' refers to structures involving the incisive, palatine, zygomatic, lacrimal, frontal and nasal bones as well as the maxillary bone proper.1,2 This anatomic region is located between two strong craniofacial bony structures, the cranial base and the mandibles.3 Biomechanically, the maxillofacial structure can be thought of as a strong, but lightweight, frame made of thin bones and large air spaces of the nasal cavity and paranasal sinuses.4 The maxillofacial frame is strengthened by the buttresses, or projecting support, that maintains the position of the maxilla in the appropriate relationship with the base of the skull and the mandibles.5,6,7 These buttresses in the dog and their importance in maxillofacial reconstruction have been characterized.1,5 Miniplates have been used for maxillofacial osteosynthesis in people since the 1970's.6-8 These special plates are ideally suited for comminuted or simple maxillofacial fracture repair because of their size and ease of contouring. Plates are typically applied using a tension-band principle and provide buttress support in maxillofacial fracture repair.9 Moreover, the transition from using stainless steel plates to titanium has advanced the field of reconstruction as titanium miniplates are lightweight, possess and elastic modulus and density similar to bone, and have great biocompatibility.9 They are also not typically removed after fracture healing because of their excellent osteointegration.7,10,11 Advanced maxillofacial reconstruction is uncommonly performed in animals. Boudrieau used miniplates for maxillofacial fractures achieving excellent functionality and cosmesis,5,12 and has reported important considerations in miniplate application in dogs.9 We recently reported our technique, experience, and follow-up of internal fixation of severe maxillofacial fractures using titanium miniplates in dogs.13

Preoperative Evaluation, Diagnostic Imaging and Surgical Planning

We recommend a multidisciplinary approach for systemic stabilization after trauma. Specifically, our patients evaluated by emergency medicine, ophthalmology and neurology clinicians. Computed tomography (CT) is used for diagnostic imaging. For surgical planning, three-dimensional (3D) reconstructive images are generated to assess spatial relationships of the fractured bones. The first consideration is to try and reconstruct the lateral, medial, and caudal buttresses, in that order.1 An additional important consideration is the presence of unstable bone fragments and the proximity of adjacent stable bone for securing those unstable fragments. Reconstruction of the orbit is also considered a priority, as is reconstruction of the nasofrontal vault if
compromised. Finally, if the alveolar processes were involved, restoration of normal occlusion is planned.

**Surgical Technique**

Extraoral and/or intraoral approaches can be used, depending on anatomical location.\(^\text{14,15}\) Selection of approach is based on preferred access to the fractured site. Traumatic wounds are avoided when possible and débridement of compromised soft tissues should be conservative to avoid unnecessary tissue loss. We use a variety of low profile titanium 2-mm non-locking miniplates (Synthes® Maxillofacial 2.0 mm Mandible Trauma, Paoli, PA). Plates are secured to bone with at least 2 non-locking, self-tapping titanium screws in each segment of the fracture. The sequence of plate placement is initiated from reconstruction and fixation of the buttress to serve as a base onto which other fragments are secured.

**Outcome**

We recently reported the outcome of internal fixation for maxillofacial fractures using titanium miniplates in 7 skeletally mature dogs.\(^\text{13}\) Fractures healed rapidly after reconstruction with immediate return to normal function and occlusion. Follow-up time of up to 94 months indicated excellent long-term function and general lack of complications. One dog developed nasal aspergillosis 1.5 years after surgery and the miniplates were removed without adverse consequences. We therefore concluded that internal fixation for maxillofacial reconstruction using titanium miniplates is an excellent solution for the treatment of comminuted and displaced maxillofacial fractures in dogs.

**Keywords:** Maxillofacial Fractures; Mandibular Fractures; Dogs; Cats.

**References**

Current Concepts in Palatal Surgery

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Summary
Computed tomography is indicated for palatal defects to allow for appropriate surgical planning, as the soft tissue defect of the cleft may underestimate the extent of the bony defect. Craniofacial defects with congenital palatal defects are common in the dog. Selective dental extractions before definitive surgical repair using mucosal flaps in a double-layer approach, is an effective alternative when treating complex hard palate defects in dogs. Surgical repair of clefts of the primary palate involves 3 steps: (1) repair of the palatal defect; (2) repair of the floor of the nose, gingival margin, alveolar mucosa and labial mucosa; and (3) repair of the lip and nasal plane.

Introduction
Congenital and acquired hard palate defects are common in small animals. These defects are clinically relevant because they often result in poor nursing with growth retardation and/or malnutrition, chronic rhinitis, and aspiration pneumonia.

Several surgical techniques have been described for palatal defect repair in dogs and cats. Traditionally, hard palate defects are repaired using local mucosal flaps to cover the area and anatomically isolate the oral from the nasal cavity. However, a common challenge is large defect size and a relatively small amount of surrounding soft tissues, which may impede successful repair. Definitive closure of palatal defects often requires a multi-stage approach. Successful surgical correction may also depend on the precise location and distribution of the defect, integrity and quality of the surrounding tissues (i.e. healthy, and intact mucoperiosteum versus fibrous tissues from previous failed surgical attempts), size of the associated bony defect, among others.

Failure of hard palate defect repair using mucosal flaps may occur because of excessive tension at the suture lines when the size and configuration of the flaps is not appropriate in relation to the defect size, shape, and location. Other cited causes for failure include infection of the surgical site, compromised blood supply, poor tissue quality, lack of underlying bony support at the suture lines, use of suboptimal suture materials and/or patterns, among others. When choosing the surgical technique, these factors must be considered to optimize the likelihood for successful repair.

Diagnostic Imaging
Diagnostic imaging by means of computed tomography (CT) is essential. In a recent retrospective CT study involving 9 dogs with congenital palatal defects, CT studies of the head were reviewed on a structure-by-structure basis. The soft tissue defect of the cleft was always smaller than the bony defect. Tympanic bullae were most commonly found to be abnormal, followed by nasal turbinates, nasal septum, vomer, cribriform plate, frontal sinuses and lateral ventricles. Other abnormalities were related to occlusion, teeth, incisive bones, maxillary bones, mandibles, hyoid apparatus, cranial bones and nasopharynx. We therefore concluded that craniofacial defects in dogs with congenital palatal defects are common and some
of these may negatively affect the quality of life. Importantly for surgical planning, the soft tissue component of the cleft may underestimate the extent of the bony defect, especially in failed repairs.

**Surgical Technique and Outcome - Secondary Palate**

Dental extractions in the proximity of a hard palate defect may be of value in dogs and cats with hard palate defects; the rationale is that by performing strategically planned dental extractions before definitive defect repair, larger areas of viable tissues will become available, thus allowing surgical closure in 2 layers that comply with the previously mentioned principles.

We recently reported on 6 dogs that had selective maxillary teeth extractions 4 - 8 weeks before definitive hard palate defect repair by double-layer local full-thickness mucosal flaps. All palatal defects were considered complex. Complete hard palate closure was achieved after initial attempt in 3 dogs; 2 dogs had revision surgery before complete closure, and in 1 dog, closure failed and further treatment was declined. No complications or long-term consequences were associated with selective dental extractions. We therefore concluded that selective dental extractions before definitive surgical repair using mucosal flaps in a double-layer approach, is an effective alternative when treating complex hard palate defects in dogs.

**Surgical Technique - Primary Palate**

Several techniques are available for repair of clefts of the lip and primary palate. It is best to allow the rostral maxillary permanent dentition to complete its eruption prior to definitive surgical repair. Strategic dental extractions may be necessary. Clefts of the primary palate are repaired in three steps, which include: (1) repair of the palatal defect; (2) repair of the floor of the nose, gingival margin, alveolar mucosa and labial mucosa; and (3) repair of the lip and nasal plane.

**Keywords:** Cleft Palate; Palate Surgery; Diagnostic Imaging; Maxillofacial Surgery; Dogs.

**References**


Current Concepts in Temporomandibular Joint Surgery

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Summary

The temporomandibular joint (TMJ) is a unique joint that requires special clinical and imaging considerations. Understanding the biology and mechanics of the TMJ are essential for formulating the appropriate therapeutic approach. In most cases, a conservative therapy is recommended occasionally surgical therapy is needed. The most common TMJ disorder in dogs is arthritis followed by fractures. In cats, the opposite is true. TMJ osteoarthritis is likely to occur in concert with other disorders requiring special consideration.

Introduction

The temporomandibular joint (TMJ) is a synovial joint in which the condylar process of the mandible articulates with the mandibular fossa on the squamous portion of the temporal bone, and both the condylar process of the mandible and the mandibular fossa of the temporal bone are covered with a unique fibrocartilaginous layer.¹ A fibrocartilaginous articular disk separates the TMJ cavity into dorsal and ventral compartments and fills the void between the condylar process and the mandibular fossa, which promotes the congruity of the joint. Importantly, the joint capsule attaches to the articular disk circumferentially, and a lateral ligament additionally strengthens the lateral aspect of the joint capsule. The sensory innervation of the temporomandibular joint is derived from the auriculotemporal and masseteric nerves of the mandibular branch of trigeminal nerve.

Relative to the cranium, the mandibular fossa remains stationary; it is only the mandible that moves via the TMJ. In carnivores, the TMJ moves via a hinge movement necessary for opening and closing the mouth, although in dogs it also allows for a slight laterotrusive movement. In cats, the morphology of the TMJ is more restrictive such that independent movement of the mandible aside from hinge movement is minimal.

Temporomandibular joint disorders are often debilitating and frequently require medical or surgical treatment. In a computed tomography (CT) study performed by our group that included 142 dogs and 42 cats, the most common TMJ disorder in dogs was osteoarthritis; however, in most cases, there were other TMJ disorders present in addition to osteoarthritis.² In cats, fractures were the most common TMJ disorder, followed by osteoarthritis. Other important TMJ disorders in dogs and cats were fractures, dysplasia, anklylosis, luxation, and tumors. Computed tomography is currently the mainstay tool in evaluation of the TMJ. Computed tomography is valuable for evaluation of osseous lesions as well as the spatial position of the TMJ bones, and CT images with three-dimensional (3D) reconstruction may improve understanding of the pathogenesis of TMJ lesions for selected patients.

The goal in managing any TMJ disorder is to restore pain-free functionality, mandibular symmetry and occlusion, and to prevent complications. Not all TMJ disorders warrant surgical intervention and, in fact, most disorders can be safely managed with non-surgical intervention or with minimally invasive techniques.
**Temporomandibular Joint Dysplasia**

Temporomandibular joint dysplasia typically results in subluxation and lateral and/or rostral displacement of the mandible. In turn, the coronoid process contacts the adjacent zygomatic arch and results in intermittent episodes of inability to close the mouth or joint pain without locking episodes. The diagnosis of TMJ dysplasia should heavily rely on clinical findings of ‘locking’ of the coronoid process on the zygomatic arch. This can be done safely with the patient under general anesthesia. A CT scan should complement the clinical findings. Long-term TMJ dysplasia should be managed surgically. The main aim of the surgery is to eliminate the potential for ‘locking’ of the coronoid process on the zygomatic arch. Therefore, coronoidectomy with or without zygomatectomy are the current surgical methods of choice. As TMJ dysplasia can occur bilaterally, clinical and CT evaluation should include both joints. Management of TMJ dysplasia by means of condylectomy is inappropriate and will result in even greater displacement of the mandible and higher potential for open-mouth jaw-locking.

**Temporomandibular Joint Luxation**

Temporomandibular joint luxation typically occurs in a rostro-dorsal direction.³ Due to the presence of the retroarticular process, luxation in caudal direction rarely occurs and if it does, the retroarticular process is likely to be fractured. Regardless, the direction of the luxation and the TMJ should be imaged by means of CT to confirm the disorder, direction and potential associated disorders. In most cases, management of TMJ luxation is done by closed reduction. Under general anesthesia, a fulcrum is obtained by placing a pencil (but not too rigid) transversely across the mandible as caudal as possible. While the mouth is gently closed the pencil is rotated in counterclockwise to result in the mandible moving in ventral and caudal direction and the TMJ to rearticulate. Rigid fixation by means of maxillomandibular fixation is recommended for 7-14 days. Recurrence of luxation is likely to occur if no fixation is done. A muzzle can be placed but may not have a positive outcome as the TMJ can relax spontaneously. Feeding tube placement is necessary if rigid maxillomandibular fixation is placed.

In difficult situations, open reduction may be needed. In that case, it is advised to examine the reason for the difficult reduction. Folding of the TMJ disc, foreign body or preexisting TMJ arthritis are possible reasons for difficult TMJ reduction. Reconstruction of the TMJ capsule and lateral ligament are necessary to prevent luxation.

**Temporomandibular Joint Fractures**

Temporomandibular joint fractures typically occur as a result of trauma and are often seen in combination with other maxillofacial injuries.³ We can classify TMJ fractures to be intra-articular and extra-articular. Moreover, the fracture can involve the condylar process and the mandibular head as a solitary lesion or cross the joint and involve the mandibular fossa as well. Furthermore, the fracture segments can be non-displaced or have displacement to various extents and may have fracture fragments within the joint space. Hence, it is crucial to have a full understanding of the fracture configuration prior to formulating a treatment plan. As in other TMJ disorders, fracture characterization should be made based on CT imaging. The goal of managing TMJ fractures is to restore mandibular symmetry, occlusion and function and to prevent long-term complications. In young dogs and cats as well as in most adult dogs (and based on the fracture configuration), non-surgical (i.e., conservative) therapy is the method of choice. In fact, there is excellent chance for fracture healing and regeneration of the damaged tissues as well as continuation of normal development in young dogs and cats with TMJ fractures.

Maxillomandibular fixation (MMF) can be done in either one of 2 methods: (1) Rigid: a dental composite (i.e., temporization material) that ‘cement’ the mandibular and maxillary canine teeth in a closed-mouth position and allowing 10-20 mm of mouth opening; and (2) elastic: a placement of elastic device between the canine teeth and the patient have minimal guiding function and the jaws maintain minimal mobility.

In general, if there is a detectable malocclusion, then closed reduction and rigid therapy is recommended for a period of 7-14 days in young patients and 2-4 weeks in adults. Once the rigid MMF is removed,
elastic therapy can be maintained for 2 additional weeks. Disadvantage of rigid therapy is delayed return to normal function, maintaining feeding tube, poor oral hygiene, difficulties in thermoregulation and potential aspiration. However, if the fracture is non-displaced and there is mild or no malocclusion, then elastic (functional) therapy is recommended for a period of 14 days. This will allow a more rapid return to normal function as compared to rigid therapy, allow the fracture area to receive more blood supply (due to the movement of the joint and muscles surrounding it) and decrease the chance of complications due to aspiration or thermoregulation issues. Open reduction is only recommended if there is/are fracture fragments in the joint space preventing opening or closing the mouth. Condylectomy is not recommended and should be reserved as an extreme measure in case of complete destruction of the TMJ and for fragments that prevent the joint from regaining normal function.

**Temporomandibular Joint Osteoarthritis**
Diagnosis of TMJ osteoarthritis (TMJ-OA) can only be achieved by clinical examination combined with CT evaluation. It is important to remember that while TMJ-OA is the most common TMJ disorder, it is often combined with other TMJ disorders. Once TMJ-OA as a solitary disease is diagnosed, therapy is aimed at alleviating pain and regaining TMJ functionality. Non-steroidal anti-inflammatory medications for a period of 2-4 weeks with or without opioid supplementation are recommended. In addition, 'jaw rest' can be performed for a period of 2-4 weeks by preventing rough chewing (i.e., chewing on raw hide, tug-of-war games etc.). However, physical therapy by means of opening and closing the mouth in order to exercise and stretch the joint in a controlled fashion is recommended. In addition, soft food should be given for the first few days followed by return to normal size kibble. Only in extreme situation where the pain cannot be controlled or the TMJ exhibits signs of ankylosis, condylectomy should be performed.

**Temporomandibular Joint Ankylosis**
True ankylosis of the TMJ or intracapsular fusion of the joint can be osseous or fibrous. It typically results in reduction of range of motion up to the point of complete immobility. The most common cause of ankylosis is trauma associated with the condylar process and/or the mandibular head. Other causes are previous surgical treatment that resulted in scarring and joint infection. In general, the incidence of TMJ ankylosis in dogs and cats has not been reported and in humans is about 0.4% following TMJ trauma. The treatment of choice is condylectomy or 'gap arthroplasty'. There is no need to fill the void following condylectomy with adipose tissue.

**Keywords:** Temporomandibular Joint, Condylectomy, Dogs, Cats

**References**
Recent Advances in Oral Tumor Management

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Summary
Malocclusion, discomfort and pain commonly result from bone defects following mandibulectomy. In an attempt to prevent malocclusion several innovative techniques were developed at UC Davis. A mandibular rim excision is occasionally possible and avoids malocclusion. For a total mandibulectomy malocclusion can largely be prevented by the use of the elastic training technique. Reconstruction of the mandible using titanium reconstruction locking plates or locking miniplates and screws represents the ideal solution. rhBMP-2 delivered in a compression resistant matrix (CRM) is a viable solution to regenerate mandibular bone and restore bone continuity. A single locking plate is biomechanically adequate for stabilizing mandibular defects.

Mandibular Rim Excision
A surgical technique for excision of minimally invasive mandibular tumors at the level of the premolar and molar teeth and the outcome in 7 dogs that had mandibular rim excision have been described. Using an intraoral approach to the mandible, buccal, and lingual mucosal incisions are made to obtain a 10-mm clean margin beyond neoplastic tissue. After subperiosteal soft tissue elevation, a curvilinear rim mandibulectomy is performed, leaving the mandibular canal and ventral border intact, followed by osteoplasty. The remaining attached gingiva and alveolar mucosa are sutured over the bony defect. The dogs were treated for odontogenic and early malignant neoplasms involving the mandible by mandibular rim excision. All dogs had healed, healthy gingival covering over the surgical defect, very good postoperative function, and good quality of life. Mandibular rim excision, with preservation of the ventral border and mandibular canal content, can be a good option for treatment of early odontogenic and malignant lesions of the mandible in medium to large breed dogs.

Elastic Training for the Prevention of Mandibular Drift Following Mandibulectomy
A noninvasive method for preventing mandibular drift after mandibulectomy in dogs has been described. The case series included 18 dogs that had mandibulectomy involving excision of a portion of the mandible caudal to the 2nd mandibular premolar tooth. One orthodontic button was attached to the lingual aspect of the canine tooth of the intact mandible and 1 to the buccal aspect of the ipsilateral maxillary 4th premolar tooth. An orthodontic elastic rubber chain was attached to the buttons creating tension sufficient for maintaining normal occlusion. The rubber chain was replaced weekly by the clients. Follow-up appointments were scheduled 2, 6, 10 weeks postoperatively and monthly thereafter if indicated. The appliance was removed when dogs had resumed normal occlusion of the canine teeth. All dogs maintained normal occlusion, normal jaw function, had no apparent disfigurement, and resumed preoperative activity levels while wearing the appliance. Eight dogs achieved temporomandibular joint stability and normal occlusion 4.5–6 months postoperatively and 8 did not, resulting in mandibular drift. It was concluded that elastic training using orthodontic buttons and power chain is a viable option for prevention of mandibular drift but requires good
client compliance. Elastic training is a quick, simple, cost-effective and noninvasive technique, preserving normal occlusion and function in many dogs after mandibulectomy.

Immediate Reconstruction Following Segmental Mandibulectomy

Utilizing a regenerative approach and specialized internal fixation for immediate reconstruction of critical-size bone defects following segmental mandibulectomy has been proven to be a viable and predictable method. Using a combination of extraoral and intraoral approaches, a locking titanium plate is contoured to match the native mandible. Following segmental mandibulectomy for treatment of malignant or benign tumors, the plate is secured and a compression resistant matrix infused with 0.5 mg/ml rhBMP-2 at a soak volume of 50% is implanted in the defect. The implant is then covered with a soft tissue envelope followed by routine intraoral and extraoral closure. Our experience over the past 5 years has demonstrated that dogs that had mandibular reconstruction healed with intact gingival covering over the mandibular defect and had immediate return to normal function and occlusion. Moreover, mineralized tissue formation was observed clinically within 2 weeks and solid cortical bone formation within 3 months. Computed tomographic findings postoperatively and in follow-up examinations demonstrated newly regenerated mandibular bone with a bone density and porosity comparable to the contralateral side. Hence, mandibular reconstruction using internal fixation and CRM infused with rhBMP-2 provides an excellent solution for immediate reconstruction of segmental mandibulectomy defects in dogs.

Regenerative Approach to Bilateral Rostral Mandibular Reconstruction

Extensive rostral mandibulectomy in dogs typically results in instability of the mandibles that may lead to malocclusion, difficulty in prehension, mastication, and pain of the temporomandibular joint. Large rostral mandibular defects are challenging to reconstruct due to the complex geometry of this region. In order to restore mandibular continuity and stability following extensive rostral mandibulectomy, a surgical technique was developed using a combination of intraoral and extraoral approaches, a locking titanium plate and a compression resistant matrix infused with rhBMP-2. Furthermore, the surgical planning consisted of computed tomographic scanning and 3D model printing. The regenerative surgical technique was typically done in 2 stages (i.e., delayed reconstruction). Bilateral rostral mandibulectomy is the first stage followed by a second stage in approximately 4 weeks to reconstruct the rostral mandibles. The main reason for the staging is that it allows for soft tissue healing over the rostral mandibles and prevents the occurrence of plate exposure through the mucosa. To date, all dogs treated have healed with intact gingival covering over the mandibular defect and had immediate return to normal function and occlusion. Follow-up computed tomography findings demonstrated that the newly regenerated mandibular bone increased in mineral volume with evidence of integration with the native bone. In summary, rostral mandibular reconstruction using a regenerative approach provides an excellent solution for restoring mandibular continuity and preventing mandibular instability in dogs.

Keywords: Mandibular Reconstruction, Elastic Training, Mandibular Rim Excision, rhBMP-2, Dogs

References
Oral Masses in Cats: Inflammatory or Neoplastic?

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Summary
oral masses are frequently encountered in the oral cavity of cats. They may appear as an enlargement or a proliferation of oral tissue and affect soft tissue, jaw bone or both. Due to the higher prevalence of oral inflammatory conditions and of oral carcinoma compared to dogs, thorough diagnostic investigations needs to be undertaken in order to achieve proper diagnosis and treatment.

Cats may quite frequently show various conditions resulting in enlargement of oral or perioral tissues with a soft or hard consistency. These conditions may share similar clinical features, which can lead to a wrong diagnostic unless thorough clinical examination is followed by diagnostic imaging and histopathology.

Because both benign dental diseases and cancerous lesions, such as squamous cell carcinoma, are frequently encountered in the feline oral cavity, early and thorough assessment is essential in achieving the correct diagnosis.

Diagnostic approach
Signalment and history: some disease processes are more likely to occur in young animals (pyotraumatic mucosal lesions, feline inductive odontogenic tumor) whereas others more frequently to affect older ones (malignant tumors). Breed is seldom a criterion of importance. History of previous recurrent diseases such as periodontitis, dental resorptions, eosinophilic oral lesions is to be taken into account as well as the slow or fast growing of the lesion.

Extraoral examination: when the enlargement can be seen externally, facial structures should be palpated to assess its consistency and the presence of pain on palpation. Mandibular lymph nodes should be evaluated for unilateral or bilateral enlargement. With a maxillary deformation, nostrils and nasal airflow must be inspected to assess potential nasal involvement; ocular discharge, ocular inflammation and asymmetrical eye-ball retropulsion must be assessed for potential peri-orbital diseases.

Intraoral examination: it includes both assessment of mucosal surfaces and teeth. Any mass or bump must be clinically evaluated for its size, location pertaining to gingival or other oral mucosae, consistency (soft, fluctuant, firm, hard), appearance (smooth or irregular surface, ulcerative or proliferative aspect). Extension in the sublingual area, palatal area or oropharyngeal area must be noted, pharyngeal tonsils must be evaluated. When the lesion is close to or around a tooth, a thorough clinical dental examination is performed using dental probe and dental explorer: the tooth is assessed for evidence of fracture and pulpal exposure, tooth discoloration, dental resorption or periodontal pocketing.

Cytology and histopathology: Cytology and/or histopathology are ways of achieving true diagnosis. Most
of the time biopsy followed by histology is the preferred means. Cytology may be complementary to biopsy to evaluate lymph nodes (fine needle aspiration), superficial masses (exfoliation cytology). The biopsy is performed under general anaesthesia and must sample the mass in its depth to avoid false diagnostic. When the mass is likely to invade bone structures, the biopsy is better performed after diagnostic imaging to make sure that the most representative tissue are sampled.

**Diagnostic imaging:** dental radiology is an essential diagnostic tool for assessing dental-related lesions. In cats, radiographs of teeth with clinical lesions yield additional or clinically essential information in 53.9 and 32.2%, respectively (1). Masses localized along the dental arch or along the palate are eligible to diagnostic imaging. Depending on its localization, of its potential extent and on the presumptive diagnosis following clinical examination, dental radiograph, skull radiographs, CT scan or MRI may be indicated to assess lateral and deep extent of the mass. Isolated masses on oral mucosae may not require diagnostic imaging at first until identified as a potentially invasive lesion. Furthermore, CT scan and MRI allow assessment of loco-regional extent and distant metastasis.

**Various clinical conditions**

**Eosinophilic granuloma complex:** may be found in cats in the perioral area (indolent ulcer on the maxillary lip facing the canine teeth and eosinophilic granuloma of the mandibular lip appearing as an enlarged “chin”) and in the oral cavity. Slightly raised red lesions with a yellowish patchy surface may be found on the surface of the tongue, sublingual mucosa, palatal or pharyngeal mucosa. They may appear as single of multiples nodules and be more or less spread over the mucosa.

**Pyotraumatic mucosal lesions:** chronic traumatic lesions of the oral mucosa especially in the sublingual area or on the alveolar mucosa may appear as red proliferative or ulcero-proliferative lesions. Bacterial secondary infection may also give a suppurative aspect. Origins of the trauma include foreign bodies, laceration or occlusal trauma. Brachycephalic cats have a tendency to have maxillary carnassials teeth inclined lingually which may result in a traumatic contact with the alveolar mucosa along the mandibular carnassials teeth and result in the creation of a proliferative lesion.

**Dental related lesions:** dental resorptive lesions are frequently encountered in cats; 25 to 30% of cats show at least one tooth affected in the general random feline population whereas the prevalence increase to 60-70% in cats presented for oro-dental diseases. Similarly, 70% of cats radiographically examined for oro-dental diseases show radiographic changes of periodontitis (2). Dental infections due to pulp necrosis or periodontitis as well as tooth root resorptive lesions may be associated with alveolar jaw bone osteomyelitis. Clinically, a suppurative or non suppurative aspect can be observed altogether with soft tissue swelling and bony changes. Depending on chronicity of the lesion and balance between infection and host-defenses more or less bone apposition versus bone lysis can be seen. Additionally, buccal bone expansion has been described as an idiopathic condition in cats radiographically associated with vertical bone loss and appearing as an increase in the thickness of the alveolar bone and widening of the periodontal ligament space buccal to the canine teeth. Clinically, it appears as an enlargement of the bony crest, particularly visible on the maxillary teeth. The affected tooth may also present pathological eruption due to bone remodeling and subsequent tooth extrusion.

**Oral tumors:** they comprise almost 10% of all tumors encountered in this species and the 3rd site of tumors after the hemolymphatic system and the skin. Squamous cell carcinoma is the most prevalent tumor and account for about 70% of oral tumors in cats (3,4). Other tumors comprise odontogenic tumors, fibrosarcoma. Oral squamous cell carcinoma in cats are frequently observed on the sublingual, lingual, gingival and pharyngeal areas. Macroscopically they may appear as mostly ulcerative or ulceroproliferative masses. Gingival
carcinoma show bone invasion quite frequently and may present with a osteolytic or osteolytic and osteoproliferative lesion, especially when the mandible is affected. In the caudal oral cavity, the tumor may spread to the orbital and zygomatic areas. Metastasis to the lymph nodes are observed in 30% of the cases and to the lung in 10% of the cases (5). Clinical presentation of oral squamous cell carcinoma in cats may be very confounding and can be misdiagnosed at first as an ulcerative lesion of the gingival or of the alveolar mucosa or as dental infection when bone lysis is present around the tooth. Prognosis is fair to poor depending on the local and extent of the lesion at the time of diagnosis. It is therefore essential to use a thorough diagnostic scheme to avoid misinterpretation leading to a delayed diagnosis while wrongly attempting to treat the lesion as a benign mucosal or dental lesion.

Conclusion
The oral cavity in feline is peculiar because of the numerous inflammatory processes, dental-related or not, that may be encountered and which may be confused with the development of a squamous cell carcinoma. Because of a huge difference in prognosis between these conditions, extreme care should be taken to reach as quickly as possible the correct diagnosis through a systematic diagnostic approach.

Keywords: Feline, Neoplasm, Oral Disease,

References
Update on Feline Chronic Gingivostomatitis, a Frustrating and Debilitating Disease

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Summary
Feline chronic gingivostomatitis is a debilitating and frustrating oral inflammatory condition characterized by ulcero-proliferative lesions extending on the alveolar, labial/jugal and caudal oral cavity mucosa. Its aetiology remains unknown but an abnormal individual body's response to chronic antigenic oral stimulation is suspected. Various causes of this antigenic stimulation include oral bacteria and viruses such as oral Calicivirus. Though dental extractions (full mouth or premolar/molar teeth) are mandatory in trying to achieve clinical cure, it is not 100% efficient and many cats require medical treatments for extended period of time. Newer developments have focused on immunomodulatory treatments such as recombinant omega-interferon, cyclosporine and stem cells.

Definition: what are we talking about?
Feline chronic gingivostomatitis (FCGS) is a syndrome clinically characterized by pain on eating and/or opening the mouth, pawing at the mouth, dysphagia, weight loss, bad general condition, grooming deficiency, ptyalism, and sometimes bleeding from the mouth (1,2). Typically, cats show ulcerative and/or ulcero-proliferative mucosal lesions located around the teeth (alveolar/labio-buccal stomatitis or caudally to the dental arch and lateral to the glossopalatine arches (caudal stomatitis). Cats presenting with the most severe and frustrating condition show caudal stomatitis, which may extend more caudally in the oropharyngeal area (oropharyngitis).

Hypothetical cause
FCGS is considered a multifactorial condition resulting from an inappropriate individual response to chronic antigenic oral stimulation of bacterial and viral origins. Chronic oral carriage of Calicivirus is thought to play a role in cats presenting with caudal stomatitis. Other factors acting on the host's immune response may exacerbate the expression of clinical lesions (3). Dental related conditions including periodontal disease and, possibly, dental resorptions are chronic inflammatory processes, which may play a role. However, no specific bacterium is associated with this condition. Several viruses may affect cats' oral and upper respiratory cavities. The prevalence of chronic carriage of Calicivirus in a random cat population is estimated between 20-30% according to various studies whereas the prevalence of Herpesvirus carriage seems lower (5%). Calicivirus have been known for a long time to cause acute focal or multifocal ulcerative glossitis and palatitis as well as acute upper respiratory disorders. The prevalence of Calicivirus carriage associated with “chronic gingivitis/stomatitis” has also been reported to be higher than in the random population. More specifically, it has been shown that acute caudal stomatitis (sometimes wrongly called “faucitis”) can be experimentally induced with Calicivirus strains sampled from the oropharynx of cats suffering of chronic stomatitis. However, this experimentally induced acute caudal stomatitis did not result in a chronic disease in the experiment setting. Recently, it has been shown using PCR technology that almost all cats presenting with caudal stomatitis were chronic oral calicivirus carrier whereas only 30% of cats with chronic gingivitis/stomatitis but without caudal stomatitis were. No specific biotype of calicivirus has been identified to be responsible for acute respiratory disorders, joint disease (lameness), oral vesicular
disease or feline chronic gingivo-stomatitis. The same strain can induce different clinical signs. However, there are antigenic differences between “stomatitis” related Calicivirus and acute respiratory disorders-related Calicivirus. Chronic infections isolates are antigenically more distant from the other isolates than the acute / respiratory isolates. The reason for this seems to be that antigenic changes occur during chronic infection. This may cause progressive mergerge of isolates, which are antigenically more distant from other isolates. Antigenic variations (resulting from a series of mutations) are induced by the immunologic pressure during chronic infection and constitute an escape mechanism for the virus (4)

**Diagnosis**

Clinical diagnosis through a thorough examination of the oral cavity is aimed at recognizing different clinical entities that may affect the mouth. Precise terminology should be used to avoid placing under the same headline all inflammatory processes. Gingivitis and periodontitis might be severe and develop rapidly (aggressive periodontitis) but are well know conditions associated with dental plaque accumulation. Extension of the inflammatory process on the alveolar mucosa beyond the muco-gingival line may sometimes occur associated with aggressive periodontal disease. However, so-called chronic gingivitis/stomatitis (FCGS) is only a therapeutic challenge when chronic caudal stomatitis is present. Chronic caudal stomatitis is most of the time associated with alveolar/buccal mucositis and all the time associated with some extent of periodontal disease. The term chronic gingivitis/stomatitis should not be used to designate any of these inflammatory conditions. Clinical diagnosis must be completed, under general anaesthesia, by radiographic identification of the extent of periodontitis and of the presence of dental resorptions. Biopsy of the mucosa should be performed when the aspect, the extent or the severity of the lesion is unusual (for example, severe unilateral inflammatory process) to rule out a neoplasia (e.g. carcinoma). A biopsy report concluding to a lympho-pasmocytic infiltrate is totally non-specific and only indicates a chronic inflammation in the mouth. PCR technology can be used to identify oral calicivirus carriage.

**Treatments**

**Dental extractions:** various treatments have been advocated for this condition. Most of them have been aimed at reducing the inflammatory process, decreasing discomfort and allowing better nutrition. 80% improvement has been reported with the use of long-acting steroids, megestrol acetate and Gold salt. However, no publication has shown that cats could be cured and side effects with these drugs are important. The current therapeutic approach to chronic gingivitis/stomatitis with chronic caudal stomatitis is to eliminate all sources of infection and of chronic inflammatory processes from the mouth in order to facilitate the action of body’s defence system against Calicivirus. There is still some debate on which teeth need to be extracted as some practitioners advocate full-mouth extractions instead of a more selective approach, which mostly result in extraction of premolar and molar teeth. The results of dental extraction in calicivirus positive cats with chronic caudal stomatitis can be globalized as such: 50-60% of cats are cured, 25-30% are markedly improved and about 15% are totally non-responsive (2,5).

**Adjunct treatments:** they are used both in the post-operative phase after dental extraction and for cats which are insufficiently improved after extractions. Choice of the protocol is individually-based and depends on clinical signs (bacterial surinfection within oral cavity and pain) more than on appearance of the lesions. Appearance of the lesion is however recorded to correlate it with clinical signs in order to evaluate improvement and cure. Various drugs can be used to improve the cat clinically.

**Antibiotics:** most commonly used drugs include amoxicillin-clavulanic acid, clindamycin, doxycycline and spiramycin-metronidazole. A three-week course is prescribed in addition to specific dental treatments in order to decrease the oral bacterial load over a significant period of time and to improve the oral confort. Additionally, refractory cases may be partially improved by courses of several weeks of antibiotics in order to decrease oral surinfection. Azithromycin has been suggested in Bartonella-positive cats with FCGS though recent studies (6) failed to find any correlation between FCGS and Bartonella sp. and treatment with such a drug is reported unrewarding.
Pain killers: ppioids and Non-Steroidal Anti-Inflammatory Drugs (NSAID) are used in the peri- and post-operative periods. Other drugs such as gabapentin and amantadine may also be used in the post operative period and for refractory cases.

Anti-inflammatory drugs: glucocorticoids can be used but high dosage are best avoided in calicivirus-positive or herpes-positive cats. A tapering three-week course can be prescribed. As far as possible, NSAID are better used inflammation control in calicivirus positive cats. When insufficient effect is observed, minimum dose (to effect) of corticosteroids can be used and in our experience has not been shown no negatively affect the outcome.

Supportive therapy: vitamins, fatty acids and specific food for critical care patients are used as necessary. Tube feeding can be done through an oesophageal tube after surgery for the most debilitated cats but is rarely necessary in our experience. A recent study has shown that changes in omega6/omega3 fatty acid ratio did not result in less inflammation or improvement of the healing (7).

Immunomodulating drugs: recent randomized double-blind controlled-studies have shown that a daily oral administration of a diluted dose of Feline Recombinant Interferon omega (Virbagen omega® Virbac) and daily administration of ciclosporin had a significant effect on cats with refractory cases of chronic caudal stomatitis (not improved after extraction) compared to control (8,9). Recently, treatment with fresh autologous mesenchymal stem cells has been tried in 9 cats; of the 7 cats that completed the study, 3 showed complete clinical remission and 2 substantial clinical improvement (10). Further studies on immune-modulatory treatments are necessary.

A recent systematic review of the literature has been published and provides useful highlights on the extent, depth and relevance of knowledge on this feline condition (11).

Keywords: Feline, Oral Diseases, Stomatitis, Gingivitis

References


Diagnostic Approach to the Pruritic Dog

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Summary
Though there are many causes of canine pruritus, this is a clinical presentation that lends itself well to a systematic, stepwise approach. The author suggests a 3-step approach, in which the first step is to identify obvious causes such as parasites; step 2 is to eliminate secondary complications such as bacterial and yeast skin infections; and step 3 is to carefully observe the remaining clinical signs. This in combination with the patient's history often helps guide further diagnostic evaluation to find the true cause of the itch.

Step 1. Identify the Obvious Things
The first step is to consider common and usually obvious things like parasites. A careful search for fleas, mites, lice, etc. should be made with a combination of the following steps:

- Flea combing and visual examination
- Check for history of application of flea preventive products and other antiparasitics
- Superficial and deep skin scrapings; trichogram (hair pluckings)
- Empirical treatment for scabies (very hard to find mite) – usually one of the avermectin or isoxazoline drugs

Step 2. Extinguish the Fires
Most patients with pruritic skin disease of any severity have their primary disease complicated by one or more “layers” of secondary complications. This often makes it impossible to see what the true nature of the primary disease is. Therefore, it is critically important as a next step to identify and treat any and all possible secondary complications. This should be addressed on the first visit. The most common pruritic secondary complications are infections. These can be identified by physical examination of the skin, in association with skin cytology where necessary.

- Superficial staphylococcal pyoderma can be recognized by the typical appearance of papules, pustules, and epidermal collarettes. There is typically pruritus, ranging from mild to severe. All of these lesions, and in addition any draining or ulcerative lesions, should be assessed by cytology, looking for typical cocci organisms. If present, treat with appropriate antibiotic (or topical chlorhexidine) for 4 weeks. If the pet has been treated with multiple courses of antibiotics in the past, a culture and susceptibility test is recommended to detect multi-drug resistant staphylococci.
- Malassezia yeast dermatitis is usually very pruritic, and there is a greasy, moist, or waxy appearance to the skin, with odor. The pruritus is often nonresponsive to corticosteroids. With chronic disease, there is lichenification and hyperpigmentation to form an appearance of "elephant skin." The most common areas are the feet, legs, neck, axillary, inguinal, and perineum. If these clinical signs are present, look for yeast by cytology. Remember that the condition is actually a hypersensitivity reaction to the yeast, so the number of yeast may be very small. If there is any doubt, TREAT! For initial
treatment, the author prefers oral ketoconazole (or any other oral azole), 5-10 mg/kg/d for 2-4 weeks.

- Remember to treat any otitis externa if present. This is a common secondary problem in many dogs and contributes greatly to the patient's discomfort.

- If staphylococcal or yeast infections are being treated, avoid use of systemic and topical corticosteroids. There are 4 reasons this is important: (1) they may lengthen or alter the course of staphylococcal pyoderma; (2) they don’t usually help yeast dermatitis anyway; (3) the anti-inflammatory effect may reduce the severity of lesions, resulting in the infection looking better, which may cause the owner to stop treatment prematurely; and (4) a major diagnostic goal early on is to determine if pruritus persists after elimination of secondary infections. It is acceptable to use antihistamine medications here – they won’t interfere with resolution of infection, may cause slight sedation to help the pet sleep, and give some ‘psychological’ comfort to the owner.

**Step 3. What Remains?**

Following this, *the patient’s response to infection control* is a valuable clue to the underlying disease and will aid greatly in planning logical diagnostic evaluation for each patient. Here, the clinician will have treated with infection and parasite control ALONE, for 3 to 4 weeks, and observe the clinical response. We can then propose 4 groups of possible underlying causes, depending on response.

- **If the response to infection control is a complete clearing of lesions, yet with substantial remaining pruritus,** (implying the underlying disease is in the “pruritic but not lesional” group) allergic causes such as adverse food reactions or environmental allergy should be strongly considered. Here, application of “Favrot’s Criteria” to make a clinical diagnosis of canine atopic dermatitis may apply. If the dog has any five of the following eight criteria present AFTER control of infections and parasites, there is a high likelihood that atopic dermatitis is present:
  - Age of onset < 3 years
  - Dog lives mostly indoors
  - Corticosteroid-responsive pruritus
  - Chronic or recurrent yeast infections
  - Affected front feet
  - Affected ear pinnae
  - Non-affected ear margins
  - Non-affected dorsal lumbosacral area

- **If the response to infection control is a partial clearing of the lesions,** but the skin is not completely normal and pruritus remains, this implies that the underlying disease is in the “pruritic and lesional” group. Possible diagnoses to consider include parasitism, adverse food reactions (some of which include lesions), primary seborrhea, and dermatophytosis. Diagnostic steps in this case might include repeated skin scrapings and trichogram, empirical treatment for scabies mites, a hypoallergenic diet trial, and fungal culture. If the underlying cause is not forthcoming, a later step might be skin biopsy. Biopsy can often shed light on the cause of scaling, seborrheic diseases. Along with these differential diagnoses, the possibility of inadequate initial treatment should be considered.

- **If there is little or no clinical response** to infection control, factors such as antibiotic resistance or poor client compliance should be considered. It is also possible that the diagnosis of pyoderma was not correct—non-pyoderma pustular diseases like pemphigus foliaceus may be present. Bacterial culture and susceptibility testing, fungal culture, and skin biopsy might be considered with this response pattern.

- **If the response to infection control is a complete clearing of both lesions and pruritus,** you must thing about what went wrong with the skin that made it more susceptible to developing infection. Especially in an older dog, consider underlying systemic disease. In a younger dog, many authors believe that very early allergic disease can first manifest as increased susceptibility to skin infections, without little or no pruritus remaining after infection control. In this event, diagnostic evaluation
consists of evaluation for systemic disease with blood and urine analyses, and possible evaluation for allergic disease.

By applying these diagnostic principles to canine pruritus, your chances of reaching the proper diagnosis, and therefore administering the proper treatment, will be greatly enhanced!

**Keywords:** Pruritus, Canine, Allergy, Infection, Parasitism
Diagnostic Evaluation of Feline Pruritic Skin Disease

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Summary
Presentation of a cat where the primary owner complaint is pruritus is common in general veterinary practice. Feline pruritic skin diseases tend to occur as “reaction patterns” (eosinophilic granuloma (1), miliary dermatitis, ventral symmetrical alopecia, etc.), but each of these reaction patterns tend to have the same list of underlying causes associated with it. Thus, for any feline pruritic condition (regardless of lesions) it is reasonable to use a uniform, systematic approach in an attempt to determine the underlying cause. Causes may be parasitic, infectious, or allergic; allergy is generally a diagnosis of exclusion in cats.

Is It a Parasite?
A first consideration may be to convince yourself and the owner that the cat is indeed pruritic and removing hair intentionally, rather than the hair “falling out by itself,” by performing a trichogram. Broken hair tips suggest removal by self-trauma of licking and may convince the owner, especially if the cat has been hiding in order to remove hair. Start with careful examination and thorough flea combing. The finding of fleas or flea stool prompts an immediate diagnosis of flea allergy dermatitis until proven otherwise. In heavy flea areas, it may be necessary to provide continual monthly flea preventive as a “diagnostic therapy” test. Remove the hair and scale from the flea comb and pull it apart with the fingers, allowing scale and debris to drop onto the table. Collect this material from the table and examine in mineral oil for mites such as Cheyletiella. In cats, a proper parasite examination should consist of superficial scrapings, deep scrapings, and hair pluckings, each examined microscopically in mineral oil. The recent finding of three different species of Demodex mites in cats makes this especially important (2). When in doubt, provide monthly applications of fipronil or selamectin, which will control all parasites except Demodex, or if available use an isoxazoline antiparasitic, which will also control Demodex.

Is It Infection?
The next step is skin cytology. Surface material can be collected by direct impression (as with moist lesions) or by collection of material with a small spatula or with cellophane tape. Fortunately for cats, bacterial skin infections are uncommon, except as surface infections when the surface is moist and exudative; most infections are with cocci. Many dermatologists have seen rather bizarre, pruritic dermatoses in cats with a moist surface, cocci on cytology, and a surprisingly dramatic response to antibiotics. Therefore, the finding of surface cocci should prompt a 2-4 week course of oral antibiotics with assessment of response.

Yeast overgrowth with Malassezia is more common, and the finding of yeast should prompt a trial of antifungal treatment. Because dermatophytosis “can look like anything” dermatophyte culture is typically considered a basic diagnostic test that should be performed in all feline dermatoses, and is most conveniently performed using the toothbrush technique.
Whatever the cytologic findings, following treatment a recheck should be scheduled in about 2 weeks. Nearly all skin infections are secondary, and the recheck examination provides the opportunity to see the “real” disease without being masked by infection.

What About Blood Tests? Should I Biopsy It?
Though routine hematologic evaluations are typically normal in most feline dermatoses, a blood count and serum chemistries should be considered in any older cat with recent onset of disease; or any pruritic dermatosis that has an unusual appearance. Occasionally, pruritus can be a manifestation of internal disease or of a paraneoplastic syndrome. In addition, future steroid therapy may be contemplated and it is wise to evaluate any middle- to older-age cat systemically prior to such treatment.

The decision to biopsy is often a difficult one, mostly due to cost-benefit considerations. The most common feline pruritic dermatoses (parasites, allergies, infections) are not readily diagnosable by biopsy. Rather, the purpose of biopsy is to rule out other, uncommon to rare feline dermatoses such as pemphigus or epitheliotropic lymphoma. Therefore, biopsy should not be considered unless the clinical appearance is unusual, the disease is especially severe, or the disease has been recalcitrant to treatment. In any event, do make sure all secondary infections are cleared up prior to biopsy, as these may influence the histologic results.

Is It Allergy?
Before deciding that allergy is the cause of a cat’s pruritus, all of the above more common causes of itch should be ruled out.

Is it Food-Related? Initial “allergy evaluation” typically begins with a dietary restriction-provocation trial in cats. There is no clear advantage of a home-cooked vs. a commercial hypoallergenic diet; the most important factor is that compliance with the dietary restriction is complete. Thus, choose a diet that the owner is happy to feed, and the cat is happy to eat. There is currently no evidence to support the use of serologic tests to support a diagnosis of food allergy! These tests are fraught with false positive and false negative results, and are therefore useless for initial diagnosis. The initial trial should be for 4 weeks, with an additional 4-8 weeks if improvement is occurring. Most importantly, if response is apparent, challenge with the original food to prove the food is responsible and not some other coincident factor.

Is it Environmental Allergy? Diagnosing a cat with environmental allergies is difficult at best. There are no good, uniform criteria for “feline atopic dermatitis” as there are for the canine disease. Diagnosis rests primarily on exclusion: the finding of a pruritic cat where all other diagnostic and therapeutic measures have been exhausted, and there is simply no other choice left. Allergy testing, in particular, is problematic. Intradermal tests are difficult to perform and interpret in cats. Most important for the practitioner to understand is the use of serologic IgE tests in cats. Studies generally demonstrate that normal cats, or pruritic but nonallergic cats (for example, cats with fleas) are positive on these tests just as often as “truly allergic” cats [3]! Thus, serologic tests must never be used to make a diagnosis of allergy. Rather, their only benefit is to attempt to determine what the relevant sensitivities may be in order to provide allergen immunotherapy as a treatment.

Keywords: Pruritus, Feline, Allergy, Infection, Parasitism

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Treating Canine Atopic Dermatitis: 
Pillars of Medical Management

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Summary
Atopic dermatitis (AD) is a complex disease with a multifactorial pathogenesis. There is no single medication or treatment that will provide complete and lifetime control of AD. Rather, successful treatment depends on thoughtful use of a multimodal treatment approach, combining several therapies that encompass treatment of both the primary disease and of secondary complications.

Start With Parasite and Infection Control
Many dogs with AD will have concurrent flea sensitization. Thus, routine prophylactic antiparasitic treatment (for example, the monthly topical or oral preparations) is advised for all canine AD patients. Most dogs with AD are very susceptible to secondary infection with both staphylococci and yeast organisms. Treatment must focus on not only initial elimination of the infections, but prevention of future episodes of infection. This is most often accomplished by frequent use of topical antimicrobial therapy. Shampoo or spray application of chlorhexidine or chlorhexidine/azole preparations weekly is recommended.

Benefits of Topical Therapy
Bathing helps! Studies have shown that frequent (weekly to twice weekly) bathing with a non-irritating, emollient shampoo can have a limited antipruritic effect; it also washes away debris, environmental allergens, and organisms from the skin. No single topical formulation has shown clear superiority in this regard. Since recurrence of secondary infections is often a problem, the choice for a maintenance shampoo often dictates use of an antimicrobial formulation, as above.

Antihistamines: Do They Really Help?
Medical management of the AD often begins with the safest drugs to administer longer-term. Though from an evidence-based standpoint we have no confirmation that antihistamines are beneficial in AD, many clinicians believe that they benefit a small number of patients, and that they are always worth trying. Recent, better-designed and controlled studies have shown some success in dogs with the antihistamines dimetindine, fexofenadine, and a combination of hydroxyzine and chlorpheniramine. However, the magnitude of relief that antihistamines appear to provide remains minimal. Combination with anti-inflammatory fatty acid supplements may provide additional benefit. There is no evidence that the newer, nonsedating antihistamine drugs now commonly used in human allergy have any additional benefit for animal use.

Anti-inflammatory Fatty Acid Supplementation
EFA supplements remain a cornerstone of conservative management. Their effects may occur through their weak anti-inflammatory effects, or through possible effects on epidermal barrier function. Though as sole therapy they have minimal efficacy, studies demonstrate they may have synergistic effects with antihistamines...
(improved efficacy) and with glucocorticoids (dose-sparing effect). Because they are relatively inexpensive, safe, and easily administered, many dermatologists advise that ALL atopic pets should receive enhanced levels of EFA. Dose is typically based on the total anti-inflammatory content, which includes EPA, GLA, and DHA. The total (DHA+EPA+GLA) should be administered at a dose of at least 25-35 mg/kg/d. This can be accomplished with supplements, or increasingly, with EFA-enhanced diets. The latter may be a more cost-effective approach that enhances client compliance, but it is difficult to ascertain which foods are “high enough” in EFA to provide the required dose. Clients must be warned that many over-the-counter supplements (such as “salmon oil”) have only very small amounts of the desired EFA. It is also important to advise clients that beneficial effects take at least 1-2 months to be seen.

Addressing Epidermal Barrier Function
There is no question that the epidermal barrier functions are abnormal in atopic people. From a clinician’s standpoint, the obvious question becomes “can we improve clinical signs of AD by somehow improving barrier function? In human AD, application of emollient preparations to the skin is an important and basic element of treatment, and unquestionably helps relieve symptoms over time. In dogs, studies have shown that the lipid composition of the stratum corneum can be modified by either dietary or topical means. Manipulation of the diet by altering its fatty acid composition affects the composition of skin lipids and may possibly help barrier function. Topical modification of barrier function is an active area of research in veterinary medicine. Initial research has shown that application of topical lipid preparations can result in improvement of the intercellular lipid lamellar structure and composition and can be associated with limited clinical improvement, though such improvement typically takes months.

Corticosteroids
Atopic dermatitis is generally quite responsive to corticosteroid drugs. They may be preferred in pets with highly seasonal disease, and are an excellent short-term treatment for acute flares. Oral prednisolone, prednisone, or methylprednisolone at 0.5 -1 mg/kg/d are the preferred treatments; repeated injections of long-acting corticosteroids should be avoided. The chief disadvantages of longer-term use of corticosteroid drugs include development of steroid resistance or “tachyphylaxis,” and adverse effects (of both annoying and medically-serious varieties). Animals receiving longer-term oral corticosteroids should have a urine culture performed twice annually to identify silent urinary tract infections. It is wise to check liver enzymes annually. Topical hydrocortisone aceponate spray is also very useful. This corticosteroid is metabolized entirely in the skin, and is not absorbed into the systemic circulation, thus sparing the pituitary-adrenal axis and liver. It can be used for “trouble areas” of atopic dermatitis, such as the ventrum, feet, anal area, etc., or can be sprayed over broader areas of the body.

Ciclosporin A Modified (CsAM)
The calcineurin inhibitors work by inhibiting production and action of cytokines, and through other mechanisms as well. Clinical trials of ciclosporin in dogs with AD demonstrate that this drug has efficacy equal to that of oral prednisone. The starting dose is 5 mg/kg/day, which can be given as a single dose or divided into multiple doses. After the first month of treatment, in some dogs the dose can be decreased. Perhaps 25% of patients will have some initial gastrointestinal discomfort from CsAM. In most cases, this will abate within a few weeks. Therapy with CsAM is remarkably free from long-term adverse effects. Gingival hyperplasia is a known adverse effect of longer-term, higher-dose CsAM therapy, but occurs only rarely (1-2% of patients) at the doses typically necessary for AD. Protocols for combination of CsAM with ketoconazole for long-term management of AD have not been developed. A major concern here would be potential for development of hepatotoxicity with long-term ketoconazole treatment. Long-term use of CsAM combined with systemic corticosteroid drugs has been associated with development of fatal opportunistic fungal infections, so should be avoided. CsAM typically acts relatively slowly, often taking 2-4 weeks to reach maximum effectiveness. Therefore, many dermatologists begin a short (2-week) tapering course of oral prednisolone along with
CsAM for faster patient relief; this appears safe to do. Therapeutic monitoring (serum chemistries, blood counts, or CsA serum concentrations) is neither recommended nor necessary when using CsAM for AD patients.

Oclacitinib

Oclacitinib, a JAK1 inhibitor, has been studied in a variety of models of pruritus and has shown the ability to suppress pruritic responses rapidly and effectively, in some cases better and quicker than prednisolone. At therapeutic doses, oclacitinib inhibits predominantly JAK1 and spares JAK2-dependent processes such as hematopoiesis. Controlled clinical trials of oclacitinib in the treatment of both canine allergic dermatitis (flea, food, contact) and canine AD showed promising results. Results in head-to-head studies against either prednisolone or ciclosporin shows the drug to be equally effective in control of itch and inflammation, and to have a very rapid onset of action with relief sometimes apparent within hours of oral administration. The drug became available in the 2015-16 period as Apoquel (Zoetis). Apoquel is indicated for control of acute or chronic pruritus in dogs over 12 months of age. Recommended dosing consists of twice daily administration for UP TO 14 days (important language, as this means twice daily for anywhere between 0 and 14 days), followed by once daily dosing for longer term use. A few dogs seem to worsen slightly when switched from twice to once daily, probably related to the short half-life of the drug (4 hr in the dog). Overall, it appears that at least 60-70% of allergic dogs receiving the drug have rapid, substantial, and prolonged relief of their clinical signs. Veterinary dermatologists involved in early clinical trials often comment that the drug is most useful as PART of a multimodal treatment approach, and that some dogs with even very recalcitrant disease have shown remarkable response. Short-term adverse effects have been limited to GI disturbance in a very few dogs, though the adverse effects occur nearly as frequently in placebo-treated dogs. There is no specific organ toxicity associated with Apoquel, therefore no specific laboratory monitoring is advocated, though good medical practice dictates that all dogs receiving this drug should be examined at least once annually. Apoquel has been administered for as long as 5 years in some dogs; in longer-term studies, occasional patients have developed benign or malignant neoplasms, but no more often than would be expected for dogs in the studied age range. The drug has been limited to use in dogs 12 months or older, mostly because in one high-dose safety study with 6 month old laboratory dogs, generalized demodicosis developed in some patients that were given 5X the label dose. Oclacitinib has not been evaluated in combination with other drugs such as systemic corticosteroids, but recent experience suggests clever ways to use these treatment in stepwise fashion. Apoquel can be used safely along with antibiotics, antihistamines, antifungal drugs, NSAIDs, allergen-specific immunotherapy, and many other medications, and vaccination of treated dogs is effective. Apoquel does not appear to interfere with serologic or intradermal allergy tests. As with other treatments targeting the immune system, it should not be used in the face of a severe infection, demodicosis or with active malignancy.

Recent practical experience with Apoquel by US dermatologists have provided insights as to how best to use the drug. First, two rare adverse effects have been seen, even at the label dose: demodicosis, and slightly lowered WBC count. Until we know more, most dermatologists advise the following examinations if Apoquel will be used longer term: (1) Recheck exams at 2 months, 6 months, and then annually; (2) at each recheck exam, check for lesions or alopecia and if any are present, scrape for Demodex mites; (3) serum chemistries and urinalysis are not necessary, though are often recommended as part of general annual health evaluation. Apoquel may not work very well on the pruritus associated with skin infection, either staphylococcal or yeast. Therefore, it is important to treat these infections before using Apoquel (or at the same time) because you will not be able to adequately judge response if infection is present. Also, think of Apoquel as an antipruritic drug, useful against allergic itch – and not a drug for “any dog with skin disease.” It has no effect on noninflammatory alopecia. It is not a substitute for steroids in autoimmune diseases such as pemphigus or autoimmune hemolytic anemia. Some clinicians have observed that it is not always useful in conditions where there is inflammatory swelling, such as severe otitis externa.
On the Horizon
Therapies continue to become more and more targeted. The newest type of therapy in veterinary medicine is monoclonal antibody (mAb) treatment. This therapy has been used in people for about 20 years, and was always considered something that would never be financially practical for dogs or cats. However, improvements in techniques and processes now make it completely possible for this new and dramatically different type of treatment to be accessible for pets. Monoclonal antibody treatments involve treatments with injections of laboratory-produced antibody proteins that target harmful molecules in the pet's body. Because these are proteins that exist normally in the pet, they are not rejected by the immune system, and are not toxic to any organ. They persist for many weeks or even months in the pet's body after subcutaneous injection. These treatments are being developed for a variety of pet diseases such as cancer, pain, and inflammatory disease. Recently, the first anti-itch mAb was approved in the USA for treatment of canine atopic dermatitis. The treatment, named Cytopoint, targets and eliminates IL-31 from the dog's body. After a single injection, the treatment can work in only a few days and can last as long as 8 weeks. Much more is to be learned about these wonderful treatments as they receive approval in other countries.

Keywords: Atopic Dermatitis, Drugs, Corticosteroids, Oclacitinib, Ciclosporin
Otitis: The Rules for Diagnosis and Treatments

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Summary

Otitis is inflammation of the ear canal. Depending on the associated structures, otitis divided into otitis externa (vertical and horizontal ear canal), media (tympanic bulla) and interna (cochlea, vestibule, semicircular canal). Otitis externa (OE) is most common in dogs and cats. Otitis media (OM) is usually secondary to an accompanying OE. Likewise, Otitis interna (OI) is generally result from exacerbation of OM. Causes of otitis are various and mostly have underlying factor that changes normal ear canal environment. Clinical signs are head shaking, rubbing ear, malodor, loss of hearing and exudate of ear canal. Treatment of otitis involves ear cleaning and the applying of topical medications basically. Systemic therapy is rarely needed in many of OE but should be considered in OM and OI.

Etiology

We can classify causes of otitis into primary, predisposing, perpetuating factors and secondary infection. Primary factors directly provoke inflammation of ear canal. Predisposing factors are responsible for increasing probability of secondary infection but not a direct cause of infection. Perpetuating factors independently involve in chronicity and recurrence of otitis.

Table 1 Etiological factors of otitis externa

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<th>Primary</th>
<th>Predisposing</th>
<th>Perpetuating</th>
<th>Secondary infection</th>
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<td>Ectoparasites</td>
<td>Temperature</td>
<td>Otitis media</td>
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<td>· ceruminous gland</td>
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<td>Yeast infection</td>
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<td>(esp. Cocker spaniel)</td>
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Diagnosis

· Otoscopic examination: Check point→Size of ear canal, Parasites, Character of exudate, Hairs, Foreign materials. Color of epithelium, Ulcers or masses, Integrity of tympanic membrane
· Cytologic examination (Ear wax smear, Direct smear): Check point→Type of infectious agent (bacteria
coci or rod? yeast? parasite?), Existence of inflammatory cells (severity of inflammation)
· Bacterial culture and Antimicrobial susceptibility test: When treat chronic and unresponsive otitis caused by bacterial infection, bacterial culture and antibiotic susceptibility test provide solution for selecting appropriate antibiotics. But if you confirm rod shaped bacteria (gram-negative) on ear waxy smear, I recommend to request susceptibility test immediately regardless of acute and chronic stage.
· Diagnostic imaging: Severe stenosis of the ear canal accompanied by chronic otitis needs X-ray or CT scan. But solitary plain X-ray imaging is not sufficient to examine middle ear. Check point—Increased density or any irregular change of tympanic bulla, Calcification, Opening of ear canal

Topical therapy
· Ear cleaning: Ear cleaning is an essential method to treat any case of otitis. Daily repeated ear cleaning emulsifies, soften and break up waxy debris and exudate. For good therapeutic outcome, both in-hospital and at-home treatment are should be conducted together. If integrity of tympanic membrane is not known, saline and squalene are recommended for ear cleaning because they don't have ototoxicity.
· Antibiotics, Antifungals: Polymyxin B, Aminoglycoside and fluoroquinolones (amikacin, gentamycin, marbofloxacin, enrofloxacin, ofloxacin, etc) mainly used for topical antibiotics. Especially, polymyxin B has good effect against Pseudomonas. Most antibiotics are deactivated by purulent debris. So, ear need to be kept clean during apply topical antibiotics. Tris-EDTA solution can promote effect of antibiotics by chelating calcium ion in pus and damaging cell wall of gram negative bacteria. Important point is that topical antibiotics can be administrated on very high concentration (100 to 1000 times higher than plasma level of antibiotics administrated systemically) because drugs directly contact to surface of bacteria. Therefore, frequently topical antibiotics overcome antimicrobial resistance determined by in vitro antimicrobial susceptibility test. Antifungal agents (miconazole, clotrimazole, etc) are used in otitis caused by Maassezia or Candida. Commercial products generally contain antibiotics and antifungals together.
· Glucocorticoid: In many case of severe acute otitis or chronic recurrent otitis, ear canal becomes edematous and erythematous. Glucocorticoid can reduce swelling, inflammation and glandular secretion and this help to resolve ear canal stenosis and pruritus. Because of low accumulation ratio in comparison with conventional glucocorticoid, hydrocortisone aceponate can be used safely with good topical glucocorticoid.

Systemic therapy
Situations need to consider administering oral antibiotics: 1) Moderate to severe thickening of the canal/pinna, 2) Periaural skin dermatitis, 3) Ulceration in the ear, 4) Large number of inflammatory cells in cytology, 5) Concurrent otitis media. Oral glucocorticoids (antinflammatory dose) can be effective option to calm down ear canal swelled by severe inflammatory reaction and control otitis externa related to allergic dermatitis. But, if any medical treatments are unresponsive and ear canal is obstructed completely by irreversible pathologic change (eg. calcification), surgery of ear canal should be considered (Lateral ear canal resection, vertical or total ear canal ablation).

Otitis media
General causes of otitis media are extension of otitis externa and pharyngeal infection through the auditory tube (In cat, upper respiratory tract disease). Occasionally, hematogenous spread of pathogen trigger otitis media and interna. Clinical sign often includes facial nerve injury signs because facial nerve is close to tympanic bulla. But in some cases, recurrent otitis externa is only clinical sign of otitis media.
· Facial nerve injury sign: Drooping of the upper lip and ear, Drooling of saliva, Decreased or absent palpebral reflex, Horner's syndrome, Keratitis sicca
In otoscopic examination, abnormal tympanic membrane is strong evidence of otitis media. Cloudy, bulging and color change (blue, red, white, amber) are symptoms representing abnormal tympanic membrane. Radiography and CT scan should be performed to evaluate tympanic bulla. Myringotomy is useful for relieving pain and collecting samples for culture. But myringotomy and deep otic flush rarely cause neurological
complications (Horner's syndrome, facial nerve paralysis, vestibular disturbance and deafness). If integrity of tympanic membrane is not known, saline, squalene and fluoroquinolones are recommended for topical therapy because they don't have ototoxicity. Treatment must include systemic antibiotics or oral glucocorticoid. Surgical intervention is considered in unresponsive or recurrent case of otitis media.

**Otitis interna**
Clinical signs of otitis interna typically occur associated with peripheral vestibular syndrome. If otitis patient show head tilt, circling, falling or rolling, nystagmus, positional strabismus and asymmetric ataxia, you should examine about otitis interna. But absence of neurologic sign doesn’t rule out otitis interna and brain stem disease also cause similar clinical sign. CT and MRI are helpful in investigating pathologic change of inner ear. CT is better to define bony structure, while MRI is outstanding for detecting soft tissue changes. Therapeutic plan is similar to when treat otitis media. Otitis interna needs more systemic therapy than otitis media.

**Keywords:** Otitis, Dog, Cat
Treating Canine Atopic Dermatitis: Allergen-Specific Immunotherapy & Sublingual Immunotherapy in Theory and Practice

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Summary

Allergen-specific immunotherapy (ASIT) is a treatment for atopic dermatitis in dogs and cats wherein extracts of allergens to which the patient is sensitive are injected, in gradually increasing amounts, to lessen the hypersensitivity state. It is the only proven treatment for allergies that actually works against the underlying pathogenesis of the disease, instead of merely covering up clinical signs with anti-inflammatory therapies. ASIT has a strong advantage of being nearly free of adverse effects in the great majority of dogs and cats, even with prolonged use. Disadvantages include the fact that it takes several months or more to begin working, that it does not always work, and that it may be relatively expensive.

How Does ASIT Work?

Various theories as to how ASIT works have been advanced, and mechanisms are still not completely understood. The “blocking antibody theory” was popular for many years, which considered primarily the effect of ASIT on B-lymphocytes, but has been augmented by the “helper T lymphocyte subset” theory. In this view, the “normal” response is called “T-helper-1” (Th1). When the allergen reaches the immune system, lymphocytes produce cytokines such as interleukin-2 and interferon-gamma. These cytokines direct B-lymphocytes to differentiate towards cells that produce IgG antibodies. We aim for this type of response when we vaccinate pets. In allergy, the lymphocyte response is instead “T-helper-2” (Th2). In this case, the lymphocytes produce cytokines such as interleukins 4, 5, and 13. These cytokines direct B-lymphocytes to differentiate towards cells that produce IgE antibodies. The immune response exists in a kind of “balance” between Th1 and Th2, and many things can potentially influence which response predominates. Modulating this IgG/IgE “balance” and returning it towards the “normal” IgG bias is the target of ASIT. Recent studies have also stressed the importance of downregulating mast cell and other effector cell reactivity; induction of tolerance via regulatory T cells, IL-10, and TGF-beta; and the importance of dendritic cells in the uptake and processing of allergens. Most effects of ASIT are thought to be allergen-specific, rather than nonspecific. Thus, accurate testing to identify the offending allergens in each patient is of paramount importance to successful immunotherapy. In particular, the clinician must strive to avoid ‘false positive’ allergy test results, which would result in including an allergen in the patient's mixture that is not relevant to that individual's disease.

How Do I Choose the Correct Allergens and Protocol?

Selection and formulation of an ASIT mixture is both an art and a science. Experimental observations that large doses of allergen evoke Th1 (IgG) responses and small doses of allergen evoke Th2 (IgE) responses suggest that there is some minimum, fairly large dose of each allergen necessary in a mixture to achieve
benefit. Thus, many allergists limit the number of extracts used in each prescription to between 10 and 15 substances. When a large number of positive reactions are obtained with allergy testing, choosing the proper 10-15 substances can be based on the following: strength of the positive reaction; degree of possible exposure of the animal to the substance; consideration of patient characteristics; and botanical interrelationships of pollen allergen groups. Because proteolytic allergens present in mold extracts can degrade some pollen allergens, some allergists administer mold allergens by separate injection. Fortunately, commercial testing laboratories are knowledgeable about the details of formulating ASIT prescriptions and provide guidance.

What About Concurrent Additional Medications?
As far as is known, concurrent treatments with antihistamines, fatty acid supplements, ciclosporin, or low-dose glucocorticoids will not interfere with response; preliminary experience suggests the same is true for oclacitinib. Such treatments are generally necessary as part of the overall treatment plan, to provide immediate and short-term relief while waiting for the ASIT to work. These treatments can be slowly tapered as response to ASIT occurs. Treatment with ASIT is generally considered to be lifelong, though it is possible to attempt discontinuation after 2 to 3 years of injections if the animal has responded very well.

How Well Does ASIT Work?
Expected response rate to immunotherapy is approximately 60-70% “good-to-excellent” response (defined as at least 50% improvement in clinical signs). Response can be seen as soon as 1 month, but more typically takes 3 to 6 months to occur, and the maximum response may take 1 year or longer. Adverse reactions to allergen immunotherapy include localized itch at the injection site and transient worsening for 12-24 hours after the injection (~10% of patients). Generalized anaphylaxis occurs in less than 1% of dogs and cats; such reactions are generally mild and further reaction can often be prevented by pretreatment with an oral antihistamine 1-2 hours prior to each injection.

Sublingual Immunotherapy: A New Option
Sublingual immunotherapy (SLIT) involves administration of allergen extract into the oral cavity, under the tongue, as opposed to by injection. It is commonly used for human allergy in Europe, particularly for atopic rhinitis and asthma. Historically, there are conflicting reports of efficacy, which may be explained in part by the extreme variation in protocols used for dosing, administration, intervals, vehicle, etc. in the different studies reported. Consideration of recent evidence has led authoritative bodies to conclude that, when used correctly, it is clearly efficacious and in fact has a response rate similar to subcutaneous ASIT. Its use in animals is very new, and it has only recently become widely available for veterinary use.

There are many similarities between SLIT, as currently used for pets, and allergy shots. As with injections, SLIT formulations are typically supplied in 3 bottles of increasing concentration, and the cost of SLIT vs. shots is approximately the same. Concurrent medications do not appear to interfere with efficacy, and are typically used during the initial few weeks or months of treatment while waiting for SLIT to become effective. The mechanism(s) by which SLIT works are somewhat different than allergy shots, implying that they may be more or less effective than injections for a given patient. Major practical differences include the specific ingredients: while shots typically consist of phenol-saline based extracts, many SLIT formulations use glycerin-based extracts prepared in special vehicles which purport to stabilize the allergens and/or facilitate uptake through the oral mucosa. Stabilizers included in some formulations allow concurrent mixing-in of mold allergens, and room temperature storage. The other major difference is administration frequency: SLIT formulations are typically administered every day, often several times per day, for the duration of therapy with no tapering.

Experience with SLIT in Animals
Evidence for efficacy: Studies on SLIT and other non-injection methods of ASIT for use in pets are only
just being reported. One study in an experimental model of canine AD failed to show evidence for efficacy of orally-administered allergen in laboratory beagles experimentally sensitized to dust mite; however in this study the allergen was fed to the dog rather than applied to the mucosa (1). Another small open trial of atopic canine clinical patients with dust mite allergy treated with SLIT reported clinical benefit in 80% of dogs, and that clinical benefit was usually accompanied by measurable immunologic changes, including significant increases in allergen-specific IgG and decreases in allergen-specific IgE (2,3). Marsella et al. (4) reported some efficacy of SLIT in a laboratory model using sensitized beagle dogs, including significant changes in cytokines such as TGF-beta and IL-10 in treated animals. Finally, a multicenter, uncontrolled open trial of 217 dogs, reported preliminarily by the author, indicated approximately 60% response to SLIT therapy, including approximately 50% of “injection failure” dogs responding (5).

**Advantages and Disadvantages of SLIT:** One big advantage of SLIT is in ease of administration. We’ve found that though many owners “don’t mind” giving injections to their pets, most owners clearly don’t relish it, and are delighted to be presented with an alternative to giving injections. Most dogs accept administration easily, even viewing it as a treat, which increases compliance. On the other hand, successful SLIT requires faithful twice-daily administration, and owners with busy travel schedules may find it more convenient to give an infrequent injection. “Head-shy” dogs may also resist treatment. In human beings, anaphylactic reactions to SLIT are rare to nonexistent, and SLIT can be used in humans with a prior history of reaction to allergy shots. In our experience, the same is true for dogs; we’ve treated numerous patients with SLIT who have had anaphylactic reactions to allergy shots. Additionally, with many SLIT formulations, you can include mold extracts with pollens in the same vial without fear of losing efficacy of non-mold allergens, and SLIT treatment bottles can be stored at room temperature for a shelf-life of 6 months; refrigeration is not necessary.

**Protocols for Sublingual Immunotherapy in Dogs**

**Workup and Testing:** In summary, do what you have always done! Initial diagnosis of AD and workup to eliminate secondary infections, parasitism, and a food component of the disease is no different than in any other atopic dog. Dogs should be evaluated for different sensitivities in exactly the same manner that the individual clinician is comfortable and familiar with for treatment using injection ASIT. Following establishment of a firm clinical diagnosis of AD, any combination of serologic or intradermal testing techniques may be used to establish the individual sensitivities of each patient.

**Allergen Selection and Formulation:** Following careful testing, again, principles for choosing the allergens in the prescription are exactly the same as those employed for choice of allergens for injection ASIT mixtures, and are completely familiar to every veterinary dermatologist, including:

- History of exposure of the patient to the allergen in question
- Cross-reactivity of allergens, including consideration of botanical groups of related weed, tree, or grass pollens
- Empirical observations on the significance of a particular allergen in relation to others, such as may be suggested by the “score” of serologic or intradermal tests

A few considerations that may be unique to formulating a SLIT prescription include the following:

- SLIT prescriptions in human beings tend to follow a “less is more” principle. There is much greater use of “mixes” of related allergens rather than combining many different individual extracts that are antigenically-related, and use of fewer allergens in the mix rather than a greater number. Consider limiting the number of allergens in your prescription to a maximum of the 10-12 you believe are most important for the patient. Remember, there is substantial documentation in other species that part of the mechanism of SLIT is allergen-specific, and part is nonspecific.
- Generally, on the prescription just indicate a list of the relevant allergens; the correct dose of these allergens will be included in the final prescription. Though this may vary my manufacturer, typically
the prescriptions do not “double-up” on a particular allergen that is felt to be more “important” than others; all treatment sets are usually prepared with a uniform and standard dose of relevant allergen.

A key difference with SLIT is this basic principle of treatment: the allergen must be dosed regularly and frequently. Multiple daily administrations are required for efficacy in human beings, and we strongly recommend that owners be counseled to administer the “allergy drops” TWICE DAILY, EVERY DAY. If they forget to give a dose in the morning, give one in the afternoon and one before bed. This twice-daily dosing schedule is indefinite for the duration of therapy. The schedule does not “taper” to once daily, every other day, etc. Administration continues twice daily for the duration of treatment.

Adverse Reactions: A few dogs may rub or scratch at their mouth after administration, perhaps analogous to the oral itch that some human SLIT patients experience. Almost always, this will disappear after the first few treatments. Likewise, occasional vomiting has been observed in a few dogs for the first few doses. In a few cases with very sensitive animals, we’ve seen worsening of clinical signs with SLIT administration – actually causing a flare of the disease. If any of these reactions occur or persist, it may require lowering the allergen dose. Contact the SLIT supplier for specific instructions as to how to accomplish this for their specific formulation.

Keywords: Atopic Dermatitis, Immunotherapy, SLIT, Allergy Shots, Allergy Drops

References
Cutaneous Adverse Food Reactions

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Summary
Food allergy arises from the failure of normal immunologic tolerance to ingested substances. It has a variety of immunologic mechanisms. Clinical features in animals include both gastrointestinal disturbance and skin disease. The skin disease has many manifestations, but is usually pruritic. Food allergy alone is relatively uncommon; more commonly, food allergy coexists along with allergy to environmental or parasite allergens. Diagnosis of food allergy is made via a dietary restriction-provocation trial; laboratory food allergy tests are highly unreliable for diagnosis.

Immunopathogenesis of Food Allergy
Food in the gut is a “foreign object” to the immune system, and it is highly desirable to prevent absorption of food substances until they are completely digested into simple amino acids, fats, and sugars. However, the gut is not a perfect barrier, and every day, tiny quantities of intact food allergens are absorbed. The immune system’s job is to process these intact food allergens without developing a hypersensitivity response to them. We depend on an intact gut mucosa and the immunologic phenomenon of “oral tolerance” to prevent hypersensitivity reactions developing to these foreign allergens.

Adverse food reactions consist of both food allergy and food intolerance. In humans, approximately ¼ of reactions are immunologic (allergy) and ¾ are nonimmunologic (intolerance). We do not know these proportions for animals. The true pathogenesis of food allergy in dogs and cats has rarely been elucidated. Good evidence that an IgE-mediated mechanism may exist in some dogs comes from an experimental model study, using an inbred colony of high-IgE producing dogs. The dogs were immunized with food allergen extracts in alum. These dogs developed measurable food allergen-specific IgE, became intradermal test-positive to the extract, were positive on gastroscopic food testing, and developed cutaneous and gastrointestinal disease when fed the offending food (1). Some animals and people with “food allergy” do not have detectable food allergen-specific IgE in their serum. Non-IgE-mediated allergic reactions are possible. Hypersensitivity reactions thought to be involved less commonly in food allergy include cytotoxic reactions, immune complex-mediated reactions, or delayed-type hypersensitivity.

Food allergy generally develops to a common ingredient that has been fed for a long time, not to a “new” ingredient or to a recent dietary change. In man, food allergens are glycoproteins with molecular weights from 10,000 to about 60,000 daltons, that are resistant to heat (cooking and processing), acid (stomach), and proteolytic enzymes (gut). This includes molecules such as bovine serum albumin, casein, lactoglobulin, ovalbumin, etc. For an IgE-mediated reaction to be possible, the allergen must contain antigenic determinants that are least bivalent. Only then can the allergen molecule “crosslink” the IgE on mast cells and cause a reaction. This, therefore, requires that the allergen molecule be relatively large and complex. Common protein and carbohydrate sources that are allergenic in pets include beef, dairy, wheat, and, for cats, fish.
Food allergy to ingredients such as coloring agents, preservatives, etc. appear to be exceptionally uncommon in animals.

**Clinical Features**

Food allergy has been reported in dogs from 3 months to 14 years of age, though most cases seem to appear in “adult” dogs or young puppies. Careful prevalence studies have not been performed in animals, in part due to the difficulty in diagnosing the disease and the frequent coexistence of other allergic diseases. Nevertheless, the consensus is that approximately 1-6% of all skin disease is caused by adverse food reactions.

Dermatologists frequently state that the clinical picture of food allergy is that “food allergy can look like ANYTHING!” Common signs include nonseasonal pruritus of variable intensity, often not responding to corticosteroid or ciclosporin treatment. Lesions reported include erythema, urticarial, papules, pustules, excoriations, hyperpigmentation, pododermatitis, seborrhea, and otitis externa. Secondary infections and seborrhea are common; and bilateral recurrent otitis externa (without other clinical signs) is possible. In the cat, pruritus of the face and neck appears most commonly reported, though food allergy has also been reported as a cause of eosinophilic granuloma, miliary dermatitis, and ventral symmetrical alopecia.

Studies in dogs have indicated that from 13-30% of dogs with food allergy also have atopic dermatitis. Conversely, of all dogs with atopic dermatitis, recent surveys suggest that from 2-13% also have food allergy, and some authors have stated recently that the true figures may be even higher. Gastrointestinal signs may be present. Intermittent or persistent vomiting, soft stool, or diarrhea are the most commonly-reported GI signs. Reported prevalence of GI signs in food allergy vary widely, depending on the study, but probably are present in 25-50% of animals with food allergy.

**Diagnosis and Management**

In diagnosing food allergy, elimination of other differential diagnoses is critical. Major initial diagnoses to rule out include ectoparasites (fleas, mites); and pruritic infections (staphylococci, yeast). Intradermal testing is not reliable for diagnosis of food allergy. Serum food allergen-specific IgE tests are also not recommended for initial diagnosis of food allergy, as careful studies of these tests indicate they have poor specificity and sensitivity in animals. “Patch-testing” for food allergy has recently been proposed (2) and may have some value, though it is difficult to perform. In part, this no doubt reflects that these are IgE tests, and food allergy may not be IgE-mediated. The only valid, conclusive test for food allergy in dogs or cats is a strict dietary restriction-provocation trial, commonly known as a “diet trial.”

There is an abundance of strong opinion, and very little fact, about exactly how best to perform a diet trial and in particular which diet is best – it’s therefore easy for both veterinarians and owners to become confused. Here are some guidelines that are reasonable:

- Before starting a diet trial, clear up any concurrent problem with parasites and/or skin infections. No hypoallergenic diet will produce improvement if the pet is overwhelmed with pruritic infection!
- The first phase of the trial is “restriction” – feeding a diet to which the pet is likely nonreactive. Some clinicians recommend home-cooking a hypoallergenic diet, and references with recipes are available. However, commercial dry or canned diets may increase client compliance as they are much easier to use. Commercial diet possibilities include either “novel ingredient” diets or “hydrolysed” diets. Neither type of diet has been shown to be conclusively better for use as a hypoallergenic trial diet. There are many unknowns here – is there crossreactivity between beef and venison allergens for dogs, as there is for people? Do some hydrolyzed diets contain trace amounts of hydrolysate with slightly greater molecular weight, such that it might be allergenic? Though food allergen IgE serology is not appropriate for diagnosing food allergy, does it have a role in guiding selection of a trial diet? These questions
are largely unanswered. Thus, trial diets are best selected based on factors such as palatability, cost, and convenience.

- Always use veterinarian-supplied, “prescription” diets for a diet trial. Never use novel ingredient diets purchased at a pet store! Recent studies suggest that nearly all “pet store brand” limited-ingredient diets do contain traces of beef, soy, or other substances not listed on the label (3). The veterinarian-supplied diets are more carefully prepared to avoid contamination with other ingredients.
- Most importantly, the diet trial must be STRICT! Encourage compliance with an owner written daily log. Snacks and treats are permissible, but only if they are hypoallergenic.
- The second important phase of the diet trial is “provocation.” Only by demonstrating worsening upon challenge with conventional ingredients can food allergy be confirmed. Thus, if improvement has occurred over a 1-2 month restriction period, the owner should challenge the pet with the original diet, and relapse typically occurs within a few days.

**Keywords:** Food, Diet, Allergy

**References**


Tips and Tricks for Diagnosis and Management of Dermatophytosis

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Summary
Dermatophytosis in cats is virtually all caused by Microsporum canis, and can have a wide variety of clinical presentations. The gold standard for diagnosing dermatophytosis is a fungal culture, though newer PCR methods may be helpful in the future. Treatment protocols should always use a combination of topical, systemic, and environmental control.

Key Points: The Fungus
- Most infections are still with Microsporum canis, especially in cats
- Feline infections with other fungal species such as M. gypseum and Trichophyton mentagrophytes appear to be on the rise, especially in animal shelters
- Infections with unusual species such as M. persicolor have been reported and may be increasing, especially in some areas of the world
- Several factors are required for development of infection, beyond just the mere presence of the fungus. Host factors are important, such as youth (kittens, puppies), debilitating disease, compromised immune status (from disease or drugs), poor nutrition, or stress. These factors are supremely important when working with multiple-cat facilities.
- **KEY TAKEAWAYS:** be on the lookout for non-M.canis infections, though fortunately these are easy to treat. Always remember to consider that infections require more than just the presence of the fungus.

Key Points: Diagnosis
- Dermatophytosis has many different clinical presentations and could be considered a differential diagnosis for almost any skin disease. This is particularly true in the cat.
- Aberrant host factors can lead to development of a kerion reaction, or to dermatophytic pseudomycetoma.
- Wood’s lamp examination is a reasonable screen, but is NOT definitive. It may find its best use when working with an infected cattery or animal shelter, with a fluorescing strain involved.
- Fungal culture on DTM is the best diagnostic method, but must be done properly
  - Toothbrush collection and inoculation
  - Incubation at warm room temperature (28 C)
  - White colony with red color change at same time
  - Microscopic identification of colony
- Newer PCR based methods may become more widely available, but must be interpreted very cautiously because PCR will be positive on live OR dead fungus!
- **KEY TAKEAWAYS:** dermatophytosis can look like anything, but fungal culture is still the gold standard for diagnosis.
Hitting the Key Points: Treatment

- In most healthy animals, dermatophytosis is a self-curing disease and eventually (in perhaps 10-16 weeks) will spontaneously resolve. Proper treatment can, however, accelerate recovery and thereby help to minimize spread.

- The best treatment protocol is a combination of 3 approaches: **topical treatment** to kill infective material on the haircoat and skin, and prevent its dissemination into the environment; **systemic treatment** to shorten the disease course in the individual animal; and **environmental treatment** to help prevent recurrence of infection or spread to other animals or people in the household.

- When you use topical treatment, whole body treatment (rinsing or shampooing) is the best method, and should be performed twice weekly. Currently favored topical whole-body rinses for dermatophytosis include lime sulfur solution (all brands appear equivalent); this chemical is very safe, but the odor is very bad.

- Miconazole or ketoconazole plus chlorhexidine are synergistic, and shampoos containing these ingredients are useful in cats as an adjunct treatment to systemic therapy. In one study, cats treated with Malaseb® shampoo + griseofulvin recovered **visually** at about the same rate as cats treated with griseofulvin alone. However, treatment with the combination of shampoo and griseofulvin achieved **negative fungal cultures** much more rapidly than treatment with griseofulvin alone.

- Clipping of hair is “going out of fashion.” Very small (invisible) trauma from the clipper blade may help to spread the infection on the cat’s haircoat.

- Ketoconazole as a systemic antifungal is best reserved for infections in dogs, particularly *Trichophyton*; use at 5-10 mg/kg/day. Studies suggest a high prevalence (~25%) of hepatotoxicity in cats, and occasionally at higher doses in dogs.

- Itraconazole has become the systemic drug of choice for treating ringworm. Expense is a factor, so its use is mostly limited to cats or small dogs. Itraconazole accumulates and persists in skin following oral administration, such that a pulse-dosing schedule is rational, as is effective as daily continuous dosing for most infections, and saves money over daily dosing. The manufacturer’s recommended pulse-dose schedule in cats is 5 mg/kg once per day, orally, on an every-other-week schedule. Treatment is generally continued for three “pulses” of one week on, one week off. There are anecdotal reports of some cats’ infections not responding to the manufacturer protocol, and requiring a higher dose. Thus, if clinical response is not occurring in a cat within the first few weeks of treatment, raising the dose to 10 mg/kg once daily is recommended (and safe).

- Terbinafine, in initial studies, also seems to be effective in some situations. This drug is much less studied, but is currently enjoying great popularity because in late 2008 it became available as a very cheap generic in the USA. In one report, cats with *M. canis* “apparently resistant” to itraconazole (though it's uncertain if this is really occurs, and how often) were successfully treated with terbinafine at 10-30 mg/kg once daily. Cats treated with a bit higher dose cure significantly faster, and 30-40 mg/kg/d is thus the recommended dose for cats – anywhere in this range is fine, depending on the tablet size. This dose is much higher than human doses, due to the cat's different metabolism of the drug. There are two adverse effects commonly reported (~1/3 of cats?): malaise and elevated liver enzymes, though the two do not necessarily occur together. Monitor liver enzymes; this drug may elevate ALT in cats, though no clinical toxicity is necessarily seen if ALT is up.

- There are definite reports of failure of individual cats to clinically respond to azole drugs, including itraconazole. Whether this is true resistance of the organism, or some host factor related to poor bioavailability, is not known. In such cases, terbinafine has been clearly successful and is recommended.

- Studies over the past several years have clearly demonstrated that many disinfectants sold and labeled as effective for killing dermatophytes in the household or veterinary clinic are, in fact, not practically effective for this purpose.
• Our best current recommendations for environmental disinfection are bleach (1:100 or 1 oz/gallon) or accelerated hydrogen peroxide-based products.

• How extensively to recommend disinfection? In a typical household with one or a few cats, it may make sense to do a thorough general vacuuming and cleaning, and perhaps wipe down any “cat-intensive” areas with disinfectant if possible. This will mechanically remove most of the contamination, and the remaining spores probably don’t matter practically AS LONG AS the owner does not anticipate that new children, new kittens or puppies, or immunosuppressed individuals will be admitted into the house.

• This situation changes ENTIRELY when discussing control in a cattery or animal shelter, where environmental disinfection is supremely important, and a common reason for failure of eradication.

• A great source of information for helping with cattery or animal shelter eradication can be found at: http://www.giveshelter.org under “Maddie’s Felines In Treatment Center”

• KEY TAKEAWAYS for therapy:
  o Always use topical and systemic treatment.
  o Use lime-sulfur or azole-chlorhexidine, with little or no clipping.
  o Use itraconazole unless cost-prohibitive; otherwise use terbinafine (dog, cat) or ketoconazole (dog).
  o Environmental treatment is crucial in a cattery or shelter; less important in households.

Keywords: Feline, Ringworm, Dermatophytosis, Fungal
Why the Skin Infection is Routine Problem in Veterinary Clinics

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Bacterial skin disease in dogs is one of the most common diseases. The incidence rate varies from 20% to 75%. Typical dermal findings include papules, pustules, epidermal collarettes, crusts, and hyperpigmentation, but these are often difficult to diagnose by naked eye. In particular, bullous impetigo, exfoliative superficial pyoderma, superficial spreading pyoderma, and mucocutaneous pyoderma are not detectable only by gross finding. Recently, the concept of bacterial overgrowth syndrome (BOGS) has been used to separate from skin disease although the same bacteria cause that. Mistaken pyodermas, which is misdiagnosed as pyoderma even though it is similar to gross appearance, is juvenile cellulitis, immunomodulatory-responsive lymphocytic-plasmacytic pododermatitis, and pemphigus foliaceus.

Commensal bacteria colonize the skin surface of animals and humans, which is the infundibulum of the hair follicle and the surface of the epidermis. *Staphylococcus pseudointermedius* is the most important cause of pyoderma is and is often isolated from mucosal of oral cavity, nasal cavity, genitalia, and anus. These areas are mainly colonized by the commensal bacteria, and by licking and grooming, they can spread the bacteria to the skin and coat. It has been known as a “Good Cocci”. However, the antibiotic resistance rate has been increased recently. In dogs, the most common cause of pyoderma is physiological and anatomical factors, because the stratum corneum, which acts as a barrier to block the entry of bacteria into the deep part of the skin, is thin. In addition, it lacks the intercellular lipid layer and lacks the lipid-squamous epithelium plug in the entrance of hair follicle, and is weakly alkaline with high pH 6.5-7.1. In addition, common predisposing factors include environmental factors, allergic diseases, endocrinopathies, poor nutrition status, immunologic incompetence, Idiopathic keratinization defect, ectoparasites, and inappropriate prior therapy.

Bacterial skin infection makes anumeric and various skin lesions, so it is difficult to diagnose only with seeing the lesions. Therefore, clinician just be accustomed with the various lesions caused by bacteria and do perform the skin cytologic examination. If not clinician should be encountered with the frustrating dermatologic cases. There are some indications for culture and sensitivity test as followings: 1) Less than 50% reduction in extent of lesions within 2 weeks of appropriate systemic antimicrobial therapy. 2) Emergence of new lesions (papules, pustules, collarettes) 2 weeks or more after the initiation of appropriate AMD therapy. 3) Presence of residual SBF lesions after 6 weeks of appropriate systemic antimicrobial therapy together with the presence of cocci on cytology. 4) Intracellular rod-shaped bacteria are detected on cytology. 5) There is a prior history of multidrug-resistant infection in the dog or in a pet from the same household. Antibiotics therapy is mainstay of canine pyoderma and antibiotics can be selected empirically or by bacterial culture and sensitivity results. An antibiotic chosen empirically should have a known spectrum of activity against *Staphylococcus pseudointermedius*. Perfusion of skin is less than ideal for establishing adequate dosages of antibiotics in comparison to other body tissues. According to some studies only 4% of cardiac output reaches the skin, in contrast to 33% of cardiac output reaching muscles and even higher levels to other body organs. In addition, tissue levels of some antibiotics reach only marginal levels in the subcutis and even lower in dermal/epidermal junction. The establishment of appropriate antibiotic dosages is controversial. Dosages used should be as close to established recommendations as possible but should
not be less than recommended. Some authors have stated that in general, doses of antimicrobial agents are doubled for skin infections so that effective tissue concentrations are more likely to be achieved. Although there is not general acceptance of this view, it does illustrate that dermatologists are not unwilling to increase dosages in patients with skin infections. Higher than established antibiotic dosages are often necessary in dogs with deep pyoderma as sequestered foci of infection may be protected by granulomatous inflammation. When treating deep pyoderma with excessive scarring and sequestered infection, the author has successfully used cephalixin at higher dosages than recommended. Adverse side effects are generally uncommon. 

Antibiotic therapy should be maintained until complete elimination of bacterial infection, rather than simply transient remission is achieved. This usually necessitates a minimum of 3 weeks for superficial pyodermas and sometimes as long as 10-12 weeks for deeper pyodermas. Antibiotics are rarely used in veterinary dermatology in accordance with manufacturer's recommendations of duration of therapy. The suggested duration of therapy listed by most manufacturers fall far short of the time span actually required to insure cure for most types of canine pyoderma. Please don't forget the topical antimicrobial therapy. Topical therapy is adjunctive therapy and extremely important in the management of many dermatological conditions. Shampoos widely used in veterinary dermatology. As an adjunct treatment to systemic therapy such as antibiotics, faster and longer lasting results in the treatment of skin infection. It can reduce the cutaneous bacterial population, remove tissue debris, direct contact of the active ingredient with the organism and promote drainage.
Abdominal and Thoracic CT Angiography in the Dog and Cat

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Summary
CT provides a rapid and effective method of identifying and characterizing thoracic and abdominal vascular disease. It overcomes the superimposition and narrow contrast range of radiographs, does not suffer from the limitations of body fat that create problems for abdominal ultrasound and also is relatively uninhibited by motion artefacts unlike abdominal MRI. It is ideally suited to performing angiographic techniques such as portosystemic shunt studies and renal angiography. There is no doubt that CT will remain amongst the most useful of advanced thoracic and abdominal imaging techniques in future years and that more specialised protocols for particular abdominal pathology will be developed.

Thoracic CT Angiography
Introduction: Computed tomography (CT) is a very valuable non-invasive imaging technique and is often used to further investigate the extent and origin of a lesion diagnosed on thoracic radiographs or on ultrasound. The fast technology advancement in CT especially in the area of multi-detector scanning has also lead to a large increase in interest evaluating the heart and vascular structures of the thorax. Multi-detector scanners allow for very rapid scans; however to reduce respiratory motion while imaging the thorax and mediastinum, a breath-hold technique or inducing apnea by prior hyperventilation should be applied.
Cardiac CT: For cardiac CT a high spatial and temporal resolution is essential. Optimal imaging would be done under gating, in which the image acquisition is synchronised with the electrocardiogram. Excellent quality studies for most cardiac conditions can be achieved with 64+ slice CT units. Determination of shunt direction requires a levo and dextro-phase CT. The dextro-phase is acquired when contrast enhanced blood has entered the right heart and pulmonary arteries, but not the left heart yet. This time span is best determined with a test bolus. The levo-phase is acquired at the end of the contrast bolus run, when contrast enhanced blood has left the right heart but is still present in the left heart.
Aortic Vascular Ring Anomalies: Persistence of the right aortic arch accounts for almost all aortic vascular ring anomalies in dogs and cats. The oesophagus is most commonly affected and its compression varies in site and severity according to the specific type of malformation. It is a congenital condition that can arise due to two different mechanisms that give rise to a right aortic arch either with or without mirror image branching. Only the anomalies with mirror branching form a ring around the oesophagus and trachea and are clinically relevant. Different types exist:
- Right aortic arch and left ligamentum arteriosum (most common).
- Persistent right ligamentum arteriosum with normal left aorta
- Double aortic arch (also trachael compression)
- Persistent right aortic arch and aberrant left subclavian artery (incomplete ring)
- Persistent right aortic arch and aberrant left subclavian artery and left ligamentum arteriosum (complete ring, 2 strictures)
- Persistent right aortic arch and aberrant left subclavian artery and right ligamentum arteriosum (complete
ring, 1 stricture)

- Aberrant right subclavian artery with left aortic arch
- Other vascular malformations may result in oesophageal entrapment

95% of persistent right aortic arches consist of an aberrant right aortic arch and a left ligamentum arteriosum with no residual blood flow. A left-sided thoracotomy without further imaging would be sufficient. However, there are several conditions that might warrant further imaging if available:

- Presence of a patent ductus arteriosus. Although this can be recognized at surgery, it is helpful to know this in advance for surgical planning.
- Aberrant left ligamentum arteriosum and subclavian artery warrant a left sided thoracotomy and surgery at one or two sites.
- Double aortic arch requiring a ventral approach.

These anomalies are not common, but with modern imaging techniques it is possible to diagnose these subtypes. Multi-slice helical CT allows imaging of a relatively large area within a very short time period. The use of a power injector is required for aortic arch imaging, but a peripheral venous injection is sufficient. Proper timing of the scan post injection is required and best achieved with a test bolus. CTA allows identification of all patent vessels and the location of the oesophageal and tracheal constriction. The non-vascularized ligamentum arteriosum remains difficult to see but its location can be assumed by the location of oesophageal compression.

**Pulmonary Thromboembolism:** CT is an excellent imaging modality for the diagnosis of macroscopic pulmonary thromboembolism in dogs. A high resolution lung CT should always be performed before the contrast study, as many features, such as vascular distension and post embolic vascular collapse, areas of lung infarcts and micronodular changes and subpleural bands with small peripheral emboli are readily visible. A pulmonary arterial-phase CT angiography is performed and the timing of the phase can be determined via test bolus or automatic bolus tracking. It is essential to be able to induce some temporary apnea for this study, which can be difficult in these often hyperpnoeic dogs. A diagnostic scan will show contrast enhanced pulmonary arteries, which in case of thromboembolism will contain filling defects. Very small thrombi in the very lung periphery can be difficult to diagnose with certainty with this conditions.

**Abdominal Vascular CT**

**Introduction:** CT allows identification of a wide range of attenuation values permitting visualization of vascular contrast enhancement even after passage through and haemodilution in the pulmonary and systemic capillary bed during the first vascular cycle. Helical CT allows scanning of long body parts in a short time span. Therefore helical CT angiography enables visualisation of contrast enhanced abdominal vessels using only a peripheral venous injection of iodinated contrast medium. Thus more invasive, costly, labour and time intensive selective angiographic procedures can be replaced by CT. Multi-slice-CT improves the image quality further and allows better timing of vascular phases.

**Arterioportal hepatic fistula:** This is a rare congenital abnormality in dogs in which the hepatic arteries feed into the portal vein with a single vessel or multiple (synonym arteriovenous malformation) tortuous shunt vessels, resulting in portal hypertension, hepatic encephalopathy and ascites. They are often combined with portosystemic shunts, complicating diagnosis. CT features are:

- Almost always peritoneal effusion
- Test bolus shows portal vein time-attenuation graph similar to aorta with minimal delay.
- Single or multiple enlarged tortuous hepatic vessels that contrast enhance immediately after abdominal arteries
- May have abruptly decreasing aortic diameter caudal to celiac artery
- Distended portal vein branches
- Microhepatia

**Portosystemic shunts:** Portosystemic shunts (PSS) are abnormal communications between the portal circulation and other venous vascular systems causing hepatic encephalopathy, raised bile acids and other
abnormalities. Portosystemic shunts can be classified according to different criteria:
The classification of PSS is an evolving process, as more and more shunt types and combinations according
to the different criteria are being identified with advanced imaging modalities. PSS are relatively common
in dogs, and the most common shunt types are single extrahepatic in small breed dogs and single intrahepatic
in large breeds. The left-divisional intrahepatic shunt is synonymous with a patent ductus venosus Arantii.
PSS are rare in cats, the most common type here is an extrahepatic portoazygos shunt. Intrahepatic PSS
are usually of assumed congenital origin, whereas extrahepatic shunts can be congenital or secondary to
some form of portal hypertension. The assumption that multiple extrahepatic shunts are always acquired
is not correct.
Microvascular portal vein dysplasia is a condition where shunting only occurs at microscopic level, requiring
a liver biopsy for confirmation. CT is used to rule out a macroscopic shunt. Dynamic CT has potential
to aid in further diagnostic by calculation of the hepatic perfusion index. CT features are:
• General features
  • Microhepatia
  • Renomegaly (inconsistent)
  • Urolithiasis
  • Cachexia
  • Enlarged tortuous hepatic arteries
  • Periportal oedema (unknown significance)
  • Gastric foreign bodies (allotriophagia)
  • Reduced portal vein diameter cranial to shunting portal tributary vein (extrahepatic shunt)
  • Cessation or reduction of portal enhancement distal to intrahepatic shunt origin.
• Single right-divisional intrahepatic portosystemic shunt
  • Wide tortuous intrahepatic shunt vessels in right lateral lobe
  • Originates from right portal branch directly at liver entrance
  • Connects to CVC from right with one ore multiple connections
• Single central-divisional intrahepatic portosystemic shunt
  • Short bulbous intrahepatic shunt vessel in central part of liver
  • Connects left branch with CVC
    Usually narrowed lumen at either shunt end
• Single left-divisional intrahepatic portosystemic shunt
  • Wide tortuous intrahepatic shunt vessel in left liver half
  • Originates from left portal branch, curves lateral, then dorsal, to connect to the hepatic ampulla
• Multiple extrahepatic shunt types
  • Multiple small chaotic vessels between great vessels and left kidney and around left kidney, less
    commonly right kidney
  • Oesophageal vascular enlargement with varix formation

Segmental caudal vena cava aplasia: This is an increasingly frequently reported congenital anomaly in
dogs in which the pre-renal CVC segment between the kidneys and the liver has not been formed. Post-renal
caval blood is shunted to a right or anomalous left azygos vein. The condition can be incidental as the
cavo-azygos shunt is a functional conduit, but it is associated with significant morbidity in about 25% of
cases, due to either thrombosis in the aneurismal cavo-azygos shunt vessel or associated portosystemic
shunts. In some cases the portal vein completely connects to the azygos vein making it inoperable.
CT-angiography is therefore an excellent modality to assess the need for and possibility of surgical intervention.

**Keywords:** Computed Tomography, Vascular, Dog, Cat
References


Imaging of Canine and Feline Chylothorax

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Summary
A variety of disease processes can cause an impairment of the thoracic lymphatic drainage and subsequent chyloous pleural effusion. Most commonly the condition is idiopathic in dogs and cats. A detailed understanding of the anatomy of the lymphatic system and mediastinum is a prerequisite for successful surgical correction. Diagnostic imaging in cases of chylothorax is mainly used to diagnose or rule-out non-idiopathic forms of chylothorax. However using specific lymphangiographic studies the exact anatomy of the thoracic duct can also be demonstrated. This information can be used for ligation procedures of the thoracic duct.

This presentation will highlight the anatomic details of the thoracic duct and thoracic cavity in the dog and cat and imaging modalities and contrast procedures to elucidate the anatomy, with an emphasis on computed tomography. The imaging findings of different disease processes associated with chylothorax will be discussed.

Pleural Space Physiology
The pleural space maintains a negative pressure generated by opposing elastic forces of chest wall and lung at functional residual capacity. Normally 2-3ml of fluid are present in the pleural space. The function of pleural fluid is to lubricate moving surfaces, and maintain lung-chest wall contact even with a small perforation via the surface tension. There is a continuous turnover of pleura fluid (8-10l/ day in humans) from the parietal to visceral pleural to maintain this balance, which is influenced by capillary permeability, colloid oncotic pressure of plasma and pleural fluid, hydrostatic pressure of visceral and parietal pleural capillaries and pleural space and balance of pressures in the lymphatic drainage. Large molecules can only be resorbed by the lymphatic system. Therefore pleural exsudate and haemorrhage require lymphatic drainage.

Chylothorax
Chylothorax is defined as the accumulation of chyle in the pleural cavity. It is due to disrupted lymphatic drainage which can be caused by cardiopathies, pericardial effusion, mediastinal masses, trauma, caval thrombosis and thoracic duct trauma. Thoracic duct lesions can occur due to diaphragmatic hernia, coughing and straining, erosion by mediastinal neoplasia or obstruction due to a number of causes. Chylous effusion contains chylomicrons, which are fat globules detectable on fat stains. Chronic chylothorax causes a sterile pleuritic with fibrosis. A differential diagnosis to chylothorax is a pseudochoylous effusion, in which there are no chylomicrons present but other cell debris. Pseudochoylous effusion is usually idiopathic in dogs and has been associated with cardiomyopathy and lymphoma in cats.

Lymphatic Drainage Anatomy
The lymphatic drainage occurs via lymph capillaries which converge into lymph vessels and then lymphatic trunks. The cranial and cervical lymphatic drainage occurs via the left and right tracheal trunk, which drain
into the jugular veins. The pelvic limb and abdominal lymphatic drainage occurs via the chyle cistern which continues into the thorax as the thoracic duct. The thoracic duct runs as a single or multiple duct in the caudodorsal mediastinum and exits into the azygous or jugular veins with several possible patterns.

Thoracic CT is gaining acceptance in veterinary medicine for investigation of many pulmonary and non-pulmonary diseases due to superior image quality, abolition of superimposition and guided lesion sampling. CT permits identification of smaller pulmonary nodules than can be identified from radiographs, and as such is important in the staging of neoplastic and granulomatous diseases. Understanding when thoracic CT is most applicable to a clinical case is fundamental to prevent frustration and to provide additional information as frequently as possible. In order to optimize the use of CT for thoracic imaging, an understanding of technique and image display optimization is necessary.

**Radiographic Assessment of Pleural Effusion**

With radiography it is possible to identify the presence, amount and location of pleural fluid and differentiate it from similar conditions such as diaphragmatic hernia. On a ventrodorsal radiograph the cardiac silhouette can be identified and assessed in the presence of pleural effusion, whereas on a dorsoventral radiograph, the cardiac silhouette is often not visible. Chronic pleural effusion often shows rounded lung margins. The type of fluid cannot be determined with radiography. In the presence of pleural effusion, it can be difficult to determine other thoracic pathologies, such as a mediastinal mass lesion.

**Ultrasonographic Assessment of Pleural Effusion**

Thoracic ultrasound is helpful in determining potentially causative lesions of a pleural effusion, such as a mediastinal, chest wall or lung mass, diaphragmatic integrity or vascular thrombosis.

**Computed Tomographic Assessment of Pleural Effusion**

Thoracic CT is applicable for a range of disease processes including evaluation for pulmonary metastatic disease or mediastinal lymphadenopathy, evaluation of mediastinal, thoracic wall or pulmonary masses for surgical resection and to determine the cause of pleural effusion. It is excellent for the identification of mediastinal masses and vascular thrombosis.

**CT Lymphography**

CT Lymphography is used to identify the exact course and pattern of the thoracic duct to find an area of leakage, but more importantly, to identify a site suitable for surgical ligation for any chylothorax, regardless of its cause. Because the thoracic duct can have multiple channels in the caudal mediastinum it is essential to identify a spot where there is only one channel that can be ligated. Also due to the lateral surgical approach it is essential for the surgeon to know on which side the duct channels are. This can achieved with CT lymphangiography. The technique for this procedure is to inject 1-2ml of iodinated water soluble contrast medium into one popliteal lymph node, and to scan immediately afterwards the thorax and abdomen, and repeat if needed in one-minute intervals.

**Keywords:** Computed Tomography, CT Lymphography, Dog, Cat

**References**


A Primer on MRI Physics – Not as Scary as You Think

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Summary
Magnetic resonance imaging (MRI) is rapidly gaining importance in veterinary medicine. It is based on manipulation of hydrogen protons placed within a strong external magnetic field. Utilization of gradients and radiofrequency pulses allows spatial localization of hydrogen protons within a given patient and generation of different sequences with the goal of highlighting specific tissues and substances. MR sequences can be subdivided into spin echo (SE) sequences, modified spin echo sequences and gradient echo sequences. Spin echo sequences include T1-weighting, T2-weighting and proton density (PD) weighting which are the most basic but also the most commonly used MRI sequences. Modified spin echo sequences are based on conventional SE principles, but additional pulses are applied to selectively suppress signal from certain tissues (inversion recovery sequences) or to accelerate data acquisition (fast or turbo spin echo techniques and single shot techniques). While the generation of SE images relies on the application of pairs of radiofrequency (RF) pulses, GRE sequences utilize only one initial RF pulse in conjunction with gradient field reversals. This lecture will cover basic MRI physics and standard sequences routinely used to image small (and large) veterinary patients.

Basic MRI physics (1-4)
Hydrogen is the optimal element for MR imaging as it is the most common element in the body (found e.g. in water and fat), its nucleus consists of a single proton and has the strongest magnetic dipole moment of any element suitable for MR imaging, and most pathologic processes result in alteration of content, distribution, and ambient environment of hydrogen protons facilitating differentiation of diseased from normal tissue. Hydrogen protons in tissues are not static but spin around their axes, generating their own micromagnetic environments. In the absence of an external magnetic field the magnetic moments of the spinning protons are randomly oriented. However, when brought into a strong external magnetic field (i.e. an MR scanner) they rearrange under its influence. The magnetic field strength is denoted by the unit “Tesla” (T). The strength of clinically used MR scanners ranges from approximately 0.2T (low-field) to 3T (high-field). Although the alignment of individual protons may be parallel or antiparallel with the external magnetic field, a slight majority of protons will align with the magnetic field, generating a magnetic vector (“net magnetization vector”) which is utilized during MR imaging. The main magnetic field is denoted by B₀, the tissue magnetization vector by M₀. As long as M₀ is parallel with the much stronger B₀ it cannot be easily separated out and cannot be used for imaging. The goal of MR imaging is to manipulate tissue magnetization in a way that it can be distinguished from the external magnetic environment. In addition to a spinning motion around its own axis, hydrogen protons wobble (or precess) under the influence of B₀, similar to a spinning top wobbling under the influence of gravity. In order to manipulate tissue magnetization so it can be separated from the main magnetic field, radiofrequency (RF) pulses are applied. Once the nuclei are exposed to a RF pulse exactly matching their precessional frequency, they gain energy and
start resonating. As a result of the energy gain some protons change their alignment with the magnetic field, causing the magnetic net vector to move away from $B_0$ or “flip”. The most common flip angle of the tissue magnetization vector is 90° used in spin echo sequences. Hydrogen protons flipped into this “transverse plane” - which is in perpendicular orientation to the main magnetic field (the “longitudinal plane”) - continue to precess. According to Faraday’s law any change in the magnetic environment of a coil of wire will cause an electric signal. Strategic placement of receiver coils in the MR unit allows detection and measurement of magnetization in the transverse plane, which is the basis of image formation in MRI. After the RF pulse is switched off, the signal induced in the receiver drops off rapidly due to two concurrent processes:

- **T1-relaxation (spin-lattice relaxation)**: Excited hydrogen protons return to lower energy states, and the magnetic net vector realigns with the main magnetic field.
- **T2-relaxation (spin-spin relaxation)**: The unified front of hydrogen protons quickly loses coherence, resulting in a signal drop-off. While true T2-relaxation is solely the result of micromagnetic inhomogeneities (spin-spin interactions; i.e. interference of one spinning proton’s micromagnetic field with its neighbors), extrinsic magnetic field inhomogeneities (magnet imperfections, disruption of the magnetic field by paramagnetic or ferromagnetic substances etc.) contribute to an even more rapid loss of phase coherence. This process is called T2*-relaxation.

### Basic spin echo (SE) sequences (1-4)

These include T1-weighting, T2-weighting and proton density (PD) weighting which are the most basic but also the most commonly used MRI sequences. Each SE sequence starts with a 90° RF pulse followed by a 180° pulse applied exactly halfway between the initial 90° pulse and the generation of the signal (echo). The 180° pulse is applied to cancel out external magnetic field inhomogeneities. It essentially reverses any effects an external disturbance (e.g. a nearby flowing vessel or paramagnetic methemoglobin in a hematoma) will have on proton alignment and resultant tissue signal. The small interactions occurring between individual protons and contributing to signal loss in the transverse plane cannot be reversed, resulting in true T2 relaxation contributing to image contrast. As the MR signal generated during a single episode of proton excitation is too small to create an image, the process is repeated many times until enough data have been collected. The time between the 90° pulse and the echo is called “time of echo” (TE), the time between successive 90° pulses is called “time of repetition” (TR). The length of TR and TE determines weighting of a SE sequence.

- **T1-weighting**: A short TR is chosen to maximize the differences in T1 relaxation between tissues. This is combined with a short TE to minimize T2 effects. Fat is hyperintense, while fluid appears hypointense. Soft tissues are of medium intensity. After uptake of paramagnetic contrast agents, physiologically contrast enhancing tissues (e.g. pituitary gland) and contrast enhancing pathologic lesions (e.g. certain brain tumors) are hyperintense.

- **T2-weighting**: A long TE is chosen to maximize differences in T2 relaxation between tissues, combined with a long TR to minimize T1 relaxation effects. Fluid has a long T2 relaxation time and therefore is hyperintense on T2-W images. Soft tissues have intermediate T2 relaxation times. Fat has a short T2 relaxation time and appears hypointense on conventional T2-W images. However, as conventional T2-W SE sequences have largely been replaced with shorter fast spin echo (FSE) or turbo spin echo (TSE) sequences in which additional pulses are applied, fat typically appears hyperintense on today’s T2-W MRI studies. A T2-W sequence can be considered a “pathology” scan, because abnormal fluid collections and tissues with abnormal increased fluid content (“juicy tissue”, e.g. edema, inflammation, neoplasia etc.) will appear hyperintense compared to normal tissues.

- **Proton density (PD) weighting**: Proton density weighting is achieved by choosing a long TR in combination with a short TE to minimize T1 and T2 effects on image contrast. PD-W images are characterized by excellent anatomic detail and are very useful in orthopedic imaging.
Modified spin echo (SE) sequences (1-4)

These sequences are based on conventional SE principles, but additional pulses are applied to selectively suppress signal from certain tissues (inversion recovery sequences) or to accelerate data acquisition (fast or turbo spin echo techniques and single shot techniques).

- Inversion recovery sequences: These are characterized by an initial 180° pulse (inversion pulse). Dependent on the time elapsed between this 180° pulse and initiation of the regular SE sequence (time of inversion; TI) they result in selective suppression of fluid (fluid attenuated inversion recovery; FLAIR) or fat (short tau inversion recovery; STIR). FLAIR: A long TI prior to initiation of a SE sequence allows selective suppression of fluid. T2-W FLAIR images are useful in conjunction with regular T2-W images in characterizing T2 hyperintense lesions. Using FLAIR, pure fluid (CSF and fluid in cystic lesions) is suppressed and becomes hypointense while solid lesions remain hyperintense. Additionally, this sequence increases conspicuity of small lesions bordering a fluid filled ventricle or the subarachnoid space. Finally, FLAIR is helpful in differentiating true T2 hyperintense parenchymal lesions from pseudolesions created by inclusion of fluid-filled structures and brain parenchyma within the same slice thickness (volume averaging). STIR: A short TI prior to initiation of a SE sequence allows selective suppression of fat. This sequence is very valuable in orthopedic and spinal imaging as it allows differentiation of pathologic T2 hyperintense lesions within the spinal canal, vertebrae and surrounding paraspinal tissues from fat.

- Fast (FSE) or turbo (TSE) spin echo techniques: In conventional spin echo imaging one 180° pulse is applied during each TR, and one echo (signal) is generated. In FSE and TSE multiple 180° pulses are applied during each TR and multiple echoes are received, resulting in a decrease in scan time without compromising image quality. FSE/TSE techniques have essentially replaced conventional SE sequences in T2-W imaging. One potential disadvantage is strong hyperintensity of fat on T2-W FSE/TSE images.

Single shot techniques (e.g., Siemens “HASTE”, Philips “SSH-TSE”, GE “SS-FSE”, Hitachi “FSE-ADA” and Toshiba “(Super)FASE”): These ultrafast techniques employ a single RF pulse, further decreasing scan time. The resultant images are characterized by very strong T2 contrast and are most beneficial in imaging of fluid filled spaces. These sequences have gained popularity in veterinary medicine for spinal imaging due to their myelographic effect which can be used to classify spinal lesions, identify sites of significant intervertebral disc herniation and diagnose spinal subarachnoid diverticula.

Gradient Echo (GRE) Sequences (1-4)

While the generation of SE images relies on the application of pairs of RF pulses, GRE sequences utilize only one initial RF pulse in conjunction with gradient field reversals. GRE sequences use smaller flip angles and shorter TRs than SE sequences, resulting in shorter scan times. The lack of a 180° pulse has important implications for image weighting and quality: (1) Conventional GRE sequences can be used to acquire T1-W, PD-W and T2*-W images; acquisition of truly T2-W images is not possible; (2) GRE sequences are prone to susceptibility artifacts as there is no compensation for external field inhomogeneities. Rapid development in the field of GRE sequences led to numerous new and advanced applications of MR imaging such as motion-free (breath hold) abdominal imaging, 3D volumetric imaging, and 3D MR angiography (MRA).

- T2*-W GRE sequence: Gas interfaces, soft tissue mineralization, fibrous tissue and certain blood degradation products (e.g. methemoglobin) cause magnetic field inhomogeneities which appear as a signal void (susceptibility artifact) on T2*-W images. T2*-W is most commonly utilized to identify intracranial or spinal hemorrhage and differentiate it from other lesions.

- 2D/3D volumetric acquisitions: Specific 2D and 3D GRE sequences may be beneficial in the evaluation of small structures (inner ear, pituitary gland, osseous structures or cranial nerves) as they allow acquisition of thin slices (< 1 mm) without interslice gap and permit multiplanar reconstruction of the 3D dataset in additional planes.
**Figure 1** MR images of the brain of a cat (T2, T1, FLAIR, T2*, post contrast T1 GRE and SE) showing a large extra-axial mass (falcine meningioma)

**Keywords:** MRI Physics, Spin Echo Sequences, Gradient Echo Sequences, Weighting

**References**


MRI of Brain Tumors

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Summary

A variety of tumors can affect the brain in dogs and cats. Traditionally, intracranial masses have been subdivided into intra-axial (arising from actual brain parenchyma) and extra-axial (arising from adjacent structures). While this classification is far from perfect, it can be helpful in establishing an appropriate list of differential diagnoses. Examples of intra-axial brain tumors include glial tumors and metastases to brain. Examples of extra-axial brain tumors include meningiomas and tumors of ventricular origin. Secondary changes to brain tumors are common and include hydrocephalus, syringomyelia, perilesional edema, mass effect and brain herniation. Although some brain tumors have a fairly characteristic appearance on MRI (e.g., meningioma or pituitary tumor) it is important to realize that overlap exists between imaging features of different tumor types, non-neoplastic mass lesions, inflammatory and vascular conditions.

Brain masses: Intra- vs. extra-axial origin

Classification of a mass as intra- or extra-axial in origin can be helpful in establishing a presumptive diagnosis (1, 2). Extra-axial masses arise from tissues other than actual brain parenchyma. Many are located in the periphery of the brain and compress rather than invade brain parenchyma (e.g., meningiomas). Some are in a typical location (e.g., intraventricular choroid plexus tumors or pituitary tumors originating from the pituitary fossa), and most are strongly contrast-enhancing as they are not protected by the blood-brain barrier. Concurrent changes to the skull (e.g., cribiform plate lysis seen with nasal tumors, or hyperostosis occasionally present with meningiomas), broad-based contact of the lesion with meninges forming an obtuse angle, and meningeal thickening and enhancement adjacent to a mass (‘dural tail’) also suggest extra-axial origin. Intra-axial masses originating from brain parenchyma may be completely surrounded by normal brain tissue in which case determination of mass origin is straightforward. Peripherally located or very large masses may be in contact with overlying meninges which may make a diagnosis challenging. While presence and degree of contrast enhancement of intra-axial masses is highly variable, and while moderate to strong enhancement can be seen with both intra- and extra-axial masses, absent or poor enhancement is most consistent with intra-axial origin and protection of the mass by the blood-brain barrier.
Associated findings
A variety of pathologic sequelae can be associated with intracranial masses, including hydrocephalus, cervicothoracic syringomyelia, peritumoral edema, mass effect and brain herniation.

MRI findings in specific tumor types (1-7)
- Meningeal tumors: Meningiomas are the most common brain tumors in dogs and cats. They originate from the meningeal lining of the brain. MRI findings include round/ovoid or plaque-like usually smoothly marginated mass(es), typically in broad-based contact with underlying bone (except tumors in ventricular location). They are typically hypointense to isointense on T1W images, hyperintense on T2W/T2-FLAIR images, and strongly contrast enhancing (homogeneous, heterogeneous or ring enhancement possible). A ‘dural sign’ is occasionally seen and represents thickening and enhancement of the dura adjacent to an extra-axial mass. Less common MRI findings include mineralization or hemorrhage associated with the mass causing T2 hypointense foci and/or susceptibility artifact (signal void) on T2*W images, bone changes adjacent to the tumor including hyperostosis, pressure atrophy or tumor invasion of bone (cats), and single or multiple tumor-associated cyst-like changes (more common in dogs than cats). Cystic meningiomas occur predominantly in the rostral fossa. Other tumor types that may affect the meninges include disseminated histiocytic sarcoma, lymphoma, granular cell tumor and metastatic disease (meningeal carcinomatosis).

- Gliial tumors are fairly common in dogs but rare in cats. MRI findings in astrocytomas and oligodendrogliomas include intra-axial, often heterogeneous, ovoid to amorphous, well defined to infiltrative T1 isointense to hypointense and T2 isointense to hyperintense mass lesions with variable contrast enhancement. Masses are commonly located in cerebrum or thalamus and less commonly in cerebellum and caudal brainstem. They are typically single lesions although cases of multiple concurrent tumors have been reported. Additional findings may include cyst-like and/or necrotic regions in the mass (T2 hyperintense, T1 hypointense, non enhancing areas), intralesional hemorrhage (susceptibility artifacts on T2*W images), vasogenic edema and mass effect. MRI features of glioblastoma multiforme include typically sharply marginated but occasionally diffuse intra-axial heterogeneous T2 hyperintense and T1 iso- to hypointense mass with variable contrast enhancement. Concurrent necrosis, peritumoral edema, mass effect and cyst-like changes may be seen. MRI findings in gliomatosis cerebri/cerebelli include focal or multifocal ill-defined T2/FLAIR hyperintense, typically non contrast-enhancing areas associated with brain and/or spinal cord.
- **Ventricular tumors:** Several tumor types can affect the ventricular system including choroid plexus tumors (CPT; papillomas and carcinomas), ependymomas and meningiomas in dogs and cats and neurocytomas in dogs. MRI findings in CPT include a papilliform or globular ventricular mass in expected anatomical location of choroid plexus. The lesion is usually hyperintense on T2W images and of variable intensity on T1W images and shows strong contrast enhancement. Signal heterogeneity secondary to cyst formation, mineralization, hemorrhage, or necrosis is possible, and concurrent ventriculomegaly/hydrocephalus, perilesional/periventricular edema, mass effect and brain herniation are common. Additional findings reported in CPC include evidence of intraventricular or subarachnoid metastases. Ependymomas are less common ventricular tumors in dogs, but in cats they are more common than choroid plexus tumors. They arise from the ependymal cells that line the ventricles of the brain. These tumors may extend from the ventricular wall into the ventricular lumen or into the adjacent brain parenchyma. MRI findings include fairly well circumscribed smooth or lobulated mass in ventricular and/or periventricular location which are typically T1W isointense, T2W hyperintense and variably contrast enhancing. Associated cyst-like structure(s) and secondary hydrocephalus, mass effect and brain herniation may be seen. Ventricular meningiomas account for the majority of ventricular tumors in cats. MRI findings include smoothly margined T1 isointense and T2 hyperintense mass, most commonly within the 3rd ventricle, with strong and homogenous contrast enhancement, and secondary hydrocephalus. Other ventricular tumors are rare and include intraventricular neurocytoma and lymphoma.

- **Central primitive neuroectodermal tumors (PNETs) including medulloblastomas:** PNETs are a heterogeneous group of poorly differentiated neoplasms derived from germinal neuroepithelial cells. They have been classified based on location as peripheral (arising from nervous tissue within bone or soft tissues) or central (originating from brain or spinal cord). Medulloblastomas are cerebellar tumors arising from the external granular layer of the cerebellum, and sharing histopathologic features with PNETs. PNETs can occur anywhere in CNS, are typically intra-axial, isointense to hypointense on T1W images, hyperintense on T2W images, and variably but typically moderately to strongly contrast enhancing. Associated hemorrhage is possible and expected to result in susceptibility artifacts on T2*W images. Medulloblastomas are intra-axial and associated with the cerebellum. They are predominantly isointense to hypointense on T1W images, variably hyperintense on T2W images, and show variable contrast enhancement.

- **Central Nervous System-Associated Tumors:** This group includes tumors originating from structures adjacent to the brain and extending locally to affect the central nervous system either through compression or invasion. MRI findings in pituitary macrotumors (adenomas and adenocarcinomas) include an oval or irregular mass measuring more than 10 mm originating from the pituitary fossa. They are of variable signal intensity but are most commonly isointense on T1W images, mildly hyperintense on T2W and T2-FLAIR images and strongly homogeneously or heterogeneously contrast enhancing. Less common MRI findings include a cystic and/or hemorrhagic component, peritumoral edema, secondary hydrocephalus, invasion into surrounding structures including sphenoid, and thickened frontal bone and abnormal soft tissue accumulation in the nasal cavity, sinuses and pharynx in acromegalic cats. MRI findings in pituitary microtumors include a normal examination, increased convexity to the dorsal pituitary margin, displacement of the vasopressin-containing and thus T1 hyperintense neurohypophysis, and possibly alteration of normal pituitary enhancement pattern during dynamic image acquisition. Other masses which may occur in the sellar or suprasellar region and which may be difficult to distinguish from primary pituitary tumors include meningioma, lymphoma, granular cell tumor, gliomatosis cerebri, craniopharyngiomas, germ cell tumors, and metastases to the pituitary gland. Trigeminal nerve sheath tumors manifest as extra-axial and strongly contrast enhancing masses in the middle or caudal fossa, which may result in muscle atrophy, compression or distortion of the adjacent brain stem and enlargement of skull foramina. Olfactory neuroblastoma is a rare malignant neuroectodermal tumor derived from...
the olfactory neuroepithelium which arises at the cribriform plate and can extend both into the cranial vault and the nasal cavity. Primary nasal tumors (e.g. adenocarcinoma, squamous cell carcinoma) may invade the brain through the cribriform plate. Tumors of the skull such as multilobular tumor of bone or masses originating from adjacent structures (ear, orbit) may also extend into the cranial vault and result in compression or invasion of the brain.

- **Metastatic CNS tumors**: Many primary tumors including hemangiosarcoma, melanomas and carcinomas have the potential for wide dissemination including spread to the CNS. MRI findings include multifocal (less commonly single) lesions associated with brain parenchyma, often in close proximity to gray-white matter interface. Lesions are rounded to ovoid, distinctly or indistinctly marginated, typically iso-to hypointense on T1-W images, hyperintense on T2-W images, and strongly homogeneously or ring enhancing. Hemorrhage indicated by susceptibility artifact on T2*W images is common in hemangiosarcoma metastases. Melanoma metastases may be T1 hyperintense, T2 hypointense and associated with signal void on T2* images because of the combined paramagnetic effects of melanin and hemorrhagic changes commonly present with these lesions. Associated vasogenic edema is common. Less common MRI findings include lack of contrast enhancement of parenchymal lesions and involvement of extra-parenchymal structures, e.g., pituitary gland and meninges.

- **Other intracranial neoplasms**: Lymphoma, histiocytic sarcoma, intravascular lymphoma and granular cell tumors may affect the CNS and have variable appearance on MRI.

- **Tumor-like lesions**: Benign lesions or borderline tumors such as hamartomas, hemangiomas, cholesterol granulomas, dermoid and epidermoid tumors may affect the brain and mimic neoplastic lesions. Similarly, some inflammatory conditions (e.g., granulomas) and vascular lesions (e.g., hemorrhagic stroke) may be confused with intracranial neoplasia.

**Keywords**: MRI, Brain Tumor, Dog, Cat

**References**


Cone Beam and Flat Panel CT: Potentials and Limitations of a New Technology for Veterinarians

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Summary
Cone Beam computed tomography (CBCT) is a relatively new technology on the veterinary market. It is based on flat panel detector design and some manufacturers prefer the name Flat panel detector computed tomography (FPCT). This presentation discusses the history, basic principles, human and veterinary applications of CBCT and FPCT and the potentials and limitations of this technology for veterinary patients.

Introduction
Computed tomography (CT) has become a powerful and popular diagnostic imaging modality in veterinary praxis since the 1990ies. In particular the development helical and multi-slice technology has enabled rapid scanning of large animal body parts with thin slice width. Therefore current CT technology provides good diagnostic information imaging modality for all body parts and excellent results for most of them. However CT has still some drawbacks, which include the size of the unit, room and cooling needs, three-phase-power requirement and the need for general anaesthesia for many patients. A small, mobile CT unit that would meet the same standards but without its drawbacks would be highly desirable in veterinary praxis. Cone beam CT (CBCT) and related flat panel detector CT (FDCT) are interesting technologies that have some potential in this regard.

Cone Beam CT and Flat Panel Detector CT History
CBCT was first developed in the 1990ies as an application for fluoroscopy units, also termed as “3D Fluoroscopy” for cardiovascular imaging and stenting purposes. These units are now technologically out-dated and have not found entrance into the veterinary market. More recently CBCT has been developed as a modification of Direct Digital Radiography (DDR) units, mounting the flat panel detector panels from DDR units onto a rotating gantry device with an X-ray tube. Aptly these units are also sometimes called “3D-Röntgen”. This has become possible due to the increased affordability of flat panel detectors. The newest technology applies hybrid technology but using DDR based flat panel detectors. To encompass the entire range of these technologies, the term FDCT has been proposed for all CT technology using DDR-based detectors.

Cone Beam CT Technology
The word “cone beam” refers to the way the data are reconstructed into images, which is different from ordinary CT units. In ordinary CT (even in helical mode), image data are reconstructed slice by slice in the transverse plane, using a filtered back projection for each slice. In CBCT image data are reconstructed within a three-dimensional conical space using a convolution back projection method called FDK (after their inventors Feldkamp, Davis and Kress). CBCT solves in principle a long on-going problem in CT technology: To view images in another plane than the originally acquired transverse slice, a multiplanar reconstruction
(MPR) is performed on either the raw data or, more commonly, the DICOM transverse images. The resolution of the MPR images is rarely isometric in ordinary CT, meaning that the MPR images are of lower resolution than the original transverse images. A CT image is made of a matrix of picture elements, or pixels, with equal height and width in the sub-millimetre range. Since these images represent a slice of tissue, the elements are also referred to as volume elements, or voxels. The depth of the voxel represents the selected slice width. The height and width of the voxel are much smaller than the voxel depth. In CBCT all three dimensions of each voxel are equal because it is directly reconstructed from 3 dimensional data. Therefore CBCT delivers isometric image resolution, meaning one can view images in any selected plane in the same resolution.

CBCT reconstruction methods are mathematically much more complex than filtered back projection and the datasets are several orders of magnitude larger, requiring considerably more time and more corrections, particularly for the periphery of the cone. A typical CBCT unit may have a 1024 x 1024 detector plate and achieve an isotropic spatial resolution of 0.1 to 0.3mm, covering a 10 to 15cm area during one rotation. To process these data, high-end graphic cards from the computer gaming technology are used and the reconstruction time for the covered area is currently between 15-30 seconds.

In FDCT the X-ray tube needs normally 10 to 20 seconds to perform one gantry rotation compared to 0.3 to 1 second in ordinary CT. The main reason for this is the much slower read-out speed of DDR-based flat panel detectors compared rare-earth detectors used in ordinary CT. Therefore FDCT is more prone to motion artefacts and this is one of the limitations of FDCT which causes constraints on using it for vascular and respiratory studies. However due to the slower rotation time the power consumption is also considerably lower (1-2kW) than with ordinary CT (50kW), meaning that a standard power supply is sufficient for CBCT allowing mobile use. Ordinary CT requires 3-phase power and is stationary.

Ordinary CT has excellent scatter minimisation because of efficient pre- and post patient collimation of the X-ray beam, exposing only a thin slice of patient anatomy at any time. Therefore image noise is brought to acceptable levels and contrast resolution is relatively good. In CBCT units a wide cone of tissue is exposed creating significant scatter. Post patient collimation cannot be applied. Instead mechanical grids and scatter correction algorithms can be applied in CBCT. However, the image noise is higher and contrast resolution is poorer in CBCT compared to ordinary CT. Image noise visibility is suppressed with wide window settings, which are appropriate for structures like the nose, lungs, bone and teeth. CBCT therefore delivers good image quality for these structures. However narrow window settings are required for viewing soft tissues and for any post i.v. contrast study. The resulting image quality of soft tissue structures is therefore often poor or even non-diagnostic in CBCT units. Many efforts are currently being made by manufacturers to remedy this problem, which include use of specific algorithms and noise reduction techniques and the combined use of cone-beam and fan-beam scanning (which makes these units FDCT rather than CBCT) and some progress has been mad already.

**Human Cone Beam CT Applications**

CBCT is currently used in humans mainly in dentistry and for radiation treatment guidance and less frequently for angiography, intraoperative imaging and mammography.

Particularly in dentistry CBCT has much to offer: The units are small and mobile and can be installed in an environment where an ordinary CT would not be a cost effective option. Because dental imaging mainly relates to teeth and bone, CBCT offers adequate image quality. The extremely high spatial resolution and the lack of image distortion (as present on radiographs) are additional benefits, particularly for endodontic and implant planning. Also, the curved anatomy of the dental arcade makes curvilinear reconstructions with isometric resolution a very useful application.

In radiation treatment guidance CBCT units are small enough to fit into the linear accelerator device and are sufficient for the daily patient setup. Because most tumours are soft tissue structures, CBCT is usually not sufficient for the initial treatment planning imaging, for which ordinary CT is used.

An emerging application is intraoperative FDCT. There is particular potential to use FDCT for operative
guidance of metal implants into bone structures, allowing precise placement of often very narrow implantation corridors, such as in the vertebral column.

**Veterinary Flat Panel Detector CT Applications**

Within the last 10 years, CBCT and FDCT have found entrance into the veterinary market. First and foremost, CBCT units are being used in small animal dentistry for the same reasons they are used in human dentistry. There are early indications that CBCT is superior to dental radiography and ordinary CT in the detection of canine dental disease, however this is an on-going area of research.

Since ordinary CT units are not custom made for animal patients, but are usually human CT units, there have always been image quality issues relating to the different anatomy for which these units are not optimised. There are also limitations for the use in horses and other large animals due to the animal size. There is an opportunity for FDCT to fill this niche in providing species-optimised CT-imaging devices. There are now the FDCT units on the market, which fill this niche, such as allowing scanning of the equine neck and higher extremity parts.

Also many veterinary institutions have space and power supply limitations and are interested in a mobile device, which make FDCT attractive. However the vast majority of veterinary users need their CT-device for all body parts including soft tissues. Therefore further improvements in soft tissue detail are paramount to make FDCT an attractive option for veterinary praxis.

**Keywords:** Computed Tomography, CBCT, FPCT
Imaging of the Canine Shoulder Joint: Radiography, Ultrasound, CT, MRI

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Summary
Diseases related to the shoulder are relatively common in dogs, and less so in cats, and can present a diagnostic challenge for the orthopaedic surgeon. Many diagnostic imaging modalities have been applied for the shoulder area, including radiography, computed tomography, ultrasound and magnetic resonance imaging, each with their own advantages and disadvantages. This presentation will discuss the pros and cons of each imaging modality for diagnosing shoulder diseases in dogs and cats. Contrast-enhanced procedures will be discussed in further detail regarding their indications and technical considerations with an emphasis on computed tomography. A detailed imaging anatomy of the shoulder joint, muscles and tendons of the dog will be given.

Introduction
Shoulder lameness is common in dogs and can be difficult to localise. Diagnostic imaging is an essential part of the work of these dogs and can include a number of different imaging modalities which pros and cons.

Canine Shoulder Joint Anatomy
The canine shoulder joint is a ball and socket joint with a spacious joint capsule including large recesses, two intracapsular ligaments (medial and lateral glenohumeral ligament), an extracapsular transverse humeral ligament and a joint capsule that blends with surrounding muscles and tendons. The main supporting muscles are the supra- and infraspinatus, subscapularis, biceps brachii, teres minor and coracobrachialis muscles. The infraspinatus muscle is the only one with two bursae to support its tendon.

Shoulder Survey Radiography
Shoulder radiography standard projections include the medio-lateral scapula in dorsal over-rotation, the caudo-cranial shoulder & scapula, the medio-lateral shoulder (± pronation & supination) and the cranioproximo-craniodistal oblique projection of the humeral intertubercular groove. Shoulder survey radiography is adequate for the identification fractures and luxation, dysplasia and degenerative changes but can challenging for the identification of aggressive neoplastic and infectious lesions, full characterization of osteochondrosis lesions.

Shoulder Radiographic Positive Contrast Arthrography
This procedure is done under general anaesthesia or deep sedation with intra-articular injection of 3-5ml of iodinated water soluble contrast medium to outline the articular cartilage and synovial membrane. This technique is particularly useful to identify cartilage flaps from osteochondrosis and biceps tendon injuries.

Shoulder Ultrasound
Shoulder ultrasound provides excellent assessment of cranial, lateral and caudal soft tissue structures of
the canine shoulder joint. It is a non-invasive, dynamic and real time examination for which sedation is recommended. High frequency linear & footprint probes should be used. The main structures that can be assessed are the lateral joint capsule, supra- & infraspinatus muscle and tendons, the biceps muscle and tendon and the teres minor muscle. There is no visualization of medial joint structures.

Shoulder MRI and MR Arthography
For MRI of the canine shoulder general anaesthesia is required. A high field strength (>1 T) magnet is required for high detail assessment. T1, T2 and STIR sequences provide excellent soft tissue detail. Joint extension is best for medial glenohumeral ligament assessment and avoidance of the magic angle artefact in the biceps tendon. Transverse and sagittal planes are best for most structures. The subscapularis tendon is best assessed in transverse and dorsal planes. Intravenous Gadolinium based contrast medium injection (10 μmol/kg) is optimal for joint capsule assessment, whereas MR Arthography is required for intra-articular structure assessment. For this intra-articular injection of 4ml Gadodiamide at 0.42mmol/l and imaging with proton density pulses with fat saturation is recommended. MRI is an excellent imaging modality for assessment of soft tissue structures of the entire joint and surroundings, but it is relatively time consuming to perform.

Shoulder CT and CT Arthrography
Shoulder CT and CT Arthrography (CTA) can be performed under deep sedation or general anaesthesia. The imaging technique should include a thin slice width (0.5 to 1.25mm) with shoulder joint positioned in extension. For CT arthrography intra-articular injection of 4-5ml of iodinated water soluble contrast medium at a concentration of 60mg iodine/ ml should be injected and the dog should be placed in lateral recumbency with the injected shoulder joint down and in extension, and the unaffected shoulder joint up and flexed. This position minimises artefacts from respiration and the contralateral shoulder joint. Canine shoulder CT and CTA are quickly performed. CT is an excellent modality to assess any bony changes of the shoulder joint, whereas CT allows assessment of intraarticular soft tissue structures.

Conclusions
Survey Radiography allows only limited assessment for common shoulder conditions. Ultrasound and Contrast Arthrography allow assessment of some more conditions. MRI with IV Contrast & MRI Arthrography allow complete assessment of soft tissue pathology, but not bone. CT and CT Arthrography allow complete assessment of bone pathology and most, but not all soft tissue pathology.

Keywords: Computed Tomography, Arthrography, CT Arthrography, Dog, MRI

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Summary
Inflammatory brain diseases can affect brain parenchyma (encephalitis), meninges (meningitis) or both (meningoencephalitis). Dependent on underlying etiology, involvement of the spinal cord (myelitis/meningomyelitis) may occur. Encephalitis may cause no detectable abnormalities on MRI or may manifest as multifocal (less commonly focal) lesions associated with brain parenchyma. Meningitis may not be detected with MRI or may appear as meningeal enhancement following administration of contrast medium. Examples of infectious inflammatory brain diseases include viral, bacterial, fungal, parasitic and protozoal disease. A diagnosis of meningoencephalitis of undetermined etiology (MUE) is made in cases of inflammatory brain disease for which a causative agent cannot be identified. Examples include granulomatous meningoencephalitis and necrotizing meningoencephalitis. It is important to note that there is significant overlap in MRI findings between infectious and non-infectious meningoencephalitis cases, and between inflammatory, neoplastic, vascular and even metabolic encephalopathies. MRI is only one piece of the puzzle when working up a small animal patient with intracranial disease.

Introduction (1)
Inflammatory brain diseases can affect brain parenchyma (encephalitis), meninges (meningitis) or both (meningoencephalitis). Dependent on underlying etiology, involvement of the spinal cord (myelitis/meningomyelitis) may occur. Encephalitis may cause no detectable abnormalities on MRI or may manifest as multifocal (rather than focal) lesions associated with brain parenchyma which typically appear hyperintense on T2-W images and hypointense on T1-W images. FLAIR has higher sensitivity than conventional spin echo sequences in detecting subtle brain lesions in dogs with clinical signs of multifocal brain disease, and its use is encouraged in all of these cases. Meningitis may not be detected with MRI or may appear as meningeal enhancement following administration of contrast medium.
Infectious inflammatory brain diseases

- **Canine distemper virus** (CDV) and **feline infectious peritonitis virus** (FIPV) are the most common causes of **viral encephalitis** in dogs and cats, respectively. In acute CDV infection, T2 hyperintense lesions and loss of contrast between grey and white matter on T2-W images may be found in the cerebellum and/or brainstem, corresponding to areas of demyelination. T2 hyperintense areas are occasionally seen in the temporal lobes which may be related to infection or post-ictal edema (2). MRI findings reported in chronic distemper meningoencephalitis included essentially bilaterally symmetric T2 hyperintensity of the cortical gray/white matter junction of the parietal and frontal lobes, T2 hyperintensity of the arbor vitae of the cerebellum with partial loss of cerebellar cortical gray/white matter demarcation, subtle focal T2 hyperintensity of the pons, and meningeal contrast enhancement (3). In FIP MRI may show T2 hyperintensity and contrast enhancement of the ventricular lining, choroid plexus and meninges compatible with ependymitis, choroiditis and meningitis. Concurrent findings may include hydrocephalus, syringohydromyelia and herniation of the cerebellum secondary to increased intracranial pressure (4).

- **Mechanisms of bacterial infection** of the central nervous system in cats and dogs include hematogenous spread, contiguous infection from adjacent structures (middle/inner ear, nasal cavity, sinuses, orbit, skull and vertebrae), direct inoculation (trauma, bite wound and surgery) and migration of foreign bodies or aberrant parasites (5). In addition to meningitis, diffuse cerebritis and meningoencephalomyelitis, CNS infection may result in focal parenchymal abscesses or empyema in the subdural or epidural space. MRI features of intracranial infection secondary to plant foreign body migration, hematogenous spread from a mediastinal abscess, bacterial endocarditis, local extension from orbital disease and from osteomyelitis of the sphenoid bone, and as complication of otitis media or interna have been described in small animals. Intracranial abscesses are typically hypointense on T1-W and hyperintense on T2-W images, with strong peripheral contrast enhancement and associated brain edema. Concurrent meningitis appearing as meningeal enhancement and/or thickening is common. Dependent on lesion location and extent additional findings might include hydrocephalus and/or brain herniation.

- **Fungal infections** (Cryptococcosis, Phaeohyphomycosis, Aspergillosis, Blastomycosis, Histoplasmosis) have been reported to affect the central nervous system in dogs and cats (6). MRI findings are variable and may include intra-axial or extra-axial masses of variable contrast enhancement, gelatinous pseudocysts, meningeal enhancement, enhancement of the ventricular lining, and intra-cranial extension of nasal or retro-orbital masses. Associated findings may include edema, hydrocephalus,
syringohydromyelia and brain herniation.

- **Parasitic meningoencephalitis** in dogs and cats is caused by aberrant migration of parasites such as *Dirofilaria, Baylisascaris, Cuxerebra, Taenia, Ancylostoma, Toxascaris and Angiostrongylus*. MRI features include focal or multifocal parenchymal lesions of variable signal intensity and peripheral parenchymal and/or meningeal contrast enhancement. Intraparenchymal hemorrhage is a common feature in parasite migration, and T2-W images are especially useful in establishing a (presumptive) diagnosis (7).

- **Protozoal meningoencephalitis** may be caused by a variety of organisms. MRI features in dogs infected with *Neospora* include cerebellar atrophy with T2 hyperintense material surrounding the cerebellum and extending into the sulci, loss of contrast between cerebellar grey and white matter, T2/FLAIR hyperintensities within the cerebellum, and meningeal contrast enhancement (8). *Toxoplasma* infection in cats may manifest as multifocal indistinct T2 hyperintense contrast enhancing parenchymal lesions with associated brain edema.

**Non-infectious inflammatory brain diseases/ Meningoencephalitis of undetermined etiology (MUE)**

- Several inflammatory conditions unrelated to infectious agents have been identified in dogs which generally respond to immunosuppressive treatment suggesting immune mediated etiology. Attempts have been made to separate these into specific diseases based on breed (e.g. “Pug dog encephalitis”) and other criteria, however, due to overlap in clinical, diagnostic and pathologic findings a definitive pre-mortem diagnosis is difficult. These disorders may be summarized under “Meningoencephalomyelitis of undetermined etiology” (MUE).

- **Granulomatous meningoencephalitis (GME)** can affect any breed dog but most often occurs in young to middle-aged toy breeds. The disease can affect brain and/or spinal cord. On MRI, GME lesions can be focal or multifocal and commonly affect the brain stem. Although the disease has a predilection for white matter, it is not associated with distinct topography. Lesions are typically hyperintense on T2-W and FLAIR images, iso- to hypointense on T1-W images and variably contrast enhancing (9). Meningeal enhancement may or may not be observed. In ocular MGE MRI may show enlargement of the optic chiasm and fairly symmetric contrast enhancement of the optic nerves, optic chiasm and visual pathways.

- **Necrotizing meningoencephalitis (NME)** is characterized by cavitary necrosis in the neuroparenchyma (10). The disease was initially described in the pug breed (“pug dog encephalitis”), but similar disorders have since been reported in other small breeds including the Maltese, Chihuahua, Pekingese, French bulldog, Shi Tzu and Lhasa Apso. A distinct form of NME described mainly in Yorkshire terriers has been termed “necrotizing leukoencephalitis (NLE)”. Descriptions of imaging findings in this group of inflammatory brain disorders are not available for all breeds. NME in pugs is usually limited to the forebrain. MRI findings include diffuse asymmetric cerebral lesions which are nonuniformly hyperintense on T2-W, isointense to hypointense on T1-W images, and affect gray and white matter resulting in loss of gray/white matter distinction. Additional findings include variable degrees of contrast enhancement of brain and leptomeninges, enlarged and asymmetric lateral ventricles, mass effect, brain herniation, and T2/FLAIR hyperintensity associated with the hippocampus and piniform lobes. MRI findings reported in Chihuahuas and French bulldogs with NME are similar, however, brainstem involvement seems more common. In almost all cases of NLE reported in Yorkshire terriers, lesions are located in the cerebrum and brainstem and appear iso- to hypointense on T1-W images, hyperintense on T2-W and FLAIR images, and show variable contrast enhancement. Concurrent hydrocephalus of variable severity is possible.

**Other inflammatory intracranial diseases**

Other inflammatory brain diseases are rare and include Greyhound nonsuppurative meningoencephalitis, idiopathic eosinophilic meningitis/meningoencephalitis, cerebral extension of steroid-responsive meningitis arteritis and others.
Keywords: MRI Physics, Spin Echo Sequences, Gradient Echo Sequences, Weighting

References
MRI of Congenital, Degenerative and Metabolic Encephalopathies

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Summary
Congenital brain abnormalities are more common in small animals than traditionally believed. Some are well documented in the literature (e.g., hydrocephalus, intracranial intra-arachnoid diverticula and craniocervical junction abnormalities); reports of some other conditions are scarce. While young age at presentation should alert the clinician to the possibility of a congenital anomaly, some animals do not develop neurologic signs until adult age. Furthermore, a congenital anomaly may not be associated with significant clinical signs, and in some instances a diagnosis is made as an incidental finding when imaging the patient for different reasons. Familiarity with normal brain anatomy and variations is crucial in the imaging diagnosis of some conditions as they may only be associated with minor imaging abnormalities. A wide and heterogeneous range of diseases falls under the category of degenerative and metabolic brain diseases. While imaging findings in different disorders vary, bilaterally symmetric signal intensity changes to deep grey matter nuclei with or without abnormal contrast enhancement, symmetric and diffuse signal intensity changes to grey and/or (subcortical) white matter, and/or brain atrophy are common and, if identified, should alert the clinician to the possibility of a metabolic, nutritional or degenerative encephalopathy.

Congenital encephalopathies (1-2)
- **Congenital hydrocephalus** is predominantly seen in toy and brachycephalic breed dogs. Common MRI findings include ventricular enlargement and thinning of the cerebral cortices. Less common MRI findings may include absence of the septum pellucidum, dilation of the olfactory recesses of the lateral ventricles and the infundibular recess, atrophy of the interthalamic adhesion, periventricular edema, periventricular diverticula, tears and clefts which may be associated with parenchymal hemorrhage, empty sella (herniation of the subarachnoid space into the sella turcica), CSF signal void in mesencephalic aqueduct on T2-W images attributed to high velocity or turbulent CSF flow, subtentorial herniation of a dilated ventricle, enlargement of the calvarium +/- open fontanelles.
- In **hydranencephaly**, there is near complete destruction and/or lack of development of the neocortex due to a destructive process occurring in utero. MRI findings include reduction of size of the cerebral cortex to a thin mantle surrounding a large fluid-filled cavity contiguous with the lateral ventricle. In **porencephaly**, cystic cavities are present in the cerebrum due to cell destruction or failure of development. MRI findings include cerebral cavities of variable size with MRI signal identical to CSF.
- **Intracranial intra-arachnoid diverticula** ("cysts") arise from splitting/duplication of the arachnoid and occur in close association with the intracranial arachnoid cisterns. MRI findings include sharply demarcated lesions dorsal to the quadrigeminal plate (= supracollicular fluid collections), at the cerebellomedullary/cerebellopontine angle (= cerebellomedullary/cerebellopontine angle arachnoid cyst/diverticulum) or associated with the 4th ventricle (= 4th ventricle arachnoid diverticulum). Lesions contain fluid isointense to CSF. Dependent on size and location of the lesion, flattening, compression
or displacement of adjacent structures may be noted.

- **Intracranial epidermoid cysts (syn. cholesteatomata) and dermoid cysts** are benign slow-growing space-occupying lesions that are caused by a failure of neural tube closure when epithelial ectoderm becomes entrapped within nervous tissue and forms a slowly expanding cystic mass. MRI findings include a mass of variable size in the caudal fossa, most commonly within the 4th ventricle. Signal intensity is variable and dependent on cyst content. The mass typically sits on midline between the brainstem and the cerebellum and causes variable degrees of compression of these structures.

- **Other intracranial cystic lesions** may have congenital etiology and are of variable clinical significance.

- Protrusion of meninges alone or meninges along with brain tissue through a calvarial defect are termed **meningocele and meningoencephalocele**, respectively. Superficial meningoencephaloceles may be diagnosed clinically. Diagnosis of basal or ethmoidal (nasal) meningoencephaloceles requires advanced imaging. MRI findings include a calvarial defect with herniation of meninges +/- brain tissue in cases of superficial encephalocele, cribriform plate defect with herniation of meninges +/- brain tissue in cases of nasal/ethmoidal encephalocele, and possibly concurrent facial/calvarial deformity.

- **Holoprosencephaly (HPE)** is a failure of the forebrain to sufficiently divide into two hemispheres, and is characterized by absent or smaller midline prosencephalic structures, incomplete separation of normally paired forebrain structures, and hydrocephalus. Dependent on severity, HPE can be subdivided into alobar, semilobar and lobar HPE. In lobar HPE only the most rostral and ventral portions of the cerebral hemispheres are fused. **Agenesis or dysgenesis of the corpus callosum** is a feature of HPE but may also occur as an isolated abnormality. MRI findings include agenesis or dysgenesis of the corpus callosum, fusion of the ventral frontal lobes, cingulate gyri, lateral ventricles and part of the diencephalon with associated loss of some normal midline structures, and unusually upturned, pointed corners of the lateral ventricles.

- **Lissencephaly** is a disorder of cortical neuronal migration is characterized by paucity, absence and/or hypoplasia of cerebral gyri (pachygyria) and thickening of the cerebral cortex. The disease has been reported in dogs and cats and it appears to be hereditary in Lhasa Apsos. MRI findings include a smooth cerebral surface and a thick neocortex with absence of the corona radiata.

- **Cerebellar aplasia/agenesis** is defined as complete absence of cerebellar tissue. **Cerebellar hypoplasia** is characterized by uniform paucity of cerebellar tissue. In kittens this is most commonly observed following in utero infection with the panleukopenia virus. Although an association with parvovirus infection has been proposed, the condition is most likely inherited in dogs. MRI findings include an absent or abnormally small cerebellum with CSF filling the space normally occupied by cerebellar parenchyma.

- **Dandy-Walker malformation complex**: In dogs this term is used for cases of cerebellar vermian aplasia or hypoplasia with or without associated cystic dilation of the fourth ventricle. Eurasier dogs are genetically predisposed. Common MRI findings include hypoplasia/agenesis of the cerebellar vermis, cyst-like dilation of the 4th ventricle, and enlargement of the caudal fossa.

- A disorder similar to **Chiari type I malformation** in humans has been reported in dogs and is characterized by a relatively small size of the caudal fossa and resultant overcrowding of the neural structures. MRI features of the condition are typically best appreciated on T2-W sagittal images, and include crowding of the caudal fossa with indentation of the caudal margin of the cerebellum by the occipital bone, attenuation of the subarachnoid space caudal to the cerebellum and at the foramen magnum (impaction), herniation of the cerebellar vermis into or through the foramen magnum, which is exacerbated by neck flexion; “kinking” of the medulla or cranial cervical spinal cord, secondary syringomyelia, and less commonly, concurrent ventriculomegaly and/or occipital dysplasia manifesting as widening and abnormal shape of the foramen magnum.

- Other congenital disorders for which MRI findings in animals have been reported include **polymicrogyria**, **focal cortical dysplasia (FCD)** and **Dyke-Davidoff-Masson-like Syndrome (DDMS)**.
Normal aging changes and changes in animals with cognitive dysfunction syndrome (3)

Physiological and pathological changes occurring in the brains of aging dogs and dogs with certain storage diseases are similar to changes observed in humans, and the dog has been studied as a model for normal human brain aging as well as for pathological changes with age ranging from mild cognitive impairment to Alzheimer’s disease/dementia. Although neurobiological changes and imaging findings tend to be more severe in animals with cognitive dysfunction, there is considerable overlap with “normal” older pets. MRI findings in normal aging dogs and dogs with cognitive dysfunction include cerebral atrophy (widening of the cerebral sulci and ventriculomegaly), small size of the interthalamic adhesion, white matter abnormalities (regional bilateral decline in volume, spontaneous development of T2 white matter hyperintensities particularly adjacent to the lateral ventricles attributed to perivascular demyelination and axonal loss), focal or multifocal lesions related to vascular damage (lacunar infarcts commonly affecting the caudate nucleus and thalami and microbleeds (silent cerebral hemorrhages)). Although there is also clear evidence of age-associated brain pathology in cats, reports on related imaging changes are scarce. MRI findings include cerebral atrophy (decrease in both grey and white matter volume) with increased ventricular size and widening of sulci, small multifocal areas of decreased signal intensity on T1-W images, predominantly in the piriform lobes, and magnetic resonance spectroscopy alterations.

Metabolic, Nutritional and Degenerative Encephalopathies (4)

- **Lysosomal storage diseases (LSDs)** comprise a wide variety of abnormalities which are characterized by the intracellular accumulation of one or more products of an interrupted metabolic pathway. For several of these conditions only individual case reports or findings in research animals are reported. *Globoid cell leukodystrophy* and *neuronal ceroid lipofuscinosis* are well documented in animals, however. *Globoid cell leukodystrophy* is caused by mutations in the gene for galactocerebrosidase and has been reported in a variety of dog breeds and cats. MRI findings include changes consistent with diffuse, symmetrical white matter disease, mild hydrocephalus, symmetric contrast enhancement of the corpus callosum, internal capsule, and corona radiata, and hypointensity of white matter tracts on magnetization transfer-weighted images consistent with demyelination. *Neuronal ceroid lipofuscinosis (NCL)* is characterized by the abnormal accumulation of lipoprotein pigment within cellular lysosomes. The disease has been reported in cats and a variety of dog breeds and has been studied in canine models of human NCLs and aging. MRI findings include widening of the cerebral sulci and cerebellar fissures and ventriculomegaly indicative of brain atrophy, and an abnormally small corpus callosum.

- **L-2-Hydroxyglutaric Aciduria** is an autosomal recessive inborn error of metabolism caused by failure to break down L-2-hydroxyglutaric acid, causing levels to rise in urine, plasma and CSF. MRI findings include bilaterally symmetric grey matter abnormalities (poloencephalopathy) affecting the cerebrum, cerebellum, diencephalon, mesencephalon and metencephalon (abnormal swelling, T2 hyperintensity and T1 isointensity or mild hypointensity of grey matter with no contrast enhancement, symmetric changes to grey matter nuclei, T2 hyperintensity of peripheral subcortical white matter.

- **Mitochondrial encephalopathies** resembling subacute necrotizing encephalomyelopathy (Leigh syndrome) in humans have been reported in a variety of dog breeds. MRI findings include bilaterally symmetric abnormalities (T2 hyperintensity and T1 iso- or hypointensity without evidence of contrast enhancement) of various brain and brainstem nuclei, symmetric spinal cord lesion(s) affecting the grey matter (same signal characteristics as brain lesions).

- **Thiamine deficiency** results in insufficient ATP production in the brain with subsequent neuronal dysfunction. In addition to the possibility of a deficiency in commercial pet foods, a deficiency may arise in dogs and cats with medical conditions. MRI findings include bilaterally symmetric multifocal abnormalities affecting nuclei of brain and brainstem.

- **Failure of the liver to remove toxic substances absorbed from the gastrointestinal tract may result in hepatic encephalopathy.** Portosystemic shunts are the most common cause in veterinary patients. Brain atrophy is the most common MRI finding. Less common findings include bilaterally symmetric T1
hyperintensity to the lentiform nuclei attributed to increased concentration of manganese, and bilateral extensive T2 hyperintense lesions along the cerebral cortex.

- **Myelinolysis/Osmotic Demyelination Syndrome** is typically caused by rapid correction of chronic hyponatremia and is most commonly seen in human patients with electrolyte derangements (alcoholics, liver transplant recipients, and patients taking diuretics). MRI findings include bilaterally symmetric T2/T2-FLAIR hyperintense non-enhancing lesions within the thalamus, caudate nuclei, and along the cerebrocortical grey-white matter junction.

- A series of progressive neurological diseases have been summarized under the nondescript term “spongy degeneration”. These conditions are primarily, but not exclusively, diseases of white matter, may be hereditary, and may result in a variety of clinical manifestations. MRI findings include large, ovoid, bilaterally symmetric T2 hyperintense and T1 hypointense non-enhancing lesions in the region of the deep cerebellar nuclei and smaller lesions within the thalamus ventromedial to the lateral ventricles.

- **Cerebellar Cortical Abiotrophy (Cerebellar Cortical Degeneration)** is characterized by degeneration of initially normal neuronal cell populations within the cerebellar cortex after birth and can be considered a subcategory in the complex group of “canine hereditary ataxias”. MRI findings include small size of the cerebellum indicated by increase in fluid separating the folia.

- Other metabolic and nutritional disorders for which MRI findings in animals have been reported include *kernicterus*, hypoglycemic encephalopathy, hypocobalaminemic encephalopathy, neuroaxonal dystrophies and idiopathic superficial neocortical degeneration and atrophy in young adult dogs.

**Keywords:** Congenital Encephalopathies, Metabolic Encephalopathies, MRI, Dog, Cat

**References**


(Original references available upon request)
Imaging of Canine and Feline Dental and Masticatory Diseases

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Summary
Dogs and cats can suffer from problems associated with closing or opening their mouth, pain on mastication and other problems such as halitosis. Diagnostic imaging is an important part of the diagnostic workup of masticatory conditions. Radiography and with increasing frequency computed tomography are the imaging modalities of choice. Intraoral radiography and computed tomography are the primary diagnostic imaging modalities for masticatory and dental disorders. Intraoral radiography offers the maximum image resolution that is often required for specific dental assessments. Computed tomography also provides very good image detail and in addition enables assessment of the entire masticatory apparatus.

Introduction
Dogs and cats can suffer from problems associated with closing or opening their mouth, pain on mastication and other problems such as halitosis. Diagnostic imaging is an important part of the diagnostic workup of masticatory conditions. Radiography and with increasing frequency computed tomography are the imaging modalities of choice.

General Radiography
Radiography of the head has traditionally been used to assess the dental apparatus, mandible, maxilla, mandibular symphysis and temporomandibular joint. Standard dorsoventral and lateral projections of the head supply some information, but suffer from superimposition of bilateral skull structures. Oblique projections for each mandible and each maxilla with the mouth maximally opened are necessary to assess all four dental arcades. For the temporomandibular joint numerous specific oblique projections have been published, which are different for the cat and the dog. These views are often difficult to accomplish and are not ideal for complete assessment. The masticatory muscles and nerves are also not assessed with radiography. The use of digital radiography has facilitated better radiographic exposure, but has not remedied the positional difficulties.

Intraoral Radiography
Intraoral radiography is essential for dedicated veterinary dentists. Modern computed or direct digital systems provide devices that are small enough to be placed intraorally in dogs and cats. Dental radiography systems provide the highest resolution of any radiographic technique, which is necessary for detailed assessment of all dental and periodontal structures. Multiple views are needed to cover the entire dental arcade. Intraoral radiography can be used to image the temporomandibular joints.

Computed Tomography
Computed tomography is an ideally suited imaging modality for dogs and cats with masticatory problems. Modern computed tomography technology allows submillimeter slice thickness images, providing excellent
anatomical detail of teeth, bones and joints. It also allows assessment of masticatory muscles and nervous system. The entire dental apparatus can be scanned within less than one minute. Images can be reviewed in transverse or orthogonal planes. Curvilinear reconstructions allow a panoramic view of all teeth. Given the fact that general anaesthesia is required for all imaging modalities and that computed tomography is the fastest imaging modality assessing the entire head, it makes sense to use computed tomography as the first line diagnostic imaging tool for all masticatory conditions, except for specific localised dental diseases.

**Imaging Features of Dental and Masticatory Disease Processes**

**Head Trauma:** Radiography allows a relatively quick assessment of the skull, but not the soft tissue structure of the head. Proper positioning and interpretation can be time consuming. Computed tomography is the modality of choice for assessment of head trauma. It is quick and very detailed for all osseous, dental and soft tissue structures. Temporomandibular joint luxation can be monitored pre- and post-reduction. Intravenous contrast medium application is usually not necessary.

**Paraoral Foreign Bodies and Abscessation:** Computed tomography is the modality of choice for suspected foreign bodies, abscessation and wounds of the oral cavity wall, masticatory muscles and retropharyngeal space. Intravenous contrast medium application is essential to outline abscess walls and foreign body reaction. Small foreign bodies from plant material may not be visible directly, however the reactive tissue surrounding it usually is.

**Craniomandibular osteopathy:** This is an inflammatory condition in young West Highland White terriers and less commonly other dogs leading to pain and reduced ability to open their mouth, associated with mandibular and temporal periosteal reaction. The condition is self-limiting and the role of imaging is to rule out other diseases and monitor its progression. Radiography is usually sufficient.

**Masticatory Myositis:** This is an autoimmune disease in young to middle aged large breed dogs against the 2M fibres of the masticatory muscles leading to pain on mastication and later inability to open their mouth and muscle atrophy. Computed tomography is the imaging modality of choice, allowing to rule out other dental and masticatory problems, identify abnormal masticatory muscles based on size, shape, density and contrast enhancement, and to select appropriate sites for a muscle biopsy, which is required for ultimate confirmation of this condition.

**Oral and Masticatory Neoplasia:** Oral neoplasia is relatively common in dogs and cats. Radiography allows only a crude assessment of bony involvement. Computed tomography is best suited to assess the tumour margination, bony involvement, local (lymph nodes) and distal (lung) metastases. It is also required for all radiotherapy cases.

**Temporomandibular Joint Diseases:** Computed tomography is best suited for assessment of the temporomandibular joints. A closed and a maximally opened jaw view should be obtained. Subluxation and luxation are easily visible. In cats a chronic irregular ankylosis can sometimes be seen. Irregular joint surfaces are often visible in dogs. These can be caused by osteoarthritis and osteochondrosis. Neoplastic conditions show often marked osteolysis and swelling.

**Open Jaw Lock:** Dogs and cats with a locked open jaw have a displacement of one coronoid process lateral to the zygomatic arch. This can be demonstrated radiographically quickly and easily. However, to establish the exact cause of open jaw lock, computed tomography is highly efficient. This can demonstrate mandibular symphyseal laxity, temporomandibular joint laxity or abnormal shape of the mandible or zygomatic arch and muscular changes.

**Jaw drop:** Dogs that cannot actively close their mouth, but the jaw can be closed by the examiner usually suffer from idiopathic trigeminal neuritis. Other differentials to consider are neoplasia or infection of the central nervous system. Magnetic resonance imaging is best suited to rule out central nervous system conditions.

**Dental Diseases:** Intraoral radiography has traditionally been the modality of choice for diagnostic imaging of dental diseases in dogs and cats. It allows detailed assessment of individual teeth and allowing specific treatment decisions. Computed tomography has become a useful adjunct to intraoral radiography, as
it provides an almost as detailed depiction of the entire dental apparatus.

**Conclusions**

Intraoral radiography and computed tomography are the primary diagnostic imaging modalities for masticatory and dental disorders. Intraoral radiography offers the maximum image resolution that is often required for specific dental assessments. Computed tomography also provides very good image detail and in addition enables assessment of the entire masticatory apparatus.

**Keywords:** Dog, Cat, Teeth, Computed Tomography
Imaging of Feline and Canine Mycobacterial Disease: An Emerging Challenge for Veterinarians

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Summary

Tuberculous mycobacteria such as M. bovis and M. microti, as well as non-tuberculous mycobacteria such as M. avium, are increasingly recognized as pathologic agents in cats. Less commonly mycobacteriosis is observed in dogs. This is a worldwide phenomenon, and is a particular concern in the British Isles. The route of infection determines the initial manifestation of disease such as ingestion for alimentary disease, inhalation for respiratory disease and fight-and-bite injuries for cutaneous disease. Haematogenous dissemination of disease can then lead to a variety of clinical signs including dyspnoea, and non-specific signs such as weight loss and anorexia. Radiography is an efficient general screening tool but computed tomography allows a more comprehensive and detailed assessment of the disease process and treatment monitoring. Most radiographic and computed tomographic changes represent multi-systemic disease, with pulmonary infiltration, lymphadenomegaly and organomegaly which are also seen in other feline diseases, such as neoplasia (lymphoma or mast cell disease), chronic inflammation/infectious processes (feline infectious peritonitis or systemic mycosis), hypereosinophilic syndrome and amyloidosis. Mycobacteriosis should be considered as a differential diagnosis in such cases. In patients with diagnosed mycobacteriosis the potential for widespread clinical and sub-clinical abnormalities must be considered and investigated in full.

Introduction:

Mycobacteria of importance to companion animals, i.e. cats and dogs, include i) obligate pathogens which can cause tuberculosis (TB), and ii) non-tuberculous mycobacteriosis (NTM), these can be divided into iia) facultative pathogenic opportunistic saprophytes that can be grown in laboratory, and iib) a subgroup of NTMs that are difficult to grow, so their environmental niche cannot be determined - feline leprosy syndrome (FLS) and canine leproid granuloma syndrome (CLGS). Regardless of which mycobacteria are involved, most cats present with cutaneous disease which can sometimes progress to pulmonary or systemic disease; only occasional cases present with primary systemic disease. In comparison, most canine cases of TB have disseminated disease at the time of diagnosis, while CLGS are cutaneous.

Diagnostic Tests:

A confirmation of mycobacterial is done histologically with a Ziehl-Neelsen stain. However, for further speciation interferon gamma, molecular PCR or culture are required. However cultivation is very slow at best (minimum of 3 months). In the UK and most countries some types of mycobacteriosis are a notifiable disease.

Radiography and Computed Tomographic Features:

In cats, radiographic changes are most commonly affecting the thorax, with bronchial, alveolar or nodular interstitial lung patterns, frequently mixed. Perihilar and sternal lymphadenopathy is commonly seen. Skeletal
changes are less common and are typically osteolytic. Occasional cases show soft tissue calcification, hepatomegaly or hepatosplenomegaly. Computed tomographic abnormalities with feline mycobacteriosis are most commonly seen in the thorax, consisting of bronchial, alveolar, ground glass or structured interstitial lung patterns as well as lymphadenomegaly. Less commonly seen are abdominal or peripheral lymphadenomegaly, hepatosplenomegaly, mixed osteolytic/osteoproliferative skeletal lesions, and cutaneous or subcutaneous masses. In dogs, mycobacterial disease is much more rarely diagnosed and therefore detailed imaging studies on contemporary infection are not available. In the few cases the authors have seen, there were extensive osteolytic and proliferative bone lesions.

**Imaging-based Treatment Control:**
Computed tomography in consciously restrained cats is a very useful CT studies can aid in decision making regarding tapering of antibiotic protocols, or reintroduction of therapy in cases of recurrence or reinfection. In some cases, persistent abnormalities can be detected by CT and therefore resolution of CT pathology should not be a goal in the management of feline tuberculosis.

**Conclusions:**
Most radiographic and computed tomographic changes in feline mycobacteriosis represent multi-systemic disease, with pulmonary infiltration, lymphadenomegaly and organomegaly which are also seen in other feline diseases, such as neoplasia (lymphoma or mast cell disease), chronic inflammation/infectious processes (feline infectious peritonitis or systemic mycosis), hypereosinophilic syndrome and amyloidosis. Mycobacteriosis should be considered as a differential diagnosis in such cases. In patients with diagnosed mycobacteriosis the potential for widespread clinical and sub-clinical abnormalities must be considered and investigated in full. Radiography is an efficient general screening tool but computed tomography allows a more comprehensive and detailed assessment of the disease process and treatment monitoring.
MRI of the Spine – An Update

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Summary
Spinal MRI is commonly performed in veterinary patients and is considered the gold standard for most indications. Multiple new MRI sequences and techniques have been developed in recent years. Unfortunately, studies comparing diagnostic value of these different sequences when imaging the canine and feline spine are scarce, and a recommended “standard MRI protocol” as customary in human radiology has to date not been published. The purpose of this presentation is to give an overview over MRI sequences used in spinal imaging, present case examples of more or less common diseases, discuss recent developments, and introduce open questions and topics for discussion.

MRI sequences for spinal imaging
According to the American College of Radiology Practice Guidelines (1) the minimum recommended sequences for evaluating the spine in human patients include sagittal T1-W, sagittal T2-W and T2*-W, and axial (transverse) T1-W, T2-W and/or T2*-W sequences. Dependent on indication and clinical suspicion other recommended sequences include STIR, fat-suppressed T2-W FSE (or other fat suppressed acquisitions), and post contrast T1-W images +/- fat suppression. A review paper discussing “Optimal MRI of the Spine” in veterinary patients (2) suggests the following protocol: Dorsal T2-W; sagittal T2-W; transverse T2-W through any suspected abnormalities or according to neurolocalization; pre and post contrast T1-W (+ FatSat) and/or GRE on a case-by-case basis and based on initial findings; and dorsal STIR to look for paraspinal soft-tissue pathology in patients with back pain and no visible spinal lesions. Other publications support acquisition of transverse imaged throughout the area of interest (3), sagittal STIR (4), sagittal single shot techniques (5-7), fat suppressed post contrast T1-W images (8), and T2*-W images (9). In recent years, multiple additional techniques and sequences have been developed (e.g. diffusion/perfusion techniques, functional MRI, magnetic resonance angiography, spectroscopy) the majority of which are more commonly used for brain than spinal imaging. Diffusion tensor imaging (DTI) is a specialized diffusion technique which utilizes strong multidirectional gradients to map white matter tracts. Initial studies proved feasibility of this technique when imaging the spine in dogs (10, 11). This technique may ultimately aid in the diagnosis of white matter disease and facilitate surgical planning.
Figure 1  MR spin echo images of a dog with a spinal tumor (sarcoma)

Figure 2  T2-W vs. STIR images in a dog with multifocal bone lesions

Figure 3  T2-W vs. single shot ("HASTE") images in 2 dogs demonstrating intervertebral disc herniation (left) and subarachnoid diverticulum (right), respectively
Figure 4 Hematoma within the spinal cord in a dog. Note hypointense appearance of the lesion on T2*-W image due to susceptibility artifact (bottom right).

Controversial topics and open clinical questions
Considering the plethora of MR sequences available and the lack of a standard protocol the following questions will need to be answered:
1. Which sequences are really needed to achieve a diagnosis in a given patient?
2. How much time should an MRI examination of the spine take?
3. Do we give too much contrast medium, and are there any adverse effects we should be aware of?
4. What criteria do we implement in deciding if there is spinal cord compression or not, and which sequence(s) do we most rely on? In a dog with multiple intervertebral disc herniations, how do we decide which is the most important?

Keywords: Spine, Vertebral Column, Intervertebral Disc Disease, Dog

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MRI and CT of the Brain – Are These Lesions Significant?

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Summary
When evaluating imaging studies of the canine and feline brain it is important to be familiar with common artifacts, anatomic variants and incidental findings and to distinguish those from significant lesions. This presentation will cover CT artifacts and pseudolesions, MRI artifacts and pseudolesions, and give an overview over incidental findings/variants and how to identify potentially significant abnormalities. In any case, it is important to remember that the diagnosis of a serious brain lesion is not made based solely on imaging. Confidence that a lesion found on CT or MRI is indeed responsible for an animal’s clinical signs depends on the degree of correlation of this lesion with the results of a thorough neurologic examination and additional testing (e.g., CSF tap).

Introduction
Advanced imaging of the head (Computed Tomography, CT; Magnetic Resonance Imaging, MRI) is increasingly performed in veterinary patients. Regardless if the animal is actually presented for central nervous system signs, or if the brain is included in the scan field of view when imaging other anatomic areas (nose, tympanic bulla or orbit), it is important to be familiar with common artifacts, anatomic variants and incidental findings, and to distinguish those from significant brain lesions.

Important artifacts and pseudolesions in brain imaging
- **CT artifacts and pseudolesions (1):** Choice of a high detail reconstruction algorithm for bone imaging (e.g. when scanning the tympanic bullae) results in a very grainy appearance of soft tissue structures and may mimic multifocal punctate brain lesions. Partial volume averaging results from inclusion of structures with different density (e.g. bone and soft tissue) in a single slice, creating areas of artificially high or low attenuation and possibly pseudolesions. Beam hardening artifact occurs adjacent to the highly attenuating temporal bones and results in a hypoattenuating bar over the brainstem and cerebellum. Motion of the patient results in streaking, blurring or ghost images. The dorsum sellae in cats is prominent and may mimic a small pituitary mass.
Figure 1 CT images of the brain in a dog. While a high detail algorithm is appropriate to evaluate bony structures (left) it is inadequate for the evaluation of soft tissue (right).

- **MRI artifacts and pseudolesions (2):** Motion artifacts may occur secondary to patient movement or physiologic movement (e.g. pulsating blood). They manifest as “ghosts” of the moving structure, blurring and/or parallel bands. A flow void artefact appears as artificial loss of signal from cerebrospinal fluid on T2-W images and is attributed to rapid or turbulent flow of CSF (3). Presence of materials with differing magnetic properties in the field of view results in susceptibility artifacts (4, 5). These may be beneficial (e.g. in the identification of haemorrhage) or may interfere with interpretation (e.g. adjacent to gas filled frontal sinuses or in the vicinity of metal). Partial volume averaging occurs when materials of different intensity are included in the same slice thickness and their intensities are averaged. As a result, hyperintensities adjacent to fluid filled structures (subarachnoid space, ventricles) on T2-W images resulting from averaging of brain and CSF signal may be misinterpreted as parenchymal lesions. The petrous temporal bone contains a variable amount of fat which may be misinterpreted as a lesion. Additional normal findings that may be confused with pathologic lesions include variable hyperintensity of the neurohypophysis on T1-W images and physiologic contrast enhancement of trigeminal nerves, choroid plexus and pituitary gland.

Figure 2 Transverse T2-W image of a cat head. The “ghosting” of the eye balls is caused by motion.

**Incidental findings and variants vs. clinically significant brain lesions**

Intracranial anatomy is fairly similar across domestic feline breeds. However, marked differences exist in brain anatomy between dog breeds, most notably in the rostral fossa. Olfactory bulb angle and orientation, cribriform plate and ethmoid turbinate position differ between dolichocephalic, mesaticephalic and...
dolichocephalic dogs. The normal neonatal brain exhibits differences from that of the mature brain (reverse relative gray matter to white matter signal intensity, lack of cerebral sulci). Prominence of the cerebral sulci, mild ventriculomegaly, and a decrease in size of the interthalamic adhesion occur with age related brain atrophy which may or may not result in significant clinical signs. A mild to moderate degree of ventricular dilation (hydrocephalus) is common in small breed dogs and may not be associated with clinical signs. Similarly, mild crowding of the caudal fossa with flattening of the caudal cerebellum is seen in some small breed dogs without evidence of associated clinical signs or secondary syringomyelia. Especially in small breed dogs a mid-sagittal fluid pocket caudal to the pituitary gland may be noted. This is believed to represent a prominent suprasellar cistern and a variation of normal. Intracranial arachnoid diverticula, especially supracollicular, can reach considerable size without causing clinical signs. Similarly, fluid replacement of absent brain parenchyma (porencephaly) may be clinically silent.

Most brain parenchymal lesions are considered clinically significant. Possible exceptions include punctate susceptibility artefacts within brain parenchyma ("microbleeds" or "silent cerebral hemorrhages") and mild bilaterally symmetric intensity changes to the cerebral white matter which are believed to be a component of (age related) demyelination. Other parenchymal lesions including focal or multifocal masses, diffuse infiltration and brain swelling are indicative of serious brain disease and warrant further evaluation (e.g., CSF tap). Contrast enhancement of structures other than physiologically enhancing tissues (vessels, pituitary gland, choroid plexus, trigeminal nerves and to some degree meninges) is abnormal and consistent with a lesion that either originated outside the blood brain barrier or has resulted in its' disruption. Parenchymal periventricular hyperintensity in animals with ventriculomegaly is abnormal and may allow differentiation of insignificant and significant hydrocephalus. Edema along the cerebral white matter tracts, mass effect and brain herniation may be observed as secondary signs of serious intracranial lesions. Ultimately, the decision if a brain lesion is significant will not be based solely on imaging but will include neurologic examination findings and results of other testing (6).

Figure 3 Probably incidental (left, chronic infarct) and clinically significant (right, glioma) brain lesions in 2 different dogs.

Keywords: CT, MRI, Artifact, Pseudolesion, Anatomic Variant

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Gastritis and Gastric Ulceration in Small Animals – How Should We Treat?

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Summary

Gastric disease in dogs and cats can either occur in isolation or can be associated with small intestinal disease (e.g., inflammatory bowel disease). The two most common gastric diseases in dogs and cats are gastritis and gastric ulceration. Gastritis may have many different causes, such as Physaloptera rara infestation or Helicobacter spp. infection, but in many cases the etiology remains unknown. Gastric ulceration is often iatrogenic, but regardless of etiology, treatment involves gastric acid suppression and sucralfate. The most effective gastric acid suppression is by administration of a proton pump inhibitor given twice daily.

Introduction

Gastric disease is common in both dogs and cats and can be challenging to diagnose. Some patients have isolated gastric disease, such as gastric ulceration, gastritis, or gastric neoplasia, while other patients have inflammatory infiltration of the gastrointestinal mucosa that involves the stomach and, most commonly, the small intestine.

Gastritis

Gastritis is an inflammation of the gastric mucosa and can have many causes, some of which can be identified, such as Physaloptera rara infestation or gastric adenocarcinoma. However, in most cases the underlying cause of the inflammatory infiltration remains unclear and gastritis is assumed to be a component of idiopathic inflammatory bowel disease. As such, the management is similar to that of IBD, including dietary trials and antiinflammatory agents. In addition, antacids may also be useful in IBD patients with a significant gastritis component.

Physaloptera Rara Infestation

Physaloptera rara is the stomach worm of the dog, but can occasionally also be found in cats. Diagnosis is rarely made by fecal examination; more commonly the diagnosis is made based on seeing the worm endoscopically or by empirically treating a patient with chronic vomiting with pyrantel pamoate (in dogs: 15 mg/kg PO; repeat in 2-3 weeks), which should be a routine measure before commencing the work-up with more costly and invasive diagnostic tools, and resolution of clinical signs.

Helicobacter-Like Organisms

It is questionable whether infections with Helicobacter-like organisms can be responsible for isolated cases of chronic gastritis as most healthy dogs and cats harbor some of these bacteria in the gastric mucosa. However, when chronic gastritis is not responsive to therapy and organisms have been identified, a treatment
trial maybe indicated. Therapeutic regimens aimed at eradicating Helicobacter-like species usually include two antibiotics (e.g., metronidazole, amoxicillin, clarithromycin, or azithromycin) and either a third antibiotic, an antacid (e.g., famotidine or omeprazole), or bismuth subsalicylate.

**Gastric Ulceration**

Gastric erosions and ulcers both describe a damaged gastric mucosa. However, ulcers are deeper than erosions and by definition describe damage that reaches the lamina muscularis mucosae or deeper layers. There are a wide variety of causes of gastric ulcers, including iatrogenic (e.g., nonsteroidal antinflammatory drugs (NSAIDs) or corticosteroids), infiltrative disease (e.g., gastric neoplasia, inflammatory bowel disease), chemical toxins, gastric foreign bodies, or secondary causes of gastric disease (e.g., gastrinoma, mast cell tumors, renal failure, pancreatitis, liver disease, hypovolemia, and others).

In healthy animals the stomach is protected from gastric ulceration by several protective mechanisms, including bicarbonate-rich gastric mucous, mucosal microcirculation (mediated through the action of prostaglandins), and epithelial turnover. Only when these protective mechanisms are being overwhelmed do gastric ulcers develop.

A variety of medications can be used for the treatment of gastric ulcers. However, the first goal of therapy should be to treat the underlying cause. The most common cause of gastric ulcers is the use of ulcerogenic drugs. Thus, patients suspected of having a gastric ulcer should not receive any ulcerogenic medications. Also, any systemic complications must be addressed, such as dehydration or electrolyte and acid/base disturbances. Also, gastric ulcers can bleed and lead to loss of large volumes of blood that must be replaced through blood transfusions. In some patients ulcers can perforate, which constitutes a surgical emergency.

**Buffering agents**

Calcium carbonate, sodium bicarbonate, magnesium hydroxide, or aluminum hydroxide are all buffering agents that are aimed at buffering gastric acid directly. It has been suggested that in addition to their buffering capacity, these agents decrease pepsin activity and increase endogenous prostaglandin synthesis. However, these agents need to be dosed frequently and are poorly palatable, making these agents less desirable for the treatment of gastric ulcers.

**H₂-receptor antagonists**

Gastric acid secretion is stimulated by three types of receptors: gastrin receptors, acetylcholine receptors, and H₂-receptors. Thus, antagonism to H₂-receptor stimulation decreases gastric acid secretion, but not completely. Traditional H₂-antagonists, such as cimetidine and ranitidine, have been largely replaced by more modern drugs, such as famotidine (0.5-1.0 mg/kg PO or IV q 12-24 hrs) or nizatidine (5.0 mg/kg PO q 24 hrs). All of these medications may provide some degree of gastric acid suppression and have been successfully used in both dogs and cats with gastritis or gastric ulcerations. However, more recent studies assessing gastric pH after use of H₂-receptor antagonists have shown no to minimal efficacy in raising gastric pH in dogs.

**Proton-pump inhibitors**

The proton-pump (H⁺/K⁺-ATPase) is the central mechanism for all gastric acid secretion, regardless of the receptor that has been stimulated to activate gastric acid release. Thus, proton-pump inhibitors very effectively block gastric acid secretion. Omeprazole is the most widely used proton-pump inhibitor in both dogs and cats and is used at a dose of 0.7 mg/kg q 12-24 hrs. A recent study suggested that in order to maintain gastric acid blockage, omeprazole had to be administered twice a day. In human medicine and a wide-variety of other proton-pump inhibitors, such as pantoprazole or lansoprazole, but these agents have only been used in isolated cases in veterinary medicine and there is limited clinical experience with these drugs. The advantage of pantoprazole is that it can be administered intravenously at 0.7-1.0 mg/kg
q 12-24 hrs, while omeprazole is only available as an oral formulation. Long-term use of proton-pump inhibitors can lead to dramatic hypergastrinemia, which can lead to excessive release of gastric acid when the drug is suddenly discontinued. Thus, a decrease in dosage may be prudent before stopping a proton-pump inhibitor after even short-term use of this medication.

Sucralfate

Sucralfate is considered a gastromucosal protectant. After oral administration, the drug dissociates into aluminium hydroxide and sucrose octasulfate, the main active ingredient of sucralfate. When exposed to gastric acid, sucrose octasulfate forms a paste that adheres specifically to damaged tissue. In general, sucralfate cannot be activated in a neutral environment. However, some studies have shown that sucralfate can be given together with antacids without loss of function. More importantly, sucralfate will inhibit the absorption of many other medications so that sucralfate should be administered at least two hours apart from other medications.

Misoprostol

Misoprostol is a synthetic prostaglandin E₁ analog. Since it is a hormone, prostaglandin first has to be absorbed from the gastrointestinal mucosa before it can have any activity. Misoprostol stimulates gastric mucosal perfusion and mucous production. It is unclear whether misoprostol is effective in treating existing ulcers, but there is good evidence that misoprostol is highly effective at preventing gastric ulceration in patients receiving NSAIDs or glucocorticoids. It should be noted that in some countries misoprostol has been legally used for inducing abortions. Thus, owners must be informed of the dangers of inadvertent exposure with this medication and the medication should only be handled with gloves when handled by women in a child-bearing age.

Keywords: Gastritis, Gastric Ulceration, Proton Pump Inhibitor, Sucralfate
Diagnosing Pancreatitis in Dogs and Cats – Anything New?

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Summary
Pancreatitis is common in both dogs and cats, but remains challenging to diagnose because many patients do not present with classical clinical signs, but may have less typical clinical signs, or may even be subclinical. Abdominal ultrasound can be useful for the diagnosis of pancreatitis, but diagnostic success is dependent on severity of disease, expertise of the ultrasonographer, the quality of the equipment, and the level of suspicion by the ultrasonographer. Several different substrates are available for the measurement of serum lipase activity, but none are exclusively specific for the measurement of pancreatic lipase activity and thus may also detect other lipases in serum. Also, lipase activity assays maybe severely affected by lipemia, icterus, and/or hemolysis. Serum pancreatic lipase immunoreactivity (PLI as measured by Spec PL® and SNAP PL) is the most sensitive and specific diagnostic test for pancreatitis currently available for both dogs and cats.

Introduction
The incidence of exocrine pancreatic disorders is quite large in both dogs and cats. The most common pancreatic disease is pancreatitis in both dogs and cats, and while it has traditionally been assumed that acute pancreatitis is more common in dogs and chronic pancreatitis more common in cats, recent data would suggest that in both species chronic pancreatitis is about two times more common than acute pancreatitis. Also, it has recently been suggested that the true prevalence of pancreatitis in dogs and cats is by far greater than previously believed. In one study of 208 dogs undergoing necropsy at the Animal Medical Center in New York City for a variety of reasons more than 21% had macroscopic lesions suggesting pancreatitis. Pancreata from all dogs were sectioned every 2 cm and a total of 64% had histological lesions of acute and/or chronic pancreatitis. These data suggest that pancreatitis is far more common in dogs than previously suspected. However, the data also suggest that infiltration of the pancreas with inflammatory cells is not necessarily clinically significant and more research is needed to better characterize clinically significant disease. Similar data have also been reported for cats. In a study of 115 cats submitted for necropsy at the University of California in Davis, 75.7% showed lesions suggestive of acute and/or chronic pancreatitis. This would suggest that, similarly to dogs, feline pancreatitis is far more common than previously expected, but also that more work is needed to clearly characterize clinically significant disease.

According to the current classification system of human pancreatitis acute pancreatitis is an inflammatory condition of the pancreas that is completely reversible after removal of the inciting cause. Chronic pancreatitis is characterized by irreversible histopathologic changes of the exocrine pancreatic tissue, such as atrophy or fibrosis. Both forms can be mild or severe. Mild forms of pancreatitis are associated with no or little pancreatic necrosis and systemic effects and often allow recuperation of the patient. In contrast, severe forms of pancreatitis are associated with extensive pancreatic necrosis, multiple organ involvement, and often a poor prognosis.
Clinical picture

Clinical signs in dogs with pancreatitis depend on the severity of the disease. Mild cases may remain subclinical. More severe cases may present with anorexia (91%; data from a study of 70 dogs with fatal pancreatitis), vomiting (90%), weakness (79%), abdominal pain (58%), dehydration (46%), and diarrhea (33%). Severe cases can present with systemic clinical signs such as fever or even cardiovascular shock. Cats, even with severe pancreatitis, present with even less specific clinical signs than do dogs. In one study of 40 cats with severe pancreatitis the following clinical signs were reported: lethargy (100%), anorexia (97%), dehydration (92%), hypothermia (68%), vomiting (35%), abdominal pain (25%), a palpable abdominal mass (23%), dyspnea (20%), ataxia (15%), and diarrhea (15%). Especially remarkable is the low incidence of vomiting and abdominal pain, both of which are common clinical signs in human and canine pancreatitis patients. Clinical signs in patients with pancreatitis are due to pancreatic inflammation or systemic effects of the pancreatic inflammation.

Imaging

Radiographic changes seen in some cases include a decreased contrast in the cranial abdomen and displacement of abdominal organs. However, these changes are rather subjective and abdominal radiography is non-specific for canine or feline pancreatitis.

Abdominal ultrasound is useful in the diagnosis of pancreatitis in dogs and cats. The sensitivity of abdominal ultrasonography is dependent on operator experience and has been reported to be up to 68% in dogs and up to 35% in cats. Changes identified include pancreatic swelling, changes in echogenicity of the pancreas (hypoechogenicity of the pancreatic parenchyma in cases of pancreatic necrosis and rarely hyperechogenicity of the pancreatic parenchyma in cases of pancreatic fibrosis) and of peripancreatic fat (hyperechogenicity in cases of peripancreatic fat necrosis), fluid accumulation around the pancreas, and less frequently a mass effect in the area of the pancreas. Other findings that have been described are a dilated pancreatic duct or an enlarged duodenal papilla.

Abdominal computed tomography is a routine procedure in humans suspected of having pancreatitis, but appears to be very insensitive for the diagnosis of pancreatitis in the cat and has never been systematically evaluated in the dog.

General clinical pathology

Complete blood count and serum chemistry profile often show mild and nonspecific changes. More severe changes can be observed in patients with severe forms of pancreatitis. Thus, findings from general clinical pathology are not clinically useful for establishing a diagnosis of pancreatitis, but are extremely useful to rule out other differential diagnoses and to evaluate the patient for systemic complications of pancreatitis.

Serum amylase and lipase activities

Serum amylase activity can be measured by use of enzymatic assays, but both sensitivity and specificity are poor and the diagnostic value of measurement of serum amylase activity in dogs and cats is very limited. Serum lipase activity can be measured by three different methods all using a different substrate. Most assays utilize a 1,2-diazyglycerol as a substrate. These assays have been shown to have a limited specificity (approximately 50%) for the exocrine pancreas and a limited sensitivity (also approximately 50%) for canine pancreatitis. Over the last 20 years a synthetic substrate, resorufin (DGGR), has been used as an alternative substrate in both human and veterinary medicine. While some studies would suggest a higher specificity for the exocrine pancreas than 1,2-diazyglycerol-based assays, other studies did not confirm these findings. Overall, the clinical utility of DGGR-based assays is probably better than those that are based on 1,2-diazyglycerol, but more studies are needed to confirm these results, and this substrate is by no means specific for the exocrine pancreas in dogs or cats, which has been suggested by some recent studies. A point of care assay has been described that uses triolein as a substrate. However, so
far the only studies that are available show this assay to correlate with the measurement of serum pancreatic lipase immunoreactivity when exclusively evaluated in serum samples that are neither lipemic, icteric, or hemolized, changes that are rather common in dogs with pancreatitis. Thus, overall, serum amylase and lipase activities are of limited clinical value for the diagnosis of pancreatitis in dogs or cats.

Trypsin-like immunoreactivity

Trypsin-like immunoreactivity is specific for exocrine pancreatic function. However, the sensitivity of serum TLI concentration for pancreatitis in dogs and cats is only approximately 30-60%, making it a suboptimal diagnostic test for pancreatitis in both species. However, serum TLI concentration remains the diagnostic test of choice for the diagnosis of EPI.

Pancreatic lipase immunoreactivity (PLI)

Specific assays for the measurement of pancreatic lipase immunoreactivity in dogs and cats (cPLI and fPLI, respectively) are available. Many different cell types in the body synthesize and secrete lipases. In contrast to catalytic assays for the measurement of lipase activity, use of immunoassays does allow for the specific measurement of lipase originated from the exocrine pancreas. Serum cPLI was measured in a group of dogs with exocrine pancreatic insufficiency and the median serum cPLI concentration was significantly decreased compared to clinically healthy dogs. In addition, serum cPLI concentration was non-detectable in most of the dogs and minimal serum cPLI concentrations were observed in the rest of the dogs, indicating that serum cPLI concentration originates from the exocrine pancreas and is specific for exocrine pancreatic function. In another study serum cPLI was evaluated in dogs with experimentally induced chronic renal failure. While serum cPLI was significantly higher in dogs with experimentally induced chronic renal failure than in clinically healthy dogs, most dogs had serum cPLI concentrations within the reference range and none of the dogs had serum cPLI concentrations that were above the currently recommended cut-off value for pancreatitis. These data would suggest that serum cPLI concentration can be used as a diagnostic test for pancreatitis even in dogs with renal failure. Also, long-term oral administration of prednisone did not have any effect on serum cPLI concentration.

The sensitivity of different minimally-invasive diagnostic tests was compared in dogs with proven pancreatitis. The sensitivity of serum TLI concentration was below 35% and that of serum lipase activity was less than 55%. In contrast, the sensitivity for serum cPLI concentration for pancreatitis was above 80%. More recent clinical studies show similar results, suggesting that the measurement of Spec cPLI concentration is the most sensitive and specific diagnostic test currently available.

Clinical studies in cats have shown similar results. In a group of cats with experimentally induced pancreatitis both serum fTLI and fPLI concentrations did increase initially but serum fPLI stayed elevated much longer than did serum fTLI concentration suggesting that, as in the dog, serum PLI concentration is much more sensitive for pancreatitis than serum TLI concentration. In another study of cats with spontaneous pancreatitis serum fPLI concentration was more sensitive and more specific than serum fTLI concentration or abdominal ultrasonography. Thus, in both dogs and cats serum PLI concentration is the most sensitive and specific diagnostic test for pancreatitis currently available. Commercial assays for the measurement of cPLI and fPLI, Spec cPL™ and Spec fPL™, respectively are now available. Also, patient-side tests for the semi quantitative assessment of pancreatic lipase are now available. These SNAP assays are useful to rule out pancreatitis in dogs or cats with suggestive clinical signs when the test is negative. Also, a positive test result suggests the presence of pancreatitis. However, a serum sample should also be sent to the laboratory for measurement of Spec cPL/Spec fPL to confirm the diagnosis and to get a baseline value that can then be used to monitor the progression of the disease.

Cytology and histopathology

Cytologic evaluation of a fine-needle aspirate of the pancreas is a great diagnostic modality to confirm a diagnosis of pancreatitis. Various studies have shown that if care is taken there is little risk of a fine
needle aspiration of the pancreas. The presence of pancreatic acinar cells confirms the successful aspiration of the pancreas and presence of inflammatory cells in the same aspirate confirms the presence of pancreatic inflammation. However, in patients with severe pancreatic necrosis only cellular debris may be aspirated and the cytological evaluation may be inconclusive. Also, lack of inflammatory cells in the infiltrate does not rule out pancreatitis as the inflammatory lesions may be highly localized.

Traditionally, a pancreatic biopsy has been viewed as the most definitive diagnostic tool for pancreatitis. Pancreatic biopsies can be collected during abdominal exploratory or by laparoscopy. The presence of pancreatitis can easily be diagnosed by gross appearance of the pancreas in many cases. However, the absence of pancreatitis can be difficult to prove and even if multiple biopsies are being collected, pancreatic inflammation, especially in cases of chronic pancreatitis, may easily be missed. It should also be noted that while a pancreatic biopsy in itself is not associated with many complications, many patients with pancreatitis have a higher anesthetic risk than a healthy patient.

**Keywords:** Abdominal Ultrasound, Serum Lipase Activity, Triolein, DGGR, Pancreatic Lipase Immunoreactivity, Pancreas Specific Lipase, PLI, Spec PL, SNAP PL
Treatment of Pancreatitis in Small Animals – Successful Approach for a Difficult Disease

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Summary
To date there is no specific treatment for pancreatitis and treatment of patients with pancreatitis is mostly supportive. The first step for any patient with pancreatitis is to try to identify the underlying cause, such as hypertriglyceridemia, hypercalcemia, or drugs. In patients with acute severe disease aggressive fluid therapy, close monitoring for the development of complications, analgesic support, antiemetics, and alimental support are all equally important. Withholding food from patients with acute pancreatitis is no longer recommended and early nutritional support is key in treatment success. Routine use of antibiotics is also discouraged. In patients with mild chronic pancreatitis an evaluation for concurrent conditions, such as inflammatory hepatic disease, IBD, and diabetes mellitus should be undertaken. An ultra low-fat food in dogs and avoiding a high-fat diet in cats are also important. Immunosuppressive therapy maybe successful is one group of patients with chronic disease. Monitoring is important in both patients with acute and also with chronic disease.

Treatment of Inciting Cause
Whenever possible the inciting cause of the disease should be removed or treated. However, this may be difficult to accomplish as most cases of canine and feline pancreatitis are considered idiopathic.

Several diseases and risk factors have been associated with pancreatitis. Dietary indiscretion is considered to be an important risk factor for pancreatitis in dogs. Also, severe hypertriglyceridemia (statistically significant increase in risk for serum triglyceride concentrations > 850 mg/dL) is considered a risk factor for pancreatitis. Pancreatitis is especially common in the Miniature Schnauzer and recently 3 different mutations have been identified in the SPINK-1 gene of affected dogs. This gene has also been associated with hereditary pancreatitis in humans. Traumatic pancreatitis (due to road traffic accidents in both dogs and cats or falling from heights in cats) has been reported. Surgical trauma can cause pancreatitis, but many human patients that undergo surgery of organs distant from the pancreas have also been shown to be at an increased risk for pancreatitis suggesting that hypoperfusion of the exocrine pancreas during anesthesia may be of bigger concern than surgical handling of the organ itself. Infectious agents have been shown to cause feline pancreatitis, with the strongest causal relationship for Toxoplasma gondii, and rare cases of Amphimerus pseudofelineus infestation in cats. Babesia canis has been reported to be associated with pancreatitis in dogs. Weaker evidence has been presented for feline panleukopenia virus infections in kittens and infections with feline herpesvirus I and feline infectious peritonitis virus. Two cases of feline pancreatitis after topical use of fenthion, an organophosphate cholinesterase inhibitor, have been reported. Many other pharmaceutical compounds have been implicated in causing pancreatitis in humans and dogs, but no cases have been reported in the cat. Hypercalcemia or calcium infusions can also be associated with pancreatitis. Chronic hepatitis in dogs and cholangitis in cats may coexist in patients with pancreatitis, but there is no evidence
that they play a causative role.

A serum chemistry profile should be performed to rule out hypertriglyceridemia or hypercalcemia. Exposure to unnecessary drugs, especially those implicated in causing pancreatitis in dogs, cats, or other species, should be avoided. Thus, a careful, drug history should be taken and the clinician should carefully determine whether treatment is still needed. For example, a patient that is treated with an anticonvulsant medication may need to be maintained on some anticonvulsant therapy, but, if being treated with potassium bromide and/or phenobarbital, the patient should be switched to another anticonvulsant medication.

Supportive Care

Aggressive fluid therapy is the mainstay of supportive therapy for dogs and cats with severe forms of pancreatitis. Fluid, electrolyte, and acid-base imbalances need to be assessed, and corrected as early as possible. This is especially important since systemic complications are associated with a worse outcome and many of the systemic complications, once established, are difficult to treat. Recent studies in humans have shown that minimal differences in blood urea nitrogen concentrations at time of admission to the hospital and also minimal changes of BUN during the first 24 to 48 hours after admission to the hospital can have a dramatic impact on the outcome in humans with acute pancreatitis.

Traditionally, dogs and cats with pancreatitis have been held off food, but over the last 10 years this practice has been questioned based on experiences in human patients with pancreatitis. There is good evidence in humans with severe forms of pancreatitis that alimentation is crucial to counterbalance the catabolic effects of pancreatitis. Also, it has been shown in several studies that enteral nutrition is superior for the nutritional management of human pancreatitis patients. A recent study has made similar observations in dogs. While there was no difference in mortality between dogs fed by esophagostomy tube or total parenteral nutrition, dogs fed by esophagostomy tube improved significantly faster than dogs fed parenterally. Also, studies in humans have shown that alimentation that enters the digestive tract before the duodenal papilla is not associated with a worse outcome when compared to patients fed by a jejunostomy tube. In fact, feeding patients through nasogastric tubes has been shown to be quite effective in humans with pancreatitis. Thus, in general dogs and cats with pancreatitis should be fed whenever possible. An ultra low-fat diet should be chosen in dogs and a moderately fat-restricted diet in cats. If patients are not interested in food feeding by gastrostomy, esophagostomy, or nasogastric tube should be attempted. If the patient vomits relentlessly a jejunostomy tube should be placed or the patient should be fed by partial or total parenteral nutrition.

Analgesia

Abdominal pain is the key clinical sign in human patients with pancreatitis and is recognized in excess of 90% of all pancreatitis patients. Abdominal pain is much more commonly recognized in dogs than in cats with pancreatitis, but even in dogs the reported rate of abdominal pain is only approximately 58% and is even lower in cats. It is unlikely that abdominal pain occurs less frequently in dogs and cats than in humans and it is much more likely that abdominal pain remains unidentified in veterinary species. Thus, the presence of abdominal pain should be assumed and analgesic drugs are indicated in all small animal patients with pancreatitis. Meperidine, butorphanol tartrate, morphine, fentanyl, or combinations of multiple analgesic drugs can be used in hospitalized patients. Outpatients can be treated with oral butorphanol, tramadol, or a fentanyl patch.

Antiemetics

Until recently, the choices for antiemetic agents for use in dogs and cats with pancreatitis was limited. Metoclopramide, a dopamine inhibitor, was most widely used. However, its effect on splanchnic perfusion remains in question and the author does not like to use metoclopramide in dogs or cats with pancreatitis. Fortunately, several other antiemetic agents have become available over the last few years. Most recently, a new drug, maropitant, an NK1 antagonist has become widely available for use
in dogs and cats. Maropitant is a highly efficacious antiemetic agent through both peripherally and centrally-mediated mechanisms and can be used both parenterally in patients that are actively vomiting and orally in patients that appear mostly nauseous.

Dolasetron and ondansetron are 5HT3 antagonists and are also very effective antiemetic agents in both dogs and cats. The injectable formulation of dolasetron can be used for intravenous, subcutaneous, or oral administration and is being used at 0.3-0.6 mg/kg q 12-24 hr in both dogs and cats. Since maropitant and 5HT3 antagonists work through different mechanisms both drugs can be combined.

**Fresh Frozen Plasma**

Studies in dogs suggest that when a2-macroglobulin, one of the scavenger proteins for activated proteases in serum, is depleted death ensues rapidly. Fresh frozen plasma and fresh whole blood not only contain a2-macroglobulin, but also albumin, which has many beneficial effects in patients with severe pancreatitis. Plasma also contains both anticoagulant and coagulation factors. In clinical trials in human patients with acute pancreatitis there was no benefit of plasma administration. Also, in dogs the benefit of plasma has not been demonstrated. However, anecdotally, fresh frozen plasma is believed to be useful in dogs with severe forms of pancreatitis.

**Antibiotics**

In contrast to humans, infectious complications of pancreatitis are rare in dogs and cats with pancreatitis. Also, even though such complications occur frequently in human pancreatitis patients and are estimated to be responsible for approximately 25-50% of all deaths associated with acute pancreatitis, a clear advantage of antibiotic use has not been demonstrated to date. Therefore, the use of antibiotic agents should be limited to those cases when an infectious complication can be identified or is strongly suspected.

**Antiinflammatory Agents**

Glucocorticoids have not shown any benefit in human patients with acute pancreatitis that do not have autoimmune pancreatitis and their use should be limited to canine and feline patients with cardiovascular shock. Nonsteroidal antiinflammatory agents all have been implicated in potentially causing pancreatitis and also did not show any benefit in human studies.

**Other Therapeutic Strategies**

Many other therapeutic strategies, such as the administration of trypsin-inhibitors (e.g. trasylo), platelet activating factor inhibitors (PAFANTs), dopamine, antacids, antisecretory agents (i.e., anticholinergics, calcitonin, glucagon, somatostatin), or selenium, and peritoneal lavage all have been evaluated in human patients with pancreatitis. With the exception of PAFANTs and selenium, none of these have shown any beneficial effect at this point. The efficacy of selenium, which has also been shown to decrease mortality in dogs in a single uncontrolled study, needs to be further evaluated before its use can be recommended. Also, dopamine has been shown to be useful in preventing progression of pancreatitis when administered within 12 hours of initiating pancreatitis in cats. While this time-limit would preclude dopamine to be effective in routine therapy of pancreatitis, patients with pancreatitis that have to undergo anesthesia may benefit from treatment with dopamine during the procedure.

**Mild Chronic Pancreatitis**

It should be noted that many small animal patients, both canine and feline, have mild forms of chronic pancreatitis. Often times these patients have concurrent conditions, most notably IBD. Very little is known about appropriate therapy for these patients and management is often limited to evaluation and treatment of the concurrent condition, and careful monitoring of the pancreatitis.

Serum calcium and triglyceride concentrations should always be evaluated in these patients in order to identify any risk factors that can potentially be addressed therapeutically.
Also, the use of low fat diets is recommended in these patients. This is especially important in dogs where an ultra-low-fat diet should be chosen.

Over the last two decades a new form of pancreatitis, autoimmune pancreatitis has been described in humans. Autoimmune pancreatitis is now more commonly recognized in humans and is characterized by a lymphocytic-plasmacytic infiltration of the pancreas. Human patients with autoimmune pancreatitis respond favorably to the administration of corticosteroids. Recently, several clinicians have started to cautiously treat canine and feline patients with chronic pancreatitis with corticosteroids and have found this treatment strategy to be beneficial in a portion of cases. Also, successful treatment of a canine patient with chronic pancreatitis with cyclosporine has been reported in the literature. However, further studies are needed before these treatment strategies can be recommended for more routine use in dogs and cats.

Regardless of the management, progress of the disease should always be monitored. Just as in human patients with chronic pancreatitis, canine and feline patients with chronic pancreatitis are at risk for developing episodes of severe pancreatitis at any time or exocrine pancreatic insufficiency and Diabetes mellitus later in life.

Prognosis

The prognosis for dogs and cats with pancreatitis is directly related to disease severity, extent of pancreatic necrosis, occurrence of systemic and pancreatic complications, duration of the condition, and the presence of concurrent disease. Several prognostic systems have been developed to predict the outcome of pancreatitis in human patients early after admission to the hospital. All of these systems are aimed to identify high-risk patients early on and to be able to aggressively treat these patients. Several of these systems have been adapted for use in small animals and evaluated in dogs or cats. Unfortunately, none of these prognostic systems have been proven useful in a clinical setting in small animals. However, some of them may be useful for use in clinical studies.

Keywords: Supportive Care, Analgesia, Antiemetics, Alimentary Support, Ultra Low-Fat Diet
Exocrine Pancreatic Insufficiency in Small Animals

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Summary
Exocrine pancreatic insufficiency (EPI) is a syndrome cause by insufficient amounts of digestive enzymes in the small intestine and is usually associated with weight loss, soft voluminous stools, and steatorrhea. The gold standard test for the diagnosis of EPI is measurement of serum concentrations of trypsin-like immunoreactivity (TLI), which is a species-specific diagnostic test that is severely decreased in dogs and cats with EPI. Many if not most dogs and cats with EPI are cobalamin deficient and treatment should not only include pancreatic enzyme replacement therapy, but also cobalamin supplementation. Prognosis for dogs and cats with EPI is usually good.

Etiology and Pathogenesis
Exocrine pancreatic insufficiency is a syndrome, which is caused by an insufficient amount of digestive enzymes of exocrine pancreatic origin in the small intestine. Pancreatic acinar atrophy is considered to be the most common cause of EPI in the dog, followed by chronic pancreatitis. In contrast, chronic pancreatitis is the most common cause of EPI in cats and humans. Other, less common causes of EPI in dogs and cats are pancreatic or extrapancreatic masses that lead to an obstruction of the pancreatic duct, congenital pancreatic hypo- or aplasia, as well as deficiencies of individual pancreatic digestive enzymes or of enteropeptidase may also lead to EPI, but have not been reported in dogs and cats.

In humans the exocrine pancreas has been shown to have a remarkable functional reserve. Ninety percent of its functional reserve must be lost before clinical signs of EPI develop. Digestive enzymes of pancreatic acinar origin play an integral role in the assimilation of all major components of food stuff and a lack of pancreatic digestive enzymes leads to malassimilation. The nutrients remaining in the intestinal lumen lead to loose voluminous stools and steatorrhea. At the same time, a lack of nutrients causes weight loss and may lead to vitamin deficiencies in some cases. Serum cobalamin (vitamin B12) concentrations are undetectable or markedly decreased in almost all cats and a more than 80% of dogs with EPI. Serum folate concentrations in dogs with EPI are often increased suggesting the presence of complicating small intestinal dysbiosis (formerly also known as small intestinal bacterial overgrowth or SIBO). In contrast, serum folate concentrations in cats with EPI are often decreased, indicating concurrent small intestinal disease. Secondary vitamin K responsive coagulopathy is rare in dogs and cats with EPI, but has been reported and should be considered if a bleeding tendency is present.

In patients with EPI caused by chronic pancreatitis, destruction of pancreatic tissue may not be limited to the acinar cells and concurrent diabetes mellitus (DM) may be observed. In most human patients with chronic pancreatitis, both exocrine and endocrine functional reserves are ultimately lost. However, overt DM usually appears later in the disease process than does malassimilation. At this point it is unknown whether dogs and cats with chronic pancreatitis follow the same pattern of progression, but it is intriguing to speculate whether EPI may be more common, but often undiagnosed in those dogs and cats with DM that are difficult to regulate.
Clinical Picture and Diagnosis

Dogs with EPI due to PAA are most often young adult German Shepherd dogs, but dogs and cats with EPI due to other causes are usually middle-aged to older and can be of any breed. Clinical signs most commonly reported in dogs and cats with EPI are polyphagia, weight loss, and loose stools or diarrhea. Vomiting and anorexia can be observed in some patients with EPI and may indicate the presence of a concurrent condition, such as inflammatory bowel disease, rather than a primary clinical sign of EPI. All of the clinical signs observed in patients with EPI are non-specific and are also seen in other disorders more commonly seen in middle aged to older dogs and cats.

The feces from small animal patients with EPI are most commonly pale, loose, voluminous, and may be quite malodorous. In rare instances these patients may also develop watery diarrhea. The high fat content of the feces can lead to a greasy appearance of the hair coat, especially in the perianal and tail region of cats.

Results from routine blood tests are within the normal range in most cases. Several tests have been recommended to estimate exocrine pancreatic function in dogs and cats. The bentiromide absorption test, commonly known as PABA test, plasma turbidity test, microscopic examination of feces for undigested fat, starch, or muscle fibers, and fecal proteolytic activity (FPA), all have been recommended for the diagnosis of EPI. With the exception of FPA, all of these tests are rather unreliable or impractical and are therefore not recommended. Fecal proteolytic activity can be determined by either azocasein- or azoalbumin- based methods, or by a radial enzyme diffusion method, but at least three stool samples from consecutive days should be evaluated and feces should be frozen immediately and shipped on ice in order to prevent loss of FPA in the samples, making this test not very practical. Currently, evaluation of FPA is only recommended in species for which a serum TLI assay is not available.

Immunoassays for the measurement of serum trypsin-like immunoreactivity (TLI) in dogs and cats have been developed and validated. These tests are highly specific for EPI in both dogs and cats. Dogs with a serum cTLI of £ 2.5 mg/L and cats with a serum fTLI £ 8.0 mg/L can be diagnosed with EPI, respectively. A recent report has shown that some German Shepherd dogs have subclinical EPI with severely decreased serum cTLI concentrations. These dogs have a lack of exocrine pancreatic tissue at biopsy, but no or only intermittent clinical signs of EPI. This highlights the remarkable functional reserve of the exocrine pancreas and the entire gastrointestinal tract in dogs.

More recently, an assay for measurement of fecal elastase in dogs has been developed and validated. Unfortunately, some normal dogs or dogs with chronic small intestinal disease may have a decreased fecal elastase concentration. Because of the low incidence of dogs with EPI a few false positive results of the assay lead to an overall large number of false positive dogs (low positive predictive value) making this test unreliable.

Treatment

Most dogs and cats with EPI can be successfully treated by dietary supplementation with pancreatic enzymes. Dried extracts of bovine or porcine pancreas are available (e.g. Viokase® or Pancrezyme®). The clinical impression in dogs and cats that powder is more effective than tablets, capsules, and especially enterico-coated products has also been substantiated in human patients with EPI. Initially, one teaspoon per 10 kg body weight and meal should be given in dogs and one teaspoon per cat and meal in cats. Oral bleeding has recently been reported in 3 of 25 dogs with EPI treated with pancreatic enzyme supplements. The oral bleeding stopped in all 3 dogs after the dose of pancreatic enzymes was decreased. Moistening the food pancreatic powder mix also appears to decrease the frequency of this side effect. If the owner has access to fresh pancreas this may be a viable alternative to use of pancreatic powder. One to three ounces (30-90 g) of raw chopped pancreas can replace one teaspoon of pancreatic extract. Raw pancreas can be kept frozen for several months without loss of enzymatic activity. Preincubation of the food with pancreatic enzymes, supplementation with bile salts, or concurrent antacid therapy are unnecessary in most canine and feline patients with EPI. When clinical signs have resolved the amount of pancreatic enzymes given can be gradually
decreased to the lowest effective dose, which may vary from patient to patient, and from batch to batch of the pancreatic supplement.

Even though pancreatic enzyme supplementation causes the clinical signs to subside in almost all patients, it has been shown in humans and dogs with EPI that nutrient absorption, and particularly fat absorption are not normalized by enzyme supplementation. This is thought to be due to the low pH in the stomach leading to irreversible damage of some of the pancreatic lipase contained in the supplement. The use of omeprazole has recently been shown to be efficacious in human patients with EPI and may be tried at a dose of 0.7-1.0 mg/kg q 12 hrs PO if the patient does not respond to routine therapy. Some authors have suggested feeding low fat diets in order to accommodate impaired fat digestion. However, this may even further decrease fat assimilation and may potentially lead to serious complications associated with hypovitaminoses of fat-soluble vitamins and conditions associated with a lack of essential fatty acids. Some types of dietary fiber interfere with pancreatic enzyme activity. Therefore, a diet low in insoluble or non-fermentable fiber should be fed.

Response to enzyme supplementation alone may not be satisfactory in some canine or feline patients with EPI. This is not surprising if one considers that many dogs and especially cats with EPI also have a serious depletion of total body cobalamin stores. Serum cobalamin and folate concentrations should be routinely evaluated in small animals with suspected EPI, and dogs and cats with decreased serum cobalamin concentrations should be treated with cobalamin parenterally. Hypovitaminoses of other fat- and water-soluble vitamins are also common, but most patients are not routinely treated with vitamin supplements and respond well to therapy otherwise. Also, as indicated vitamin K deficiency may occur in rare cases and should be anticipated as a potential complication.

Some dogs and cats do not respond to enzyme supplementation and cobalamin application. These patients likely have concurrent small intestinal disease. In dogs with EPI small intestinal dysbiosis is common and may need to be treated with antibiotic therapy (antibiotic of choice: tylosin at 25 mg/kg q 12 hrs PO for 6-8 weeks). In cats with EPI, inflammatory bowel disease can frequently occur concurrently.

**Prognosis**

EPI is associated with an irreversible loss of pancreatic acinar tissue in most cases, and complete recovery is therefore extremely rare. However, with appropriate management and monitoring these patients usually gain weight quickly, pass normal stools, and can go on to live a normal life for a normal life span.

**Keywords:** Exocrine Pancreatic Insufficiency, Trypsin-Like Immunoreactivity, Chronic Pancreatitis, Cobalamin, Pancreatic Enzymes
Diagnosis and Treatment of Canine Lymphoma

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Summary
Canine lymphoma (LSA) is a heterogenous hematologic malignancy that affects mainly medium to large-sized dogs. It is the most common canine hematologic malignancy, comprising ~84% of all tumors. Although high-grade nodal LSA is characterized by the expansion of large lymphoblasts originating in lymph nodes, there are many variants of this disease, whereby different organ systems or different subtypes of lymphocytes can be affected. These subtypes can explain, in part, the biological heterogeneity of LSA, and the explanation of all of these variants is beyond the scope of this presentation. Almost all variants of LSA are treated with systemic chemotherapy. This seminar will describe the diagnosis and treatment options of canine high-grade B- and T-cell lymphoma.

Diagnosis
The typical dog with LSA will present with generalized (or less commonly regional) lymphadenopathy. Differential diagnoses for generalized lymphadenopathy can include Ehrlichiosis or other immune-mediated diseases, systemic mycosis, severe pyoderma or other skin disease, and reactive hyperplasia. The simplest way do discern the cause for lymphadenopathy is via fine needle aspiration cytology of an affected lymph node. If possible, the submandibular lymph nodes should be avoided due to the likelihood of some component of reactive hyperplasia being present due to drainage from the mouth and ears. Although many clinical pathologists are able to confirm a diagnosis of canine LSA cytologically, a biopsy of an affected lymph node provides the most information and is considered the “gold standard” of diagnosis (as with all tumors). It is critical that empiric prednisone therapy not be given prior to diagnosis if lymphoma is a differential, since steroids may mask the signs of illness and have the potential to induce resistance to other forms of chemotherapy (See below).

Complete clinical staging helps to ascertain the extent of disease, ensures that other types of medical problems are not present, and can provide prognostic information for the client. Complete staging should include complete blood count, serum chemistry panel, urinalysis, thoracic radiographs, abdominal ultrasound, a bone marrow aspirate, and immunopheotyping. The World Health Organization has developed a clinical staging system for dogs with multicentric LSA, which takes into account the number and location of involved lymph nodes, presence or absence of hepatosplenomegaly, and the presence or absence of disease in the bone marrow, central nervous system, or other extranodal sites. In addition, a substage is assigned, (a) representing a patient without clinical signs of illness, and (b) representing a patient with clinical signs (anorexia, lethargy/weakness/ depression, significant weight loss, vomiting, diarrhea, etc.) (See Table 1). Most dogs that present are WHO Stage IIIa or IVa.
Table 1 WHO staging criteria for canine lymphoma.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
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<tbody>
<tr>
<td>I</td>
<td>Disease confined to a single lymph node.</td>
</tr>
<tr>
<td>II</td>
<td>Regional lymphadenopathy (confined to one side of diaphragm).</td>
</tr>
<tr>
<td>III</td>
<td>Generalized lymphadenopathy.</td>
</tr>
<tr>
<td>IV</td>
<td>Hepatosplenomegaly (with or without lymphadenopathy)</td>
</tr>
<tr>
<td>V</td>
<td>Bone marrow, CNS, or other extranodal site involvement</td>
</tr>
<tr>
<td>Substage a:</td>
<td>No clinical signs</td>
</tr>
<tr>
<td>Substage b:</td>
<td>Clinical signs of illness</td>
</tr>
</tbody>
</table>

Complete staging allows a thorough assessment of factors that may help to predict the outcome with treatment for an individual patient. Factors that have historically carried the most prognostic significance for remission duration and survival include presence of clinical signs at presentation (substage b), presence of hypercalcemia (usually associated with T-cell LSA), mediastinal lymphadenopathy (usually associated with T-cell LSA), significant bone marrow infiltration, and immunophenotype. Immunophenotype (ie T-cell vs B-cell LSA) is a very powerful predictor of outcome, as is the first response to chemotherapy. These prognostic factors do not typically alter the likelihood that a patient will achieve a complete response (CR); they do however, affect the likely duration of that response.

More on immunophenotyping

Immunochemistry & Immunocytochemistry: Most veterinary pathology laboratories are capable of immunophenotyping lymphomas with the use of immunochemistry (gold standard), performed on biopsy samples. Antibodies against CD3 will generally stain T cell lymphomas, and antibodies against CD79a or PAX5 will generally stain B cell lymphomas. Many laboratories are now also offering immunocytochemistry, which applies a similar staining technique to air-dried LN FNAs on slides.

Flow cytometry: Several US universities can perform immunophenotyping on fresh fine-needle aspirates using flow cytometry. This method is more sensitive than PARR for immunophenotyping, and is also useful for confirming diagnoses in animals with suspected leukemias and distinguishing between lymphoma and thymoma in dogs with mediastinal masses. Additional information regarding prognosis may be obtained through flow cytometry; specifically, dogs with B-cell lymphoma whose tumor cells have low expression of MHC class II have a significantly worse outcome than those with higher class II expression. Furthermore, flow cytometry has recently identified a subset of T cell lymphomas that appear to have an indolent course and a good outcome either with watchful waiting or conservative oral chemotherapy. This population of lymphomas is characterized by low or absent expression of the pan-leukocyte marker CD45 and generally high expression of the interleukin-2 receptor CD25. These are generally interpreted as T-zone lymphomas histologically, 40% occur in golden retrievers, and concurrent lymphadenopathy and peripheral lymphocytosis is common.

PARR (PCR for antigen receptor rearrangements): This molecular diagnostic test evaluates the presence or absence of a clonally expanded population of B cells or T cells, and is approximately 75% sensitive and 95% specific for canine lymphoid neoplasia. An advantage of this technique is that it can be performed on almost any type of sample, including air-dried or previously stained cytology slides, effusions, aspirates, cerebrospinal fluid, frozen tissue formalin-fixed tissues, and peripheral blood. Several new publications have evaluated the utility of real-time PCR to quantify the amount of DNA possessing the lymphoma-specific clonal gene rearrangement in the blood. This correlates with remission status, and early evidence suggests that the amount of lymphoma DNA in the blood following chemotherapy may correlate with remission length as well.
Immunostaining: Immunostaining for the cellular survival protein, surviving, may be a useful prognostic factor in dogs with stage IIIa and IVa B cell lymphoma, a population for which there are no reliable prognostic factors currently. Another recent study has demonstrated that monocyte count may be an independent predictor of outcome in dogs with lymphoma: dogs with monocyte counts higher than 800 cells/dL had an outcome nearly 4 times worse than those with lower monocyte counts. These interesting preliminary findings need to be confirmed in additional studies.

Treatment

Combination Therapy: There is general agreement among oncologists that CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) protocols are most effective at inducing and maintaining remission in canine lymphoma. Among these, the most widely used version of this protocol, the University of Wisconsin, Madison 25-week protocol (UW-25). (1) This paper addressed the question of the necessity of a maintenance phase that extended up to two years, which was standard at the time. No differences were found between the new protocol and historical controls in disease-free interval (DFI) or overall survival time (OS). The complete remission rate (CR) was 92.3%, DFI was 282 days, and OS 397 days. Of great clinical interest was a 100% rate of second remission when re-induced with the same protocol after failure following completion of the first 25-week protocol. This excellent second response rate helps to tell thegue work out of rescue chemotherapy decisions when a patient has completed the entire protocol once. Toxicity was mild with approximately 40% of dogs experiencing at least one dose reduction or chemotherapy delay, and less than 10% requiring hospitalization at any point. This protocol is the author’s first choice, all other things being equal.

Chun and others reported an attempt to improve survival by dose-intensifying the UW-25 protocol. (2) Dogs were treated with a protocol similar to the UW-25, but with the dosage of doxorubicin increased to 37.5 mg/m² and dosage of cyclophosphamide to 250 mg/m². No difference was seen in DFI or OS between the experimental and the control protocol. This suggested no benefit in survival from increasing the dose of chemotherapy. Unfortunately, the odds of death due to toxicity in the experimental protocol were 8.8 times those of the control protocol. These same results were found in another study. These studies underscore the lack of benefit of increasing the dose of chemotherapy over what has been judged to be tolerable. This is possible in human medicine, where bone marrow growth factors can routinely be used, but the lack of such support in veterinary patients makes the practice too risky.

As T cell lymphomas have historically been refractory to CHOP-based chemotherapy, recent attention has been given to alkylator-based protocols for this presentation. Reported median survival in a group of dogs with T cell lymphoma was only 235 days. (3) Recent evidence showed a significantly rate of response to doxorubicin in B cell lymphomas compared to T cell lymphomas. The combination of L-asparagine, mechlorethamine, vincristine, procarbazine, and prednisone (L-MOPP) has been investigated for treating T-cell lymphomas.19 Of 50 dogs with T-cell lymphoma treated with L-MOPP, the complete remission rate (CR) was 78%, DFI was 189 days, and OS 270 days.(19) This is a moderately toxic protocol with a higher likelihood of morbidity than the usual CHOP protocols. For clients interested in treating a dog with T-cell lymphoma more aggressively, this protocol may be appropriate.

A study suggested that prednisone is not a necessary component of the CHOP protocol. In a prospective, randomized trial, there were no differences in response, response duration, or survival between dogs receiving CHOP or CHO. (4) However, due to the less tangible benefits or prednisone early in the course of therapy, most oncologists continue to include prednisone in these protocols.

Doxorubicin as a single-agent to treat canine lymphoma has been reported in several papers. Dogs that received doxorubicin had a CR of 64% yielding a median DFI of 131 days. The OS was 237 days. (5) These numbers appear shorter than those in the UW-25 study, but cannot be compared directly. Toxicity was mild with fewer than 10% hospitalized. A more recent paper by Simon and others compared single-agent doxorubicin to a CHOP protocol. The doxorubicin arm yielded a complete remission rate (CR) of 52%, a DFI of 309 days, and OS 322 days. These numbers were not statistically different from those of the

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CHOP arm of the study. For dogs who cannot tolerate vincristine, clients who need a less frequent visit schedule, and clients who need a less expensive, but effective, alternative, doxorubicin as a single agent probably represents the best option.

Tanovea™ (Tanovea™ VetDC) was discovered by Gilead Sciences, Inc., and licensed to VetDC for use in animal cancer, (previously known as VDC-1101). This agent was designed to preferentially target and attack cancer cells implicated in lymphoma. The data from studies totaling well over 330 patients have shown Tanovea™ to be highly effective against LSA with a 60-80% overall response rate. Not surprisingly, responses are higher in naïve LSA vs relapse and in dogs with a B cell phenotype. Data suggests Tanovea™ is well-tolerated with a similar side effect profile as other commonly used agents. The administration is via the intravenous route with a 1mg/kg dosing every 3 weeks. Tanovea is now available in the U.S.

**Half body Irradiation:** A number of reports have described the use of sequential half-body irradiation interposed in a CHOP-based chemotherapy protocol. Unfortunately, the total dose and dose rate of the irradiation, as well as when the irradiation is administered, has not been consistent, making comparisons between the protocols difficult. In the most promising report the median overall survival was 684 days and the 1-, 2-, and 3-year survival rates were 66, 47, and 44%.

**Autologous and Allogeneic Bone Marrow Transplantation:** The topic of another presentation!

**Keywords:** Lymphoma, Canine, Chemotherapy, Immunophenotype

**References**

Bone Marrow Transplantation for the Treatment of Canine Lymphoma

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Summary
For close to 6 decades, the domestic dog (Canis familiaris) has served as a preclinical model for hematopoietic cell transplantation (HCT). Based on limited knowledge gained in early, uniformly unsuccessful, human clinical trials and later in murine studies, dogs were identified as a suitable species for study based on their large body size, longer life span, genetic diversity, random-bred nature, ease of handling, and relatively economical colony maintenance costs. For these reasons, there is a tremendous body of literature documenting the utility of dogs for studies of HCT, total body irradiation (TBI), myeloablation, cell dose requirements, graft manipulation, engraftment, and GVHD. Therefore, the vast majority of clinical HCT problems encountered in people were solved using dogs. It is estimated that >95% of human transplant procedures were first perfected using canines.

Hematopoietic cell transplantation was revolutionized by a number of important discoveries in both humans and dogs dating back to the 1990s. First, the cloning and large-scale production of recombinant hematopoietic cytokines, including granulocyte colony-stimulating factor (rhG-CSF, recombinant human G-CSF and rcG-CSF, recombinant canine G-CSF), stem cell factor (rhSCF and rcSCF), and granulocyte macrophage colony-stimulating factor (rhGM-CSF and rcGM-CSF), that could increase circulating hematopoietic progenitors and support their in vivo growth. Second, the finding that peripheral blood contains small numbers of hematopoietic progenitor cells and that recombinant cytokines dramatically increase these numbers via recruitment from the bone marrow. Third, the production and characterization of anti-CD34 monoclonal antibodies directed against human, mouse, primate, and canine mononuclear cells that allowed the isolation of a population of cells that can reconstitute the peripheral blood cell lineages after lethal marrow ablation. And finally, the development of sophisticated continuous-flow blood separators that are able to remove and separate blood components via centrifugation on the basis of specific cell densities.

The NC State Canine Bone Marrow Transplant Unit currently utilizes refurbished TerumoBCT COBE® Spectra apheresis machines (https://www.terumobct.com/COBE-Spectra) to perform a number of apheresis procedures, including peripheral blood mononuclear cell collections for HCT. We are using HCT, in conjunction with chemotherapy, to perform both autologous and allogeneic HCT in dogs with hematologic malignancies, including high-grade B- and T-cell lymphomas and lymphoid leukemias.

Initial Studies
Our initial data was generated using a Baxter-Fenwal CS-3000 Plus with Access Management System continuous blood separator (Fenwal, Baxter Healthcare Corporation, Deerfield, IL) that were kindly donated by the Mayo Clinic, Rochester, MN. While using a slightly different technology than the TerumoBCT machines, we were able to refine our apheresis procedure and show that the use of rhG-CSF ((Neupogen, Amgen, USA: 5µg/kg SQ BiD x 5 days) clearly increased the peripheral white blood cell count and increased the number of CD34+ cells in the final harvest product.
Figure 1 Total white blood cell count of 6 normal dogs receiving rhG-CSF for 5 days. In all dogs, the target dose of 30,000/ul was reached. All dogs received a double dose of Neupogen (10 ug/kg) 2 hours before the apheresis procedure.

Figure 2 Flow cytometric analysis of the apheresis product from a normal rhG-CSF treated dog. The left dot plot represents forward scatter (x-axis) vs side scatter (y-axis) of the harvested product with gating (circle) on the cells that are analyzed for CD34/CD14 content in the right dot plot. The CD34+ percentage was 0.35% (upper left)

Figure 3 Flow cytometric analysis of the apheresis product from an rhG-CSF primed treated dog. The CD34+ % of this product was 3.5% (upper left hand quadrant). Based on the total number of cells harvested in 50 mls of product, ~1.94 x 10^7 CD34+ cells/kg were harvested.

In all 6 dogs receiving rhG-CSF, the target dose of 2 x 10^6/kg CD34+ cells/kg was easily achieved, with a range of 7.8 x 10^6/kg to 3.3 x 10^7/kg. In addition, all dogs tolerated the procedure extremely well, with no evidence of any clinical abnormalities with appropriate monitoring and support.13,14

CLIENT-OWNED DOGS WITH HEMATOLOGIC MALIGNANCIES

Autologous B-cell lymphoma: Based on our results and previous reports documenting the utility of this
procedure when treating dogs with hematologic malignancies, we began performing HCT in conjunction with chemotherapy on client-owned dogs diagnosed with hematologic malignancies beginning in November, 2008. All dogs were diagnosed with a hematologic malignancy by their referring veterinarians and received standard multi-agent systemic CHOP-based chemotherapy (cyclophosphamide, adriamycin, vincristine, prednisone).

Most of the dogs with B-cell lymphoma (24 total) were in a complete clinical remission before HCT, although five of these dogs had either relapsed with disease and driven back into remission or were not in clinical remission prior to HCT. All dogs received high-dose cyclophosphamide (300-650mg/m² IV) in an effort to clear their blood and bone marrow of all cancer cells two weeks before starting rhG-CSF for 6 days. Blood and bone marrow PARR (PCR for antigen receptor rearrangements) was performed upon presentation to NC State and all samples were negative using B cell primers. In all dogs, the target dose of >30,000 WBC/ul after six days of rhG-CSF was achieved (apheresis product analysis from 1 of these dogs are seen in Fig. 4).

![Figure 4](image)

**Figure 4** Flow cytometric analysis of the apheresis product from a dog in remission for B-cell lymphoma. A total of 4.726 x 10⁶ total cells were harvested. Based on 3% CD34+ cells, a total of 3.9 x 10⁶ CD34+ cells/kg were harvested, well above the target of 2 x 10⁶ CD34+ cells/kg.

After CD34+ cell enumeration, the apheresis products are refrigerated overnight. The following day, all patients underwent 10 Gy total body irradiation (TBI) using a previously described radiation protocol. The harvested cells were then returned to the patient intravenously immediately after TBI. The patients remained in the hospital for 2 weeks post-TBI for supportive care relating to TBI toxicities, which included variable gastrointestinal signs and severe cytopenias. All patients showed complete hematologic recovery during this time.

![Figure 5](image)

**Figure 5** Disease-free interval and overall survival time based on relapse or no relapse before HCT.

The median disease-free interval and overall survival (OS) of all dogs from the time of PBHCT was 271 and 463 days, respectively (Fig. 4). In addition, 33% (5/15) dogs who underwent HCT before relapse remain in clinical remission.

**Autologous T-cell lymphoma:** These 15 dogs received high-dose cyclophosphamide (500-750mg/m² IV) as described previously, and underwent apheresis with either the Baxter-Fenwal CS-3000 Plus cell separator (1) or the Terumo/BCT cell separator (14). Greater than 2 x 10⁶ CD34+ cells/kg were harvested from all
dogs. TBI consisted of 10 Gy (3), 11 Gy (9 dogs), or 12 Gy (2 dogs). One dog received only 8 Gy due to a dose miscalculation. HCT and hematologic recovery were similar to that previously described in the cohort of B-cell lymphoma dogs.

![Graph 1](image1.png)

**Figure 6** Disease-free interval and overall survival time of dogs with TCL.

The median disease-free interval and overall survival (OS) of the 13 dogs transplanted (2 dogs died in hospital) in first remission from the time of HCT were 184 and 240 days, respectively. Two of 13 (15%) dogs are alive 4 yrs and 5 yrs post-HCT.19

**Allogeneic HCT:** We have performed 5 allogeneic HCTs where a DLA-matched donor was located who donated peripheral blood CD34+ cells after 5 days of rhG-CSF priming (many more allogeneic HCTs have been performed in private practices). Zeke Ben-Dor: Dx splenic LGL leukemia via flow cytometry

![Graph 2](image2.png)

**Figure 7** Left: DLA-88 and DRB1 matching of Zeke and donor (#3). Right: Zeke exhibited complete donor chimerism within 2 weeks after HCT.

Zeke remains alive today (~5 yrs post-HCT) and is clinically normal.20 2/3 other allogeneic HCT dogs remain alive and in clinical remission >4 years post-HCT. One dog died of unrelated causes.

These results support the notion that canine patients with lymphoma can undergo autologous and allogeneic HCT in a clinical setting and become long-term survivors. Although we report an ~33% cure rate of canine B-cell patients (as defined as living >2 yrs post-HCT), we have much more to learn about the procedure and the long-term consequences of HCT. Therefore, more patients need to be transplanted and more time needs to elapse before we can begin to determine the true cure rate (surviving >2 yrs) of lymphoma using this technique. High-grade T-cell lymphoma remains a challenging disease to cure, even with chemotherapy and HCT. Finally, we now know that dogs with long-term GI issues, such as food sensitivity secondary to chronic allergies, dogs with chronic pruritis, and dogs with long histories of vomiting/diarrhea before HCT are at an increased risk for transplant-related mortality.

**Keywords:** Canine, Bone Marrow, Autologous, Transplantation

**References**

(20) Suter SE et al. JAVMA, Vol 246, No. 9, May 1, 2015.
Diagnosis and Treatment of Feline Lymphoma

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Summary
Feline lymphoma (LSA) is much less common in cats than dogs, although it is the most common (~25%) neoplasia in cats. It is the most common hematologic malignancy in the cat, with the GI form representing ~80% of cases. Generalized lymphadenopathy is an uncommon presentation for cats with LSA. Clinical signs are dependent on the body system affected. Common anatomic sites include alimentary, mediastinal, nasal, renal, cutaneous and multicentric. Due to the changes in FeLV testing and vaccination, there has been a shift in the anatomic distribution of feline LSA over the past 20 years. Whereas the mediastinal form, occurring in young FeLV+ cats previously predominated, we are now seeing more of the alimentary form in older, FeLV- cats. Given the anatomic distribution in cats, diagnosis is more often achieved through histopathology after exploratory laparotomy or endoscopy. Needle aspiration cytology of enlarged peripheral lymph nodes in cats can sometimes be difficult to interpret, as cats are subject to a variety of lymphoid hyperplastic conditions, such as IBD, that can mimic LSA cytologically. There is also a high degree of suspicion that chronic low-grade IBD in cats can transform into a low-grade T-cell LSA. Furthermore, treatment options and outcome may vary considerably, depending on whether a feline lymphoma is diagnosed as “small cell, low-grade” or “intermediate to high-grade”. Finally, it can be difficult to distinguish between a low-grade LSA in cats with peripheral lymphocytosis and a true chronic lymphocytic leukemia (CLL).

Diagnosis
Clinical staging in cats with LSA is very similar to that in the dog. However, addition of FeLV and FIV serology is reasonable, due to its impact on prognosis and husbandry. A pre-treatment abdominal ultrasound can be helpful to establish a pre-treatment baseline in cats with alimentary LSA.

Immunophenotyping is clinically irrelevant with regard to prognosis in cats. Interestingly, most small cell gut LSAs (duodenum, jejunum, etc.) are T-cell in origin, while most stomach LSAs are B-cell in origin. Cats can also develop high-grade GI LSAs, most of which are T-cell in origin. Regardless, the prognosis of a cat with low-grade GI LSA or CLL is much better when compared to a cat with high-grade LSA. Cats can also develop an LGL (large, granular, lymphocyte) GI LSA, which has a very guarded prognosis.

Anatomic location of the LSA is a strong prognostic indicator. (1) Common anatomic sites include alimentary, mediastinal, nasal, renal, cutaneous and multicentric. See Table 1. Due to the changes in FeLV testing and vaccination, there has been a shift in the anatomic distribution of feline LSA over the past 20 years. Whereas the mediastinal form, occurring in young FeLV+ cats previously predominated, we are now seeing a great deal more of the alimentary form in older, FeLV- cats. Given the anatomic distribution in cats, diagnosis is more often achieved through histopathology after exploratory laparotomy or endoscopy. Needle aspiration cytology of enlarged peripheral lymph nodes in cats can also aid in finding a diagnosis, although they can be difficult to interpret, as cats are subject to a variety of lymphoid hyperplastic conditions that can mimic LSA cytologically. Anatomic locations associated with a very poor prognosis include high-grade renal and spinal LSA, although this author has seen many cats with these diseases live >1 yr.
Table 1 Anatomic sites of feline LSA and FeLV status

<table>
<thead>
<tr>
<th>Form</th>
<th>Frequency</th>
<th>Avg. Age</th>
<th>FeLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediastinal</td>
<td>20-50%</td>
<td>2-3 yrs</td>
<td>80%</td>
</tr>
<tr>
<td>GI/liver</td>
<td>15-45%</td>
<td>8 yrs</td>
<td>30%</td>
</tr>
<tr>
<td>Multicentric</td>
<td>20-40%</td>
<td>4 yrs</td>
<td>80%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>25-30%</td>
<td>3 yrs</td>
<td>80%</td>
</tr>
<tr>
<td>CNS</td>
<td>5-10%</td>
<td>3-4 yrs</td>
<td>80%</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>&lt;5%</td>
<td>8-10 yrs</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

Although there are not as many anti-feline antibodies against feline hematologic antigens as in dogs, flow cytometry may also be of some value when trying to diagnose LSA/CLL in cats. Many universities and commercial laboratories in the U.S provide feline flow cytometry.

PARR (PCR for antigen receptor rearrangements) can also be a valuable tool to rule in or rule out feline lymphoma. (2) PARR can be a helpful when the cytologic results of fine needle aspirates of enlarged abdominal LNs are equivocal. The sensitivity of feline T-cell PARR is ~90%, while the sensitivity of feline B-cell PARR is much lower, at ~60%. Therefore, feline B-cell PARR will give many false negative results.

Clinical signs

The clinical presentation is dictated by the form of LSA affecting the cat. Since the abdominal/gastrointestinal form is the most common, common clinical signs include: weight loss, anorexia, lethargy, vomiting, and/or diarrhea. Multicentric cases present clinically ill with peripheral lymphadenopathy. Mediastinal LSA often presents with respiratory signs such as dyspnea (from the mass or secondary pleural effusion), difficulty resting, and regurgitation (mass effect). Signs associated with the extranodal LSA also will depend upon location: Nasal: sneezing, epistaxis or facial deformity; CNS: paresis and/or paralysis; Renal: vomiting, anorexia, palpably bilaterally enlarged kidneys.

Treatment

Surgery: The vast majority of feline LSAs are not surgical diseases. However, there are instances where surgery is useful in debulking a tumor that is causing clinical signs. The most common instance is a large, obstructing GI mass leading to severe constipation, obstipation, or, in severe cases, perforation. (3) Surgery can ameliorate the acute clinical signs of obstruction quickly. Surgery can also be performed if a GI mass is large and infiltrating all the layers of the gut wall, predisposing the cat to a GI perforation once it responds to chemotherapy. This is most often seen with intermediate/high-grade GI LSA.

Systemic chemotherapy: Chemotherapy is considered standard therapy for the treatment of feline high-grade lymphoma at any location and is either used alone or in combination with radiation therapy (nasal, CNS, abdominal). Single agent chemotherapy with agents such as prednisolone, doxorubicin, and mitoxantrone reports response rates ranging from 9-40% and associated remission durations of 3-12 months. Multiple studies have documented improved remission rates, duration of remission and survival with combination chemotherapy. The most commonly used protocols are COP or CHOP. (4,5) This author always uses a rotating protocol consisting of prednisone, L-asparaginase, vincristine, cyclophosphamide, and doxorubicin, administered over a 25-week time frame. Although the literature suggests complete response rates of 30-65% and associated remission rates of 5-9 months, I have generally found that ~1/3 of the cats will not respond at all, ~1/3 will achieve a remission but the remission will only be for a few months, and ~1/3 will achieve a remission and have a MST of ~1 year. There is also a small subset of cats that live > 2 years. The non-responders generally succumb to their disease in 4-8 weeks.

The low grade LSA form, due to its indolent nature, may not need a traditional multi-agent protocol. It is extremely important to tell owners that cats with low-grade GI LSA may never have completely normal feces and may always have bouts of intermittent vomiting. The chronic GI irritation these cats have can
lead to intestinal scarring and villus blunting that may or may not resolve with treatment. Therefore, even if treated effectively, these cats can continue to have clinical signs of GI upset, although these should be significantly reduced.

**Radiation therapy (RT):** RT may be considered for nasal lymphoma and studies have reported disease free intervals of 6-69 months. There remains some controversy concerning the use of systemic chemotherapy in these cats, however a recent study found that ~40% of cats with nasal LSA went on to develop systemic disease. Therefore, I typically advise a course of CHOP therapy after RT is completed. Cats receiving a combination of RT and chemotherapy had longer survival than RT alone with many patients failing outside of the nasal cavity.

Two studies have evaluated a small number of cats with abdominal LSA treated with fractionated radiation therapy as part of a fist-line therapy and in a rescue setting. Treatment appeared well tolerated and preliminary results were promising. (6,7)

**Prognosis**
As described previously, the overall prognosis of cats with LSA is dependent on location and grade, while immunophenotype is much less important than in dogs. In general, cats with low-grade, indolent malignancies, most of which are GI in origin, can live for many years when treated with Leukeran/prednisolone. Several studies have since shown a high response rate (80-96%) with survival times of 700-900 days. After ~6 months of treatment, many of these cats can slowly be weaned off steroids completely and receive Leukeran only 2-3 times/week. Cats with CLL can also live for many years with treatment.

Anatomic locations associated with the worst prognosis include spinal (weeks) and renal (~3.5 months). The prognosis for cats with high-grade GI LSA with treatment is ~6-9 months, although the range of survivals is large. Cats with mediastinal LSA that present with clinical signs of dyspnea, malignant effusion, etc. have an overall poor prognosis. Similarly, cats diagnosed with LGL LSA (cytotoxic T-cell or NK cell) have a very guarded prognosis. (8)

**Keywords:** Lymphoma, Feline, PARR, Chemotherapy

**References**
Diagnosis and Treatment of Canine Mast Cell Disease

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Summary
Mast cell tumor (MCT) represents the most common cutaneous tumor in the dog (~25% of all cutaneous tumors), and is more common in dogs than cats. There is a large degree of variation in the histologic appearance and biologic behavior of canine MCT, ranging from histologically and behaviorally benign to histologically and behaviorally malignant. However, 65 to 80% of MCT will remain local diseases, therefore, local control can lead to complete cures. Malignant MCT are aggressive tumors that tend to spread via local lymphatics first, and then to internal organs such as the liver and spleen. For these tumors, both local and systemic control is needed. Knowledge of the signs associated with worrisome prognosis, and the steps to take to address the potential for recurrence and/or metastasis, can help to simplify the approach to this sometimes-frustrating neoplasm. Likewise, newer information regarding local and systemic treatment of MCT has increased the management options available for veterinarians and owners to consider.

Diagnosis
Canine MCT have been referred to as “the great pretender”, because they can look and feel like anything. This can include soft, subcutaneous masses that can feel exactly like lipomas. Thus, needle aspiration cytology should be offered for any lump or bump seen. Cytology is sufficient to achieve a diagnosis of MCT in approximately 90% of dogs. The classic appearance is a population of large round cells with central nuclei and abundant cytoplasm, with characteristic blue-purple cytoplasmic granules. Granules may not be visible in approximately 10% of MCT, which may confound the diagnosis in a small number of cases. In addition, standard Dif-Quik staining solutions (Romanowsky stain) may not stain a small percentage of MCT granules.

Staging
The majority of canine MCT low-grade I & II tumors, while locally aggressive, are unlikely to metastasize. However, some of these tumors can be biologically unpredictable and, as such, knowing some criteria that may help predict this aggressiveness may be helpful in designing a preoperative staging plan.

Prior studies have identified several prognostic factors associated with MCT: (1) Histologic grade is one of the strongest -- dogs with high-grade (grade III) tumors may die of their disease rapidly despite appropriate local therapy; (2) Clinical stage - Dogs with metastasis to regional lymph nodes or other structures at presentation have a less favorable long-term prognosis; (3) Location - Tumors in the preputial, perianal, oral, subungual (nail bed) and other mucocutaneous sites classically have worse prognoses; (4) Recurrence following initial surgical excision is a negative prognostic indicator; (5) The presence of systemic signs (anorexia, vomiting, hematemesis, melena) is a strong negative prognostic indicator, as it often indicates systemic dissemination; (6) Recent rapid growth or tumor ulceration are also worrying signs.

Animals with tumors displaying these criteria may have a higher likelihood of metastasis, and thus a thorough search for disease elsewhere is reasonable prior to undertaking definitive therapy. This may
also be reasonable in lower-risk patients if very expensive or aggressive treatment is likely to be necessary, or if the tumor is in a location not amenable to wide surgical excision. In the absence of these factors, it is reasonable to proceed immediately to surgery.

Complete staging for canine MCT should include cytologic evaluation of the regional lymph node, abdominal ultrasound, and thoracic radiographs. Of these tests, abdominal imaging and lymph node cytology are the most likely to yield important results. Cytology of abnormal lymph nodes or organs in the abdomen is indicated, however aspirates of structurally normal liver and spleen are rarely useful. If radical, expensive or potentially disfiguring surgery is being contemplated, an incisional biopsy may also be considered for histologic grading. Other tests such as bone marrow aspiration cytology and buffy coat smear are generally low-yield tests unless a dog has significant cytopenias or peripheral mastocytosis.

If no evidence of metastasis is found, appropriate local therapy can be pursued. Identification of disease in the regional lymph node means that this should be removed as well at the time of surgery, and that additional systemic therapy should be considered irrespective of histologic grade. Identification of disease beyond the regional lymph node usually means that surgery will be of little or no benefit.

**Treatment**

**Surgery:**

Even well-differentiated MCT are associated with aggressive local tissue infiltration. Thus, it is necessary to include a generous margin of normal-appearing tissue on all sides of the tumor (including deep), to insure any microscopic nests of tumor are removed. The standard recommendation is to remove a minimum of 3 cm of normal-appearing tissue 360 degrees around the tumor, and at least one normal fascial plane deep. The entire specimen should be submitted in one piece, preferably with the margins marked in some fashion, so that the pathologist can assess all margins for adequacy of excision. There is accumulating information, however, that surgical margins less than 3 cm may be sufficient in “tight spots”. This seems especially true for low/intermediate grade tumors, and those that are fairly small and well-demarcated.

**Interpreting the Pathology Report**

Two equally important pieces of information need to be gleaned from the pathology report: (1) Histologic grade; and (2) Adequacy of surgical margins. Pathologists often utilize a numeric grading scheme, where “Grade I” is well-differentiated and “Grade III” is poorly differentiated, however some pathologists will now utilize words such as “low, intermediate or high-grade” or “well, poorly or intermediately differentiated” in place of a numerical scale. (1)

Low or intermediate grade MCT with complete surgical margins usually require no further therapy, as the risk of recurrence or metastasis is only approximately 10%. However, regular rechecks for recurrence, metastasis, or new cutaneous masses is indicated. Low or intermediate grade tumors with incomplete surgical margins have a high chance of recurrence, but a low chance for metastasis. Thus, further aggressive local therapy is reasonable. When possible, immediate re-excision of the surgical scar (and an additional 3 cm tissue in all directions and another fascial plane deep) is the most useful treatment. The entire excised tissue should be inked and re-submitted for histopathology.

High grade MCT with complete surgical margins have a low chance for recurrence, but a high chance for eventual metastasis. Systemic therapy (e.g. chemotherapy) can be offered in an attempt to delay or prevent this. High grade MCT with incomplete margins have a high likelihood of both recurrence and metastasis: Therapy designed to address both of these possibilities (e.g. additional surgery or radiotherapy, with chemotherapy) is indicated.

There is recent information that assessment of mitotic index (a measure of the rate of proliferation, which can be assessed on any histology slide), may be a strong predictor of outcome, identifying intermediate grade tumors at high risk of spread and, potentially, high-grade tumors at lower risk of spread. (3) This should be provided on all MCT histopathology reports. Generally, a mitotic index >5/10hpf identifies a more
aggressive tumor. (2,3)

Special Stains and Other Tests
A variety of specialized histochemical or immunohistochemical tests for assessment of proliferation (agryophilic nucleolar organizer region, or AgNOR staining, Ki67, and proliferating cell nuclear antigen, or PCNA) have been evaluated for their predictive value and have likewise been demonstrated to correlate well with postsurgical outcome. However, it is not clear as yet whether these more cumbersome assessments provide any more prognostic information than that provided by simple assessment of mitotic index.

Recently, expression of KIT, a tyrosine kinase receptor for the hematopoietic growth factor stem cell factor (SCF), has been demonstrated in canine and feline MCT. Several studies have demonstrated, in 20-40% of canine MCT, the presence of mutations in the c-kit gene, leading to constitutive activation in the absence of bound SCF. In the multiple studies, MCT possessing c-kit gene mutations or altered subcellular localization as assessed by immunohistochemistry (e.g. a shift from the normal membranous location to an intracellular location) are associated with an inferior prognosis when compared to those with wild-type c-kit and normal KIT protein localization. Both c-kit gene sequencing and KIT protein immunohistochemistry are available through multiple academic laboratories in the U.S.

Caution Owners Against a “Wait and See” Approach
The importance of addressing the potential for local recurrence the very first time the tumor appears cannot be overstated. Owners should be strongly cautioned against adopting a “wait and see” attitude, with the intent of becoming more aggressive if/when the tumor grows back. Recurrent tumors are likely to grow more quickly, invade more deeply, and are more likely to ulcerate or become painful.

Nonsurgical Therapies
In addition to aggressive local surgery, several other local therapeutic modalities have been investigated for the adjuvant treatment of canine MCT. Radiation therapy (RT) has proven to be a very effective local treatment modality when combined with “marginal” surgical excision. 2-year control rates of 85 to 90% can be expected when incompletely excised low- or intermediate-grade MCT are treated with RT. Radiation therapy to bulky tumors is consistently less effective than RT to microscopic disease, with a one-year control rate of approximately 50%. (4)

Animals with undifferentiated MCT, MCT that have metastasized, or tumors in a historically unfavorable location (see above) may benefit from the addition of systemic therapy. (5) In addition, aggressive surgery or RT may be cosmetically unappealing or financially impossible for some owners. Recently, several studies have been published investigating various systemic therapies for measurable canine MCT, summarized in Table 1.

Table 1 Response to Medical Therapy in Measurable Canine Mast Cell Disease.

<table>
<thead>
<tr>
<th>Agent(s)</th>
<th>Number Treated</th>
<th>%CR a</th>
<th>%PR b</th>
<th>%ORR c</th>
<th>Median Resp. Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>25</td>
<td>4%</td>
<td>16%</td>
<td>20%</td>
<td>NR d</td>
</tr>
<tr>
<td>Vincristine</td>
<td>27</td>
<td>0%</td>
<td>7%</td>
<td>7%</td>
<td>NR</td>
</tr>
<tr>
<td>CCNU (Lomustine)</td>
<td>21</td>
<td>6%</td>
<td>38%</td>
<td>44%</td>
<td>79 d e</td>
</tr>
<tr>
<td>Pred/Vinblastine</td>
<td>17</td>
<td>33%</td>
<td>13%</td>
<td>47%</td>
<td>154 d</td>
</tr>
<tr>
<td>P/C/V</td>
<td>11</td>
<td>18%</td>
<td>45%</td>
<td>63%</td>
<td>74 d</td>
</tr>
<tr>
<td>COP-HU d</td>
<td>17</td>
<td>23%</td>
<td>35%</td>
<td>59%</td>
<td>53 d</td>
</tr>
<tr>
<td>Pred/VBL/CCNU</td>
<td>37</td>
<td>24%</td>
<td>32%</td>
<td>57%</td>
<td>52 wks</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>46</td>
<td>4%</td>
<td>24%</td>
<td>28%</td>
<td>46 d (for PRs)</td>
</tr>
<tr>
<td>Pred/Chlorambucil</td>
<td>21</td>
<td>14%</td>
<td>24%</td>
<td>38%</td>
<td>533 d</td>
</tr>
</tbody>
</table>

a CR = Complete response  
b PR = Partial response  
c ORR = Overall response rate  
d NR = Not reported  
e Excludes patient that experienced a CR, euthanized without evidence of disease after 440 days  
f P/C/V = prednisone/ cyclophosphamide/vinblastine

255
Canine Mast Cell Tumor and KIT
The majority of canine (and human) mast cell neoplasms express the tyrosine kinase growth factor receptor KIT, and a large minority of canine MCT (20-50% depending on the study) possess a mutation in the c-kit gene coding for the KIT protein. KIT codes for a transmembrane protein that serves as the receptor for the growth factor stem cell factor, important in the maturation of normal mast cells and other hematopoietic cells. Mutations can render KIT active even in the absence of bound stem cell factor, leading to unchecked growth. New molecules have been developed that inhibit signaling through the KIT tyrosine kinase, and these compounds are able to interfere with the proliferation of canine MCT in vitro. The 2 veterinary-approved molecules in this class are toceranib (Palladia, Pfizer) and masitinib (Masivet/Kinave, AB Science). Imatinib (Gleevec, Novartis), although not approved for veterinary use, has been demonstrated to have some efficacy in canine MCT. (6,7)

Both toceranib and masitinib are associated with objective response rates of ~30% in dogs who do not have a KIT mutation and ~60% in dogs who do have a KIT mutation. (6,7) Although these are oral medications, side effects are common, consisting of mainly gastrointestinal disturbance, mild hematologic changes, and protein-losing nephropathy.

Keywords: Canine, Mast Cell Disease, Chemotherapy, Grade

References
Cardiac Auscultation and Phonocardiography

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There is no one single test that is the best test for all dogs with heart disease, but a good physical examination is vital in all cases to help refine the clinical question. History and physical examination are crucial to success. Physical examination is itself a diagnostic test, and when carefully carried out, has better sensitivity and specificity than many of our other diagnostic tests in cardiac patients. The choice of test also depends on the question being asked: if the reason for diagnostic investigations is to identify the cause of a cardiac murmur, then echocardiography is probably the test of choice. If the question is whether an animal has congestive heart failure or not, the tests of choice will be physical examination and thoracic radiography. It is essential to determine whether any heart disease is present, but it is not always necessary to prove conclusively which type of cardiac disease. If cardiac disease is present, it is nearly always necessary to establish if the animal has signs of heart failure, or whether it is compensating well for any cardiac disease, as treatment usually depends on the stage of heart disease.

Physical Examination
Cardiac auscultation yields information on heart rate, heart rhythm, and presence of abnormal heart sounds (gallops and murmurs). Many dogs in heart failure will have increased sympathetic tone and therefore an increased heart rate. Sinus arrhythmia indicates high resting vagal tone, which suggests a dog is probably well with any heart disease. A fast, chaotic rhythm with pulse deficits suggests atrial fibrillation, which is reasonably specific for heart disease. Presence of a murmur in general does not necessarily indicate heart disease, as some functional murmurs may be present in the absence of structural cardiac changes. Careful characterization of a murmur (paying attention to point of maximum intensity, timing within the cardiac cycle, intensity, character, radiation and variability) will give much better specificity. It is often possible to shorten a differential list to just a few possible causes if a murmur is well characterized.

ECG
An ECG is indispensable when an arrhythmia is present. It is not always possible to identify the precise rhythm disturbance solely from auscultation, although specificity may be reasonably good for atrial fibrillation and some complete heart block cases. Waveform changes are not often helpful in mitral valve disease or dilated cardiomyopathy.

Thoracic Radiography
Together with physical exam, thoracic radiography is one of the most useful tests for identification of pulmonary edema/congestive heart failure. The combination of left atrial enlargement, distension of the pulmonary veins, and interstitial/ alveolar pulmonary infiltrates has high specificity for indicating left-sided heart failure. Radiography is less good for identifying the specific type of heart disease.

Echocardiography
Echocardiography is the best test for identifying the type of heart disease, but only if carried out by an
experienced and skilled operator. Doppler echocardiography allows precise characterization of hemodynamic disturbances. Even in skilled hands, there is still controversy over the cut-off points for diagnosing early heart disease in asymptomatic patients. Echocardiography is less useful for identifying congestive heart failure.

Cardiac Biomarkers
Blood tests for NT-proBNP and troponin-I are becoming increasingly available, and are attractive as markers of severity of heart disease. These biomarkers are not specific for different types of cardiac disease. NT-proBNP concentrations are higher in dogs with congestive heart failure than in dogs with asymptomatic heart disease, and may be helpful in differentiating cardiac causes of respiratory distress. Troponin concentrations are increased by cardiac myocyte damage.

Which test is best in the following clinical scenarios?

Old, Small Breed Dog with a Murmur (Asymptomatic)
A left apical holosystolic murmur in an older, small breed dog is likely to indicate degenerative mitral valve disease. An echo can confirm this, but experience and skill is required, and the pre-test probability is already high that the dog has mitral valve disease. It is more important to stage the disease, and this can be done with a careful history and imaging. Either thoracic radiographs or an echo can be used to establish whether there is left heart enlargement. Dogs with normal cardiac size and no left atrial (LA) enlargement are likely to remain asymptomatic for the next 12 months or so, and do not require any treatment. Dogs with LA dilation are at higher risk of developing congestive heart failure (CHF).

Older, Small Breed Dog with a Cough or Respiratory Signs
If a typical mitral murmur is present, it is important to determine whether there are other signs consistent with CHF, and whether the mitral valve disease is sufficiently severe for there to be a risk of CHF. In most instances, thoracic radiography is the most useful test. An echo can identify LA enlargement, and normal LA size can often rule out CHF. Thoracic radiographs have the advantage of identifying primary respiratory disease, although small airway disease can be difficult to identify. The respiratory rate recorded by the owner at home can be extremely helpful in distinguishing cardiogenic pulmonary oedema from airway disease - CHF is usually associated with a resting respiratory rate of > 40/min.

Large Dog with a Murmur and/or Arrhythmia (Asymptomatic)
Dilated cardiomyopathy (DCM) is the main differential diagnosis, but other non-cardiac disease can cause both murmurs (e.g., anemia) and arrhythmias (e.g., splenic masses). If an arrhythmia is present, an ECG must be obtained. Diagnosing preclinical DCM with echocardiography can be very challenging, so this is probably a situation where referral to a cardiologist may be valuable as there are important prognostic implications. A 24-hour Holter ECG should also be considered for dogs with arrhythmias.

Large Dog with a Murmur and/or Arrhythmia and Respiratory Distress
The important question for management is whether the dog has CHF, and this can be based predominantly on physical examination (tachypnea ± crackles/absent breath sounds ventrally; ascites with jugular distension) and thoracic radiographs. However, identification of LA enlargement by echo can support a clinical suspicion of CHF, and pleural effusion can be imaged directly. A 24-hour Holter ECG may be useful for longer term management if arrhythmias are present.

Dogs with Ascites, or Hypotensive Dogs with Weakness
Pericardial effusions are easily missed. Auscultation may be normal or heart sounds may be quiet, but cardiac tamponade is likely to result in a moderate tachycardia with pulses that wax and wane with respiration.
Jugular distension is one of the most useful physical exam findings with pericardial effusions. If suspected, pericardial effusions can be imaged with ultrasound, though sometimes it can be challenging to differentiate pericardial from pleural effusions (thoracic radiographs will do this more easily).

**BRADYCARDIC DOGS**
Sinus bradycardia is rarely associated with primary cardiac disease, and an echo is not likely to be very helpful. An ECG is essential to identify the arrhythmia, and choice of subsequent tests depends on whether pacemaker implantation is being considered.

**TACHYCARDIC DOGS**
The most important test is an ECG. Irregular tachyarhythmias are most likely to be atrial fibrillation, which is usually associated with structural heart disease. Regular tachyarhythmias should be managed differently, whether they are caused by ventricular tachycardia (VT) or supraventricular tachycardia (SVT). Dogs with VT will benefit from an echo to look for structural heart disease, as this is managed differently from VT due to non-cardiac disease. Uncontrolled SVT can cause a DCM phenotype, but this can be reversible if managed appropriately.

**A PUPPY WITH A MURMUR**
A murmur in a puppy may be an innocent functional murmur, or it may be caused by life-threatening congenital heart disease. Quiet, brief murmurs are more likely to be innocent, whereas loud, long murmurs are more likely to indicate congenital disease (especially a continuous murmur, which indicates a patent ductus arteriosus, which can be corrected).
Atrial Fibrillation; Electrophysiology, Diagnosis, Treatment and Prognosis

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TYPES OF ANTIARRHYTHMIC AGENTS

A. Class I Agents
Sodium channel blockers, responsible for phase 0 of the action potential. By slowing the upstroke and prolonging repolarization the conduction of a reentrant impulse can be changed.
   - Class Ia, prolongs AP duration by prolonging repolarization and also increase the refractory period, examples include procainamide and quinidine.
   - Class Ib, shortens AP duration by shortening repolarization and also increases the refractory period, examples include lidocaine and mexiletine.

B. Class II Agents
Beta blockers. Can be selective (beta 1) or non-selective (Beta 1 and 2), commonly used include propranolol, atenolol, and carvedilol. These agents suppress automaticity and can block triggered events since high sympathetic tone predisposes to afterdepolarization.

C. Class III Agents
Block the outward potassium channel delaying repolarization. No pure Class IIs are used, sotalol does have this effect plus non-selective beta blocking property. Amiodarone is getting much attention lately. Amiodarone also has Class I, II, and IV effects.

D. Class IV
Calcium channel blockers. Non-dihydropyridines are active in the heart, the others more in the vasculature. Diltiazem most commonly used.

Atrial Fibrillation (AF)
1. Atrial fibrillation is caused by multiple simultaneously occurring disorganized atrial impulses that bombard the AV node. During AF there is rapid atrial activation and the atrial rate can range 400-600 depolarizations/minute.
2. ECG characteristics:
   a. R-R intervals are irregularly irregular.
   b. No P-wave
   c. QRS complexes are usually narrow and upright in leads II. The QRS complex can be wide in appearance if a bundle branch block is present.
   d. Atrial activity is represented by fibrillatory (f) waves of varying amplitudes.

WHAT THERAPY FOR SUPRAVENTRICULAR TACHYCARDIA?

Supraventricular Tachycardia (atrial fibrillation, atrial flutter, atrial tachycardia)
Therapeutic goals of managing SVT are to improve clinical signs and long-term prognosis either by controlling ventricular response rates (rate-control) or by converting SVT to NSR (rhythm-control). Pharmacological
rate-control for SVT with diltiazem and digoxin remains central to long-term management in dogs. These pharmacological therapies exert their beneficial effect via modulation of AV nodal conduction by prolonging the refractory period of the AV node. A resting average HR of 160 bpm or less on an ECG in the hospital has been suggested as the target HR for rate-control. Survival outcome is significantly better in dogs with adequate HR control (<160 bpm) in comparison to those with poor rate-control.

1. Diltiazem XR 1-2 mg/kg BID: Calcium channel blocker that will suppress AV nodal conduction, more powerful effect than digitalis in regard to rate control.
2. Sotalol 1-2 mg/kg BID: effectively decrease heart rate of SVT. They may decrease cardiac output so that they can compromise a patient with CHF.
3. Digoxin 0.003 mg/kg BID: Will help to suppress AV nodal conduction. Good for use in CHF since it improves cardiac function mildly. Check digoxin levels 1 week after starting. Goal used to be to have a trough level (6 to 8 hours post pill) of 1–2 ng/ml.

Transvenous electrical cardioversion (TVEC)
Large breed dogs often develop atrial fibrillation (AF) or atrial flutter (AFL) in the absence of underlying heart disease, known as lone AF or AFL. Sustained AF or AFL can cause left ventricular systolic dysfunction and chamber enlargement. Therefore, it can be speculated that lone AFL can lead to atrial structural and electrophysiological remodeling, providing a possible explanation for the progressive nature of this arrhythmia to AF. Since lone AFL itself could lead to atrial fibrosis, conversion to normal sinus rhythm (NSR) may have substantial clinical relevance and prognostic benefit by preventing irreversible atrial remodeling. Low energy TVEC is an effective technique for restoring NSR by delivering direct current to the atrial myocardium in dogs with AF or AFL regardless of underlying heart disease and chronicity of atrial arrhythmias. Theoretical benefits of conversion to NSR are to improve stroke volume and exercise performance, and to prevent AF-induced electrical and structural remodeling in the atria, which will ultimately improve long term prognosis.
Cardiac Interventional Procedures I

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Congenital Heart Disease

Prevalence
Overall prevalence of congenital heart disease in the dog has been reported to be around 0.5-0.9%. Patent ductus arteriosus (PDA) is the most frequent abnormality, followed by pulmonic stenosis (PS). In cats, the estimated prevalence of congenital heart disease is 0.2-0.4%. Ventricular septal defect (VSD) was most common.

Innocent (Puppy) Murmurs - These are a normal finding in many puppies. They are always Left Basilar, Early Systolic, Soft (Grade 1-2) and often Musical in Quality. They are due to relatively high velocity flow through the great vessels in puppies, and the fact that many puppies have a slight anaemia. Innocent murmurs should be gone by 16 weeks of age.

Congenital Cardiac Murmurs - Any murmur which has a position, timing or intensity which does not fit an innocent murmur should be investigated. Loud or split second heart sounds should also be investigated in puppies.

Patent Ductus Arteriosus

Prevalence - PDA is the most common congenital heart disease in the dog (30% of congenital defect).
Normal Development - The Ductus Arteriosus develops from the Left Sixth Aortic Arch. It is present to shunt blood from the PA to descending aorta in the fetus. Normally initial muscle contraction decreases flow dramatically within 12-14hrs after birth. Generally closure is complete by 7-8days.
Pathophysiology - Left to right shunting occurs due to the higher systemic pressure than PA pressure in most patients. This leads to continuous shunting, and thus a continuous murmur. The leads to increased pulmonary blood flow, and increased return to the LA and LV. This volume overload leads to eccentric LV hypertrophy, Dilation of the LA, Ascending Aorta, PA and overcirculation of the lungs.

Clinical Signs and Physical - Puppies can remain asymptomatic, develop left sided CHF or show poor growth. Arterial pulses are hyperkinetic and a continuous murmur loudest over the left base is heard. A systolic murmur at the left apex may also be present due to MR secondary to the left ventricular dilation.

ECG - P-Mitral (wide p-waves) and LV enlargement pattern (tall R-waves) are usually present.

Radiography - This shows pulmonary overcirculation, LA and LV dilation and on the DV a Ductal Bump (Ascending Aortic Dilation – 1 O’Clock position on the DV), PA (2 O’Clock) and Left auricular appendage dilation (3 O’Clock).

Echocardiography - LV and LA dilation, Dilation of the ascending aorta and PA dilation are all seen on 2D echo. LV systolic function can be apparently normal, mildly depressed or severely depressed (leading to a hypodynamic heart and lower than normal FS).

Natural History - With Left to Right PDA, 64% of dogs will be dead within 1 year without closure of the lesion. Some dogs with PDA can survive until maturity, and some can even live to 10 years of age, but this is the minority of cases.

Treatment - Left to Right PDA - closure of the PDA is recommended. This can be by surgical ligation
or thromboembolization (Giantrco Coil, Amplatz Canine Ductal Occluder). Generally it is not beneficial to delay the procedure. Surgical Ligation - Several publications have shown that a >85% success rate should be expected with surgical ligation of PDA's. However, there is always a small residual risk of rupture of the ductus, even with a highly experienced surgeon. Thromboembolization - Overall a success rate for closure approaching 99% should be expected. Prognosis following thromboembolism is excellent. Either technique should have a greater than 99% success rate. Trivial persistence of ductal flow (on echo) following either procedure is common, but clinically irrelevant.

**Prognosis** - Following closure of left to right shunt, prognosis appears to be excellent, with LV dilation tending to return towards normal, as well as normalization of systolic dysfunction. Mitral regurgitation, when present pre-operatively is likely to persist in the medium term at least. This leads to a left apical systolic murmur. Prognosis is guarded when advanced CHF or severe systolic dysfunction are present pre-operatively.

### Pulmonic Stenosis

**Histology** - Thickening of the valve Spongiosa and Bands of Fusiform Cells in a Dense Collagen Network are observed. These changes represent overproduction of the normal valve elements or a failure of conversion of the embryonic valve primordial.

**Pathophysiology** – Obstruction to outflow from the RV leads to Elevated RV Systolic Pressures. This then stimulates Right Ventricular Concentric Hypertrophy. The stenosis leads to elevated blood flow velocity across the valve (since Flow = Velocity/Area) and this jetting of blood leads to a Post Stenotic Dilation of the PA. RV Stiffness is increased (due to the concentric hypertrophy) and this leads to an exaggerated jugular pulsation. Elevated RV pressure leads to reduced RV coronary flow.

**Clinical Signs** - PS is common in certain breeds, including English Bulldog, Boxer, Beagle, Miniature Schnauzer, Labrador, Mastiff, Newfoundland, Terriers and Spaniel Breeds. Many dogs present with Asymptomatic Murmurs. Symptoms when present are usually due to either Low CO (Syncope, Exercise Intolerance, Lethargy) or right-sided CHF (if the tricuspid valve becomes incompetent). It is also possible for Right to Left Shunting to develop (patent foramen ovale) leading to cyanosis. **Physical Examination** - Prominent Jugular Pulse (due to enlarged a-waves), Left basilar systolic murmur and sometimes a right apical systolic murmur (tricuspid insufficiency) are present.

**ECG** - This shows a right ventricular enlargement pattern, and may show p- pulmonale.

**Radiography** - Right sided cardiomegaly is sometimes appreciated. More consistently, a Post-Stenotic Dilation of the PA is present (bulge at 2 O’clock position on the DV), with pulmonary under-perfusion. 100% of dogs with PS showed RV enlargement, 65% PA Dilation and 65% Diminutive Pulmonary Vessels.

**Echocardiography** - RV Hypertrophy, Infundibular Hypertrophy (with dynamic outflow obstruction on spectral Doppler), Dilation of PA, Pulmonary Annulus Hypoplasia and Thickening and/or Doming of the Pulmonic Valve Cusps. Doppler studies show elevated blood flow velocity at the level of the valve. Estimation of the instantaneous pressure gradient can be achieved using the modified Bernoulli Equation. PS lesions are graded according to the following scheme. Mild - Pressure gradient across the lesion of 20-50mmHg. Moderate - Pressure gradient across the lesion of 50-80mmHg. Severe - Pressure gradient across the lesion of Greater than 80mmHg.

**Natural History** - Dogs with mild to moderate PS generally live normal life expectancy. Dogs with severe PS may develop Sudden Death (arrhythmia), Syncope, Tricuspid Insufficiency (leading to right sided CHF), RA dilation and Atrial Fibrillation (due to TR or elevated RV diastolic pressure).

**Treatment** - Affected dogs should not be bred. Doppler gradients of >80 mmHg seem to be associated with decreased life expectancy, and thus balloon dilation should be recommended. Beta Blocker therapy is unproven for reducing the risk of sudden death or increasing life expectancy in dogs with PS.

**Balloon Valvuloplasty** - This is particularly successful when commissural fusion is the major underlying pathology, although some benefit may still be gained even with thickened leaflets. Continued improvement in pressure gradient following balloon dilation has been reported up to 90 days after the procedure. Balloon
dilation was associated with a 53% reduction in hazard ratio for sudden death. Median age for sudden death was 9 months in untreated dogs.

**Post-balloon valve pathology** - Following balloon, it is common to see pulmonic insufficiency. The aim at the time of the procedure is to reduce the pressure gradient by 50% or below 50mmHg. Both of these are considered to be successful results, as it is possible for pressure gradient to decrease further in the post-operative period.
Cardiac Interventional Procedures II

SeungWoo JUNG

Assistant Professor of Cardiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

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Updates of Hypertrophic Cardiomyopathy in the Cat

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Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats. The echocardiographic features of HCM are highly variable and quite heterogeneous. The hypertrophy of the left ventricle can be variable in severity and also in its location, and asymmetrical hypertrophy seems common. This makes traditional one-location measurements unreliable. Measurements are therefore often performed from a two-dimensional diastolic image and measured at different locations. Papillary muscle thickening is also a common but variable feature.

What are the most common causes of heart murmur in cats?
A murmur or a gallop sound is incidentally discovered at routine visits (vaccinations, neutering). The finding of a murmur, which raises suspicion of heart disease, may cause problems in deciding anesthetic procedures. Furthermore, a proportion of these cats are used for breeding. The presence of heart disease in these cats will inevitably be problematic for the breeder. Therefore, it is of importance to correctly identify the cause of heart murmur and heart disease in cats with suspected cardiac disease in order to establish prognosis, predict possible complications, and institute adequate therapy.

How do we determine left ventricular hypertrophy (LVH) in cats?
A simplistic cut-off value for LV wall thickness is used to define LVH in cats. However, there is poor agreement on the appropriate techniques for measuring wall thickness in cats, or the optimal cut-off values.

What are differential diagnoses for LVH in cats?
Hypertrophic cardiomyopathy (HCM) in cats is defined as the presence of left ventricular (LV) hypertrophy without other systemic causes such as hyperthyroidism, acromegaly and systemic hypertension. Diastolic function tests during echocardiography often help further characterization of HCM.

Can a cat have HCM without LVH?
Over 1,000 genetic mutations have been identified in familial HCM of humans, and some individuals are mutation-positive but have a normal cardiac phenotype. Currently only two mutations have been discovered as a cause of feline HCM. Cats negative for two mutations discovered can develop HCM, and some mutation-positive cats never develop LVH because of genetic heterogeneity.

What are clinical sequelae of HCM?
Cats with enlarge left atrium often present congestive heart failure (CHF), aortic thromboembolism and cardiac sudden death. Although the primary abnormality in HCM is diastolic dysfunction and delayed relaxation, some HCM cats develop systolic dysfunction. These cats often exhibit signs of CHF and carry poor long term prognosis.
What is steroid-induced CHF in cats with HCM?
The administration of steroids for non-cardiac purposes can lead to acute CHF in some HCM cats. These cats seem to recover clinically from CHF, and their enlarged left atrium tends to reverse-remodel back to its normal size with adequate medical management and monitoring.

What is restrictive cardiomyopathy (RCM)? Is this an aberrant form of HCM?
Restrictive cardiomyopathy (RCM) is a poorly recognized cardiomyopathy characterized by non-dilated, non-hypertrophied ventricles with diastolic dysfunction resulting in dilated atria and variable systolic function. Although RCM shows diastolic dysfunction mimicking HCM, it carries a separate entity of unknown cause from HCM. Since left atrial enlargement is one of the characteristic features of RCM, cats with RCM often undergo rapid cardiac progression and present CHF and aortic thromboembolism. There is little consensus on the diagnostic criteria for restrictive and unclassified cardiomyopathies in cats.
Myxomatous mitral valvular degeneration (MMVD) is the most common heart disease in dogs, and is characterized by valvular thickening and regurgitation, leading to secondary changes in cardiac structures and function. The prevalence of the disease has been correlated with the age and the breed. Small breed dogs, such as the Cavalier King Charles spaniel (CVKC), are predisposed to the disease and the disease prevalence in the CVKC dogs older than 10 years is greater than 90%. Given that the disease is common, but only a minority of dogs will progress to heart failure, it would be useful if there were a way in which those animals at greatest risk of experiencing progressive disease could be easily distinguished. With the identification of a high-risk subpopulation, subsequent monitoring and therapy could be more effectively targeted towards those patients at greatest risk. Early signs of decompensation could be identified, and treatment could be introduced from the earliest stage at which it is known to be effective.

Is mitral valve prolapse (MVP) the same disease as MMVD?  
In dogs, the vast majority of clinically significant mitral regurgitation (MR) is caused by primary myxomatous disease. MVP is defined by echocardiographic evidence of superior displacement of the valve leaflet above the valve annular plane. While MVP in humans is typically regarded as a separate disease entity with a natural history that is often different from that of primary MR, MVP in the dog is typically regarded as part of the clinical findings associated with MMVD.

Are characteristics of MMVD in Maltese the same as in Labrador Retriever?  
MMVD also occurs in large breed dogs, although the disease characteristics are somewhat different from small breed dogs. MMVD in large breed dogs characterizes that the degree of AV valvular thickening is less prominent, and they tend to show some degree of left ventricular systolic dysfunction on echocardiogram earlier. The interventricular septal motion is often normal to hyper-dynamic, while left ventricular free wall motion is markedly decreased. Fractional shortening (FS) and ejection fraction (EF) are lower, and end-systolic volume index (ESVI) is higher than normal limits. In comparison, small breed dogs with MMVD show hyper-dynamic left ventricular performance (higher than normal FS) and prominent valve thickening. Furthermore, episodes of cardiac arrhythmias such as ventricular arrhythmias (VA) or atrial fibrillation (AF) are frequent in large breed dogs with MMVD and anecdotally appear to adversely impact the quality of life and overall long term survival.

What are clinical risk factors for rapid cardiac progression?  
Many dogs with the disease remain at the preclinical stage, and some dogs do progress more rapidly to more severe forms than others. The risk factors associated with a more rapid progression include: breed, family history, age, left atrial size, and degree of regurgitant volume. The presence of ruptured chordae tendineae certainly also indicate a more rapid progression, although this finding is most commonly found in dogs with progressed valvular changes and severe MR. Thus, there is a need for clinical parameters that have prognostic and/or diagnostic value. The left atrial dimension, vertebral heart score, natriuretic
peptides, heart rate variability, degree of left ventricular systolic dysfunction, and severity of pre-renal azotemia are reliable parameters in predicting the presence of decompensated heart failure.

**What are clinical sequelae of MMVD?**
Early medical treatments prior to congestive heart failure (CHF) have not shown to delay disease progression. Only subpopulation of dogs with MMVD undergoes cardiac progression and develops life-threatening CHF. CHF is strong risk factor for development of AF and poor survival outcome. AF is the most commonly presented supraventricular arrhythmia in dogs. Cardiac structural changes that increase atrial wall stress predispose to AF. Hemodynamically loss of atrial contraction and shortened diastolic filling time due to irregular and typically rapid ventricular response rate in AF decrease cardiac output and elevate atrial filling pressure, both of which ultimately contributes to the further deterioration of CHF and the quality of life. Pulmonary hypertension may occur concurrently in dogs with left-sided heart disease. Hypoxia-induced pulmonary hypertension may occur in the setting of left-heart failure due to pulmonary edema.

**What is the clinical significance of pulmonary hypertension (PH) in dogs with MMVD?**
The definition of pulmonary hypertension is a mean pulmonary artery pressure of greater than 25 mmHg at rest. In veterinary medicine, pulmonary hypertension (PH) has been described as echocardiographically estimated pulmonary arterial systolic pressure based on a peak systolic tricuspid regurgitation gradient. Pulmonary hypertension can be further classified as pulmonary arterial hypertension (PAH) (i.e. pre-capillary, resulting from abnormalities on the arterial side of the pulmonary vascular system, also described as “active”) or pulmonary venous hypertension (PVH) (i.e. post-capillary, resulting from pulmonary venous hypertension associated with left-sided heart disease and leading to pulmonary capillary hypertension, also described as “passive”). Pulmonary venous hypertension is a common finding associated with left-sided heart disease, particularly MMVD in dogs. It appears that PVH secondary to left-sided heart disease is associated with high mortality. In general, MMVD-related PVH appears to be the most common cause of PH in dogs and the severity is typically mild to moderate.

**What are medical options for congestive heart failure secondary to MMVD?**
MMVD in human is considered a surgical disease as mitral valve repair is the treatment of choice in patients with moderate to severe disease. In contrast, MMVD in dogs is primarily a medical disease. Treatment of MMVD with ACEIs in dogs with earlier asymptomatic stages of disease is controversial. No data regarding treatment of dogs at risk for development of MMVD are available. CHF is a common endpoint of most cardiovascular diseases, even though medical managements with diuretic drugs, angiotensin converting enzyme inhibitors (ACEIs), positive inotropic drugs and vasodilators alleviate clinical signs and prolong survival time. Despite advances in medical therapy, the prognosis of dogs with CHF due to MMVD remains very poor. Clinical conditions such as chordae tendineae rupture, left atrial wall tear or cardiac arrhythmias have been recognized as negative prognostic factors.

It is likely that the optimal system for prediction of outcome, that will have the greatest value for general practitioners, will consist of a combination of simple-to-obtain measures that do not require advanced expertise or expensive equipment to obtain. In this setting, the combination of biomarkers with several readily identifiable clinical features of the disease is likely to be of the greatest value. The application of a validated simple-to-use system, in combination with evidence-based interventions known to improve outcome in patients with MMVD, has the potential to have a positive impact on the large population of patients affected by this condition.
Internal Medicine

August 30(Wed) 16:20~17:05, Premier Ballroom B

Diagnosis, Treatment and Prognosis of Pulmonary Hypertension

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INTRODUCTION
Pulmonary hypertension (PH) is defined as estimated pulmonary arterial systolic pressure greater than ~ 30 mm Hg. Pulmonary hypertension can be further classified as pulmonary arterial hypertension (PAH) (i.e., pre-capillary) or pulmonary venous hypertension (PVH) (i.e., post-capillary). Pulmonary arterial hypertension is influenced by pulmonary blood flow, pulmonary vascular resistance (PVR), and pulmonary venous pressure. Pulmonary arterial pressure and pulmonary capillary wedge pressure (PCWP) can be directly measured, invasively. Pre-capillary PH occurs when there is an elevation in PAP (i.e., increased mean PAP) as a result of an increased PVR with a normal left atrial pressure (i.e., normal PCWP). Diseases that result in pre-capillary PH include: idiopathic PAH, heartworm disease, congenital systemic-to-pulmonary shunts, necrotizing vasculitis/arteritis, pulmonary disease and pulmonary thromboembolic disease. In contrast, the definition of post-capillary PH is an elevated PAP as a result of increased PCWP and a normal PVR. The phenomenon of post-capillary PH occurs with mitral valve disease and cardiomyopathy.

PATHOLOGY AND PATHOPHYSIOLOGY
The normal pulmonary circulation is comprised of a vast network of thin-walled arteries, veins and capillaries characterized by a very low vascular resistance, low pressure and high capacitance. Pulmonary hypertension occurs secondary to an imbalance of endogenous and exogenous pulmonary arterial vasoconstrictors and vasodilators resulting in pulmonary arterial intimal proliferation, medial hypertrophy, vasoconstriction and thrombosis.

Endothelin Pathway
Endothelin-1 (ET-1) is released by pulmonary vascular endothelium resulting in potent pulmonary artery and vein vasoconstriction, smooth muscle cell proliferation and increased collagen synthesis. A primary stimulus for increased ET-1 concentrations is heart failure due to increased local production, decreased pulmonary clearance and upregulation of endothelin-A receptors (ETA). In addition to powerful affects influencing PH, ET-1 also results in sodium and water retention, potentiation of other neurohormonal systems and promotion of myocardial hypertrophy, fibrosis and remodeling.

Prostanoid Pathway
Prostacyclin and thromboxane A2 are endogenous arachidonic acid metabolites produces from pulmonary artery endothelial cells and platelets involved in maintenance of vascular tone in normal physiologic and pathophysiologic states. Prostacyclin is a vasodilator, inhibitor of platelet activation and has antiproliferative effects on the pulmonary artery. In contrast, thromboxane A2 is a potent vasoconstrictor and promotor of platelet activation. In cases of pulmonary hypertension, the balance of protacyclin and thromboxane A2 formation favors thromboxane A2 leading to vasoconstriction, cellular proliferation and thrombosis.
Nitric Oxide Pathway
Nitric oxide (NO) is synthesized endogenously from L-arginine and oxygen by nitric oxide synthase isoenzymes in the pulmonary artery vascular endothelium. Nitric oxide activates guanylate cyclase and subsequently cyclic guanosine monophosphate (cGMP) which results in a reduction of intracellular calcium concentration. The NO pathway enhances smooth muscle cell relaxation, inhibits smooth muscle cell proliferation and hypertrophy, and inhibits platelet aggregation and adhesion. cGMP is rapidly inactivated by phosphodiesterase (PDE), particularly PDE-5 isoenzymes. In experimental models and clinical studies of heart failure in people, a deficiency in basal NO synthesis as well as a decrease in endothelial release and response to NO has been found, suggesting that the loss of NO-dependent vasodilation and anti-cellular proliferation may contribute to the production of PH through vascular remodeling.

DIAGNOSIS

Invasive Hemodynamic Assessment
Right heart catheterization can provide multiple hemodynamic parameters that aide in the diagnosis and etiologic classification of PH. Right heart catheterization provides hemodynamic information regarding the right atrial, right ventricular, pulmonary artery and pulmonary capillary wedge pressures.

Echocardiography
Echo can estimate the systolic, diastolic and mean PAP. The peak systolic TR velocity and gradient reflects the estimated peak systolic right ventricular pressure and is used to classify PH as mild (≥ 3.0 to < 3.5 m/s, ≥ 36 to < 50 mm Hg), moderate (3.5–4.3 m/s, 50–75 mm Hg) or severe (> 4.3 m/s, > 75 mm Hg). Other parameters that echo can evaluate include: pulmonary artery systolic flow profiles, Tei index of myocardial performance, systolic time intervals and right ventricular tissue Doppler.

TREATMENT
Pulmonary hypertension treatment focuses on targeting the derangements that result in medial hypertrophy, intimal proliferation and decreased vascular compliance. Pulmonary vasodilating drugs currently in use target the pathophysiologic abnormalities associated with the pulmonary arterial endothelin pathway (endothelin receptor antagonists), prostanoid pathway (prostacyclin analogues), and nitric oxide pathway (specific or non-specific phosphodiesterase inhibitors). Sildenafil (Viagra, Revatio) is an orally active, highly selective PDE-5 inhibitor. Multiple studies have demonstrated the benefits of sildenafil in dogs with PH. Sildenafil appears to produce beneficial effects in PH by multiple mechanisms, but the primary mechanism operative in PH patients appears to be direct pulmonary artery vasodilation.
Paradigm Shifts in Modulating the Renin-Angiotensin-Aldosterone System

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Renin-Angiotensin-Aldosterone System
Control of circulating blood volume is a tightly regulated physiological process, and is critical for maintaining cardiovascular homeostasis. One of the most important neurohormonal adaptations on heart failure is activation of the renin-angiotensin-aldosterone system (RAAS), which leads to increased water retention through multiple mechanisms.

Aldosterone Escape
Interestingly, despite the use of ACE inhibitors and angiotensin-receptor blockers (ARBs), aldosterone levels increase in 30-40% patients with heart failure. This phenomenon is often referred to as ‘aldosterone escape’. Although circulating levels of aldosterone initially decrease in patients who are treated with ACE inhibitors and ARBs, in some patients’ aldosterone levels will increase to greater than pretreatment levels.

Natriuretic peptides
A variety of feedback mechanism are normally activated to offset the effects of the RAAS on sodium and water retention. Natriuretic peptides are among the most important RAAS counter-regulatory hormones. These peptides functionally unload the heart through peripheral vasodilation as well as by inducing renal excretion of sodium and water. Neprilysin is a membrane-bound enzyme that catalyzes the degradation of a number of endogenous peptides, most notably the natriuretic peptides, as well as angiotensin II and bradykinin. Inhibition of neprilysin is an attractive strategy to increase natriuretic peptide levels and achieve the vasodilation and natriuresis these peptides produce.

Angiotensin-Neprilysin inhibitor¹
In the recent clinical trial (PARADIGM-HF trial) in human cardiology, the use of an angiotensin receptor-neprilysin inhibitor resulted in striking reduction in all-cause mortality, cardiovascular mortality, and heart failure hospitalizations when compared with the use of an ACE inhibitor (enalapril). Entresto (Novartis, Switzerland) is the first of a new class of drugs referred to as ARNIs (angiotensin-receptor-neprilysin inhibitors) that contains equimolar amounts of valsartan (angiotensin receptor blocker) and sacubitril (neprilysin inhibitor). Entresto is associated with 20% decrease in the primary end point of death from cardiovascular cause of first hospitalization for heart failure compared with enalapril. In addition, Entresto reduces blood pressure in patients with hypertension and produces greater reduction in blood pressure in patients with mild-to-moderate hypertension than an equivalent dose of valsartan alone.

Reference
Small Intestinal Dysbiosis in Dogs and Cats – Is it Real?

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Summary
It has long been recognized that some dogs and cats have diarrhea that responds favorably to antibiotic therapy. More recently, it has been determined that the microbial ecosystem within the gastrointestinal lumen is important for gastrointestinal and systemic health and that alterations of the gastrointestinal microbiota (i.e., dysbiosis) can lead to clinical signs. The diagnosis of small intestinal dysbiosis can be based on measurement of serum cobalamin and folate concentrations, determination of the fecal dysbiosis index, and/or response to therapy. The treatment of small intestinal dysbiosis can include the use of prebiotics, probiotics, synbiotics, antibiotics, or fecal transplantation.

Introduction
The microbiota is the collection of all microorganisms in the GI tract, including viruses, bacteria, and fungi. A physiologic microbiota is essential for gastrointestinal and overall health of the host. Intestinal dysbiosis describes a situation where the intestinal microbiota is altered in either composition or numbers, which can lead to clinical signs of gastrointestinal disease. There are several different terms that describe similar clinical conditions: antibiotic-responsive diarrhea (ARD), tylosin-responsive diarrhea, small intestinal bacterial overgrowth (SIBO), and intestinal dysbiosis. For the purpose of this presentation antibiotic-responsive diarrhea and intestinal dysbiosis will be used interchangeably. It is however conceivable that a patient with clinical intestinal dysbiosis does not respond to appropriate antibiotic therapy.

Etiology
Antibiotic-responsive diarrhea or intestinal dysbiosis is caused by an abnormal proliferation of bacteria and/or the change in bacterial species present in the intestinal lumen. However, dysbiosis/ARD should not be considered a primary disorder in most if not all patients with this disorder. There are several protective mechanisms that prevent a patient from dysbiosis/ARD. Gastric acid, intestinal motility and antibacterial activity of pancreatic juice all limit the bacterial numbers in the small intestine. Gastric acid directly destroys bacteria that are ingested with the diet and also decreases the pH of the ingesta, leading to a lower pH in the proximal small intestine. However, the lack of gastric acid secretion alone is not sufficient for dysbiosis/ARD to develop. Propulsive movements of the small intestine are probably the most important protective factor since there is no physical barrier between the large intestine and the small intestine that would prevent retrograde cultivation of the small intestine by the large intestinal microbiota. The antibacterial properties of pancreatic juice are not well understood. Pancreatic digestive enzymes may be partly responsible for the antibacterial action of pancreatic juice. Any disease process that affects one or more of the protective mechanisms discussed can ultimately lead to intestinal dysbiosis/ARD.
Clinical Findings

Intestinal dysbiosis in dogs and cats leads to chronic small bowel diarrhea that is often intermittent. Weight loss can be present in some cases. Other clinical signs maybe due to the primary underlying disease process, such as partial obstruction, exocrine pancreatic insufficiency, or others.

Diagnosis

Part of the controversy about intestinal dysbiosis/ARD is due to the fact that this disorder is difficult to diagnose. Traditionally, the gold standard for assessment of the intestinal bacterial ecosystem is bacterial culture, but it is now recognized that this does not allow for accurate characterization of the intestinal microbiota.

Serum folate concentration - folic acid is synthesized by enteric bacteria and is available for absorption. In dogs with intestinal dysbiosis/ARD for a long period of time, serum folate concentration can increase. While an increased serum folate concentration is fairly specific for intestinal dysbiosis, it is not very sensitive. In one study only 50% of all dogs with intestinal dysbiosis had increased serum folate concentrations.

Serum cobalamin concentration - Many species of bacteria utilize cobalamin and compete with the body for dietary supplies. Unlike an increased serum folate concentration a decreased serum cobalamin concentration is not specific for intestinal dysbiosis. Any severe small intestinal disease involving the ileum can lead to cobalamin deficiency. Also, a lack of intrinsic factor and digestive proteases in dogs with exocrine pancreatic insufficiency can cause cobalamin deficiency. A decreased serum cobalamin concentration is rather insensitive for intestinal dysbiosis and in one study only 25% of dogs with intestinal dysbiosis had decreased serum cobalamin concentrations. A combination of a decreased serum cobalamin and an increased serum folate concentration is highly specific for intestinal dysbiosis but rather insensitive. These two parameters are to date the most practical diagnostic tools for the diagnosis of intestinal dysbiosis/ARD.

The GI Lab at Texas A&M University has recently developed PCR based assays that are able to measure the fecal abundances of some key bacterial groups. A mathematical algorithm is used to report these changes as a single numerical value; the so called dysbiosis index (DI). A negative DI indicates normobiosis, whereas a positive DI indicates dysbiosis. The DI was specifically trained to diagnose the dysbiosis associated with chronic enteropathy in dogs and an increased DI in dogs with chronic enteropathy provides additional information during the diagnostic work-up of these dogs.

The limitations for the diagnosis of intestinal dysbiosis/ARD are part of the reason why the term antibiotic-responsive diarrhea has been coined. If a patient with intermittent chronic diarrhea responds to antibiotic therapy the diagnosis of antibiotic-response diarrhea can be made. This maybe somewhat unsatisfactory as these patients may also respond to other therapeutic trials and as the term ARD does not define an underlying cause. However, the term ARD does represent a practical clinical diagnosis.

Treatment

The therapeutic goal in dogs with dysbiosis/ARD is the identification and treatment of a possible inciting cause. For example, serum TLI concentration should be evaluated. If a primary cause cannot be identified one of several therapeutic strategies or a combination thereof should be employed.

Prebiotics

Prebiotics are substances that preferentially support the resident bacterial ecosystem of the intestine. Basically, prebiotics are non-digestible food components (dietary fiber) that are being fermented by intestinal bacteria. This can lead to normalization of the intestinal microbiota. In one study, the use of fructooligosaccharides (FOS) in the diet showed a lasting advantageous effect. While this has not been evaluated as of yet, other prebiotics, such as inulin or beet-pulp, may also prove to be beneficial.

In one study dogs with intestinal dysbiosis diagnosed based on clinical signs and serum folate and cobalamin concentrations were divided into two groups. One group was treated with an antibiotic for 6 weeks and the other group was switched to a diet containing FOS. Both groups of dogs responded equally with normalization of fecal quality among improvement of other parameters, but many of the dogs treated
with the antibiotic showed a relapse after the antibiotic therapy was stopped, while the beneficial effect of the diet was maintained.

Probiotics
Several studies have been conducted in dogs that show that certain probiotics carry health benefits in dogs with gastrointestinal disorders. Scenarios for which there is good evidence of a beneficial effect of probiotics are the prevention of stress-related diarrhea, treatment of stress-related diarrhea, and acute non-specific diarrhea. The effects of probiotics in dogs and cats with intestinal dysbiosis/ARD have not been sufficiently studied.

Synbiotics
Synbiotics are combinations of prebiotics and probiotics. There are three different approaches to synbiotic use. Some boutique pet foods are fortified with a prebiotic and are sprayed with a probiotic. Even though most of these pet foods use bacterial spores that show much greater resilience to environmental factors than bacteria themselves, this mode is likely unrealistic as the load of bacteria is small and the bacterial spores may not be stable enough to reach the patient. Another approach is to use a pet food fortified with a prebiotic and also use a probiotic nutraceutical concurrently. This is likely the most realistic synbiotic approach. The use of a nutraceutical that contains both a pre- and a probiotic may be realistic in cats and small dogs, but in large dogs the amount of prebiotic in the supplement is likely not sufficient to show any prebiotic effect.

Fecal transplantation
In humans with chronic intestinal disease there has been some experience with fecal transplantation. Experience in dogs and cats with intestinal dysbiosis/ARD are limited and great care would need to be taken not to transplant enteropathogens, especially when donors are chosen that are subclinically infected.

Antibiotics
Tylosin (25 mg/kg q 12 hrs for 6 weeks) is the antibiotic of choice. Tylosin is extremely safe and is not used in humans for the most part — thus, creating resistant bacterial strains is not a big concern. In one study, a group of dogs was treated with 400 mg/kg daily for a period of 2 years and none of them developed any side-effects. The superb efficacy of tylosin has been well-demonstrated in studies from Finland. Some of the newer studies would suggest that smaller dosages may also be beneficial, but these findings will need to be verified. Other antibiotics, such as metronidazole can also be used. Some patients respond to therapy rapidly and do not have a recurrence. However, other patients do not respond to antibiotic therapy alone. If there is no marked improvement after 2 weeks of appropriate antibiotic therapy further work-up is necessary. Some patients may respond to therapy with a complete resolution of clinical signs but may have a recurrence of clinical signs as soon as antibiotic therapy is discontinued. These patients require further diagnostic work-up. In some of these patients a specific underlying cause of the intestinal dysbiosis can be identified and treated accordingly. However, in some patients no specific cause can be identified and prolonged, maybe even life-long, antimicrobial therapy is required.

Ancillary Therapy
If serum cobalamin concentration is decreased below the lower limit of the reference interval or in the very low end of the reference interval, cobalamin should be supplemented.

Keywords: Microbiota, Dysbiosis, Prebiotic, Probiotic, Synbiotic, Tylosin
Cobalamin Deficiency and Cobalamin Supplementation

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Summary

Cobalamin (vitamin B12) deficiency occurs commonly in both dogs and cats with chronic gastrointestinal disease and it is widely accepted that patients may not respond favorably to treatment of the underlying condition unless they are also treated with cobalamin supplementation. Traditionally, cobalamin has been supplemented by parenteral administration, but recent data would suggest that oral cobalamin supplementation may be as efficacious as parenteral supplementation. Given the ease of oral administration this mode of supplementation would be much preferable over the parenteral route.

Cobalamin Function

Cobalamin refers to a group of compounds that are exclusively derived from bacterial sources. The biologically active forms of this vitamin are methylcobalamin (required for methyl-group transfers) and adenosylcobalamin (required for adenosyl-group transfers), but there are other molecules that belong to this group of vitamins, such as hydroxocobalamin or cyanocobalamin. Cyanocobalamin does not occur naturally, but is manufactured by bacterial fermentation for treatment of cobalamin deficiency. Cobalamin has important functions in amino acid metabolism and DNA synthesis.

Cobalamin is an essential cofactor for several enzyme systems in many mammalian cells. The first enzyme system, methylmalonyl-CoA mutase, is located in the mitochondria and plays a crucial role in the transformation of propionyl-CoA to succinyl-CoA. Thus, cobalamin plays a major role in the metabolism of several amino acids.

Cobalamin is also important in the transformation of the sulfur-containing amino acids methionine and cysteine. Homocysteine is an intermediary amino acid that is formed from methionine and is not found in the diet. The transformation of homocysteine to methionine is linked to another metabolically crucial process, the generation of the biologically active tetrahydrofolate from N5-methyltetrahydrofolate. Simplistically, the cobalamin-dependant enzyme methionine synthase transfers a methyl group from N5-methyltetrahydrofolate to homocysteine, which results in tetrahydrofolate and methionine. Thus, this enzyme not only plays a role in the transformation of sulfur-containing amino acids, but may be even more important in the generation of the biologically active tetrahydrofolate, which is involved in the synthesis of both purines and pyrimidines and thus a crucial step in DNA and RNA synthesis.

Cobalamin Absorption

Dietary cobalamin is tightly bound to dietary animal-derived protein. In the stomach, dietary protein is partially digested by pepsin and HCl and cobalamin is released. However, cobalamin immediately binds to a transporter protein called haptocorrin or R-protein. Haptocorrin is mostly synthesized and secreted by the gastric mucosa. Haptocorrin in turn is digested by pancreatic proteases in the small intestine. Free cobalamin binds to intrinsic factor. In humans, intrinsic factor is mostly synthesized and secreted by the
parietal cells of the gastric mucosa, but there is good evidence that in dogs and cats most of intrinsic factor is synthesized and secreted by pancreatic acinar cells. Cobalamin/intrinsic factor complexes are absorbed by a complex receptor in the microvillus pits of the apical brush border membrane of the ileal enterocytes. Thus, the absorption of cobalamin is an extremely complex system that relies on a multitude of factors and processes. As cobalamin is being absorbed into the intestinal epithelial cells, it dissociates from intrinsic factor and free cobalamin is released into the circulation, where most of it binds to yet another protein, transcobalamin II. The main storage compartments for cobalamin in the body are the liver and the kidney, which maintain serum cobalamin concentrations by releasing cobalamin whenever needed.

**Cobalamin Deficiency**

The most common causes of cobalamin deficiency in dogs and cats are chronic and severe distal or diffuse small intestinal disease and EPI. In addition, short-bowel syndrome, an exclusively vegetarian or vegan diet, or hereditary cobalamin deficiency are less common causes of cobalamin deficiency.

A recent study has shown that 82% of dogs with EPI were cobalamin deficient. Similar studies in cats have shown that most, if not all, cats with EPI are cobalamin deficient. As discussed above, intrinsic factor in dogs and cats is mostly supplied by pancreatic acinar cells. Therefore dogs and cats with EPI may lack enough intrinsic factor for cobalamin absorption. The lack of pancreatic proteases and the alteration of the small intestinal microbiota may also play minor roles.

Dogs and cats with severe and long-standing small intestinal disease involving the ileum may also show cobalamin deficiency. In one study, 49 of 80 cats (61%) with chronic signs of gastrointestinal disease had cobalamin deficiency, as evidenced by a subnormal serum cobalamin concentration. It is interesting to note that there is one study from the UK that would suggest that cobalamin deficiency is much less common in cats in the UK than in the USA. However, there are other reports from the UK that would suggest that cobalamin deficiency does occur frequently in cats with gastrointestinal disease in the UK. These differences between studies are interesting as they point to differences in measuring serum cobalamin concentrations in dogs and cats with different assays. There appear to be considerable cobalamin stores, but it is unclear where most of the cobalamin stores are located in dogs or cats, but as in humans it takes a considerable amount of time for these body stores to be depleted if an insufficient amount of cobalamin is being absorbed.

Dogs and cats with short bowel syndrome are typically cobalamin deficient because cobalamin absorption is exclusively limited to the ileum and removal of the ileum will thus lead to cobalamin deficiency. Also, vegetarian or vegan diets do not contain cobalamin unless they have been supplemented with that vitamin. Dogs and cats fed these diets exclusively and who are not receiving any vitamin supplementation will develop cobalamin deficiency. Hereditary cobalamin deficiency has been recorded in a few dog breeds, including the Giant Schnauzer, Beagle, Border Collie, Australian Shepherd, and Chinese Shar Pei. Recently, a region of chromosome 13 has been identified that cosegregates with cobalamin deficiency in the Chinese Shar Pei, but the actual gene causing the disease has not yet been identified.

Most dogs and cats with cobalamin deficiency only show clinical signs of gastrointestinal disease, which could either be a cause or the effect of cobalamin deficiency. Other clinical signs include weight loss, central neuropathies, peripheral neuropathies, or immunodeficiencies. In a recent case report a Border Collie with selective cobalamin deficiency was described. The dog presented with hyperammonemic encephalopathy and fully responded to cobalamin supplementation. In another case report a juvenile Beagle presented with failure to gain weight, lethargy, intermittent vomiting, seizures, anemia, and leucopenia. This dog also fully responded to treatment with cobalamin supplementation. In a separate case report, a 4-year old cat presented with severe encephalopathy and was diagnosed with an organic acidemia and cobalamin deficiency. Interestingly, in contrast to the Border Collie mentioned above, this cat had a normal plasma ammonia concentration.
Diagnosis of Cobalamin Deficiency

A definitive diagnosis of cobalamin deficiency can be challenging. Clinical signs are ultimately caused by cobalamin deficiency on a cellular level. However, the cellular cobalamin status is difficult to assess. Serum cobalamin concentration has been traditionally measured to help assess cobalamin status, but some patients with cobalamin deficiency on a cellular level do not always have severely decreased serum cobalamin concentrations. Thus, in order to avoid missing patients with cobalamin deficiency, cobalamin supplementation should be considered even when serum cobalamin concentration is low normal. Several assays for the measurement of serum concentrations of cobalamin in humans are available. In order to be used in dogs and cats, these assays designed for use in humans must be validated for use in dogs and cats. The GI Lab at Texas A&M University has analytically validated an automated chemiluminescence assay designed for the measurement of cobalamin concentrations in humans for use in dogs and cats. A reference range for serum cobalamin concentration in dogs and cats was established. Reference ranges are not transferrable between labs and each lab should establish their own reference range.

Serum or urine methylmalonic acid (MMA) concentration can also be used as an indicator of cobalamin status. Cobalamin deficiency leads to accumulation of MMA and thus concentrations of MMA are often dramatically increased in the serum or urine of patients with cobalamin deficiency. Serum MMA concentrations have been shown to be increased in cats with cobalamin deficiency and have been shown to decrease with cobalamin supplementation. Also, recently dogs with severely decreased serum cobalamin concentrations were shown to have increased serum MMA concentrations. Interestingly, several dogs with low-normal serum cobalamin concentrations were also shown to have increased serum MMA concentrations, demonstrating that a severely decreased serum cobalamin concentration is not optimally sensitive for the diagnosis of cobalamin deficiency on a cellular level and that a cut-off value for cobalamin supplementation should be chosen that is in the low-normal reference range. This is especially true if one considers that cobalamin supplementation is minimally invasive, safe, and relatively cheap. As suggested by these data, measurement of serum MMA concentration may be a better diagnostic test for cobalamin deficiency than serum cobalamin concentration. However, measurement of MMA concentration in serum or urine is technically involved and expensive. Thus, MMA is currently not routinely assessed in patients evaluated for cobalamin deficiency.

Thus, the only routinely available diagnostic tool to assess cobalamin status in dogs and cats is serum cobalamin concentration, which should be evaluated in every dog and cat with chronic signs of gastrointestinal disease or with clinical signs compatible with cobalamin deficiency that cannot be attributed to other conditions (i.e., unexplained immunodeficiencies, anemias, neuropathies).

Cobalamin Supplementation

Patients with severe cobalamin deficiency often do not respond to therapy of the underlying gastrointestinal disorder unless or until cobalamin is being supplemented. As mentioned, patients with low-normal serum cobalamin concentrations should be considered for cobalamin supplementation as measurement of serum cobalamin concentration may not be optimally sensitive for the diagnosis of cobalamin deficiency and there is no indication that over-supplementation of cobalamin leads to complications. The most common form of cobalamin used for supplementation is cyanocobalamin, but hydroxocobalamin or methylcobalamin can also be used in patients that don’t respond to cyanocobalamin supplementation (most of these patients will also fail to respond to other forms of cobalamin) or those that appear to have side effects to supplementation with cyanocobalamin (side effects from cyanocobalamin administration have never been definitively demonstrated in either dogs or cats). Traditionally, the standard route of cobalamin application is by parenteral administration. This is because cobalamin deficiency has been shown to lead to cobalamin malabsorption in the ileum. However, there are recent data that show that oral supplementation may be as efficacious as parenteral supplementation. Dosing schedules for parenteral supplementation have been described. As for parenteral administration, dosing for oral supplementation is empiric with 250 µg of cyanocobalamin being administered orally once a day in cats or in dogs up to 10 kg BW, 500 µg in dogs weighing over 10 kg but less than 20 kg, and 1000 µg in dogs weighing more than 20 kg. Daily supplementation
is required and after a 6-8 week period one should discontinue supplementation for about a week and recheck serum cobalamin concentration.

In one large retrospective study of 51 client-owned dogs with low-normal or subnormal serum cobalamin concentrations patients were supplemented with oral cyanocobalamin (250-1000 µg cobalamin orally once a day) and serum cobalamin concentrations increased in all of the dogs. Interestingly, not all patients had the same underlying cause of cobalamin deficiency, suggesting that the cause of cobalamin deficiency may not play a role in determining the success of oral supplementation. Similarly, a recent study looked at oral cobalamin supplementation in geriatric cats, only some of which were actually cobalamin deficient. Similarly to the study in dogs serum cobalamin concentrations increased significantly with oral supplementation. Also, more recently a small retrospective study in 16 cats with chronic enteropathy or intestinal lymphoma and low or low-normal serum cobalamin concentrations showed dramatic increases in serum cobalamin concentrations in all 16 cats.

While prospective studies are needed and ongoing, these initial data are very promising and oral supplementation could be applied routinely unless there is evidence that a particular patient does not respond to supplementation.

**Keywords:** Cobalamin, Vitamin B12, Cyanocobalamin, Central Neuropathies, Peripheral Neuropathies, Methylmalonic Acid
Diagnosis and Treatment of Canine Osteosarcoma

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Summary
Canine endosteal osteosarcoma (OS) is the most common primary bone tumor in dogs, accounting for up to 85% of skeletal malignancies. Approximately 8,000-10,000 dogs each year develop OS in the US. Canine OS is primarily a disease of middle-aged to older dogs (median age 7 years old), although there is a large range in age on onset. Appendicular OS is classically a malignancy of large and giant breed dogs, while axial OS is much less common and tends to affect small dogs (<30lbs). Other much less common OS variants include periosteal OS, parosteal OS, mammary gland OS, and OS of soft tissues. The etiology of canine OS remains unknown, although a hereditary basis has been suspected based primarily on the large breed prevalence of the disease and the subjective assessment of an increased incidence in some related families. Primary appendicular OS originates from the endosteal surface of the marrow cavity in the metaphyseal region of long bones, with the distal radius and proximal humerus being the most common bones affected.

Treatment of osteosarcoma is aimed at both removing the painful tumor and using systemic chemotherapy to delay the growth of metastatic disease. Removal of the tumor requires amputation for the majority of affected dogs. Approximately two weeks following surgery, I recommend beginning systemic chemotherapy. Platinum drugs (cisplatin or carboplatin) and doxorubicin have efficacy against canine OS. These treatments are given at 2-3 week intervals for a total of 4-6 treatments. For dogs who do not have an amputation, I recommend pain control using either palliative irradiation, NSAIDs, aminobisphosphonates, opioids, or a combination of these. For these cases, I may also consider stereotactic radiation therapy (SRT) which appears to alleviate pain and extend life when used in conjunction with systemic chemotherapy.

Canine appendicular OS is a treatable, but essentially incurable, malignancy with reported median survivals (MS) of ~4 months with amputation alone and MS of 9-14 months when systemic chemotherapy is given after amputation. Most treated dogs develop pulmonary metastatic disease that leads to euthanasia.

Natural behavior
Endosteal and periosteal OS are very aggressive malignancies that cause local bone destruction and significant pain. At the time of diagnosis, affected dogs are considered to have micrometastatic disease, with hematogenous spread to the lung parenchyma being the most common site. Metastatic disease can also be found in other long bones, vertebrae, ribs, pelvis, and other soft tissues such as liver, spleen, or kidney. Axial OS tends to be a less aggressive disease systemically, although significant local destruction and pain can be seen. Rib/scapula OS are as aggressive as endosteal OS. Mammary gland OS is very difficult to control with surgery, so these dogs tend to be euthanized due to an inability to control the tumor locally. OS of soft tissues in very difficult to control systemically, so most of these dogs are euthanized due to overwhelming metastatic disease.
Diagnosis

Most dogs will be presented with a history of lameness and have a visible swelling at the metaphyseal region of a long bone, and/or be one of the known predisposed breeds (Rottweilers, great Danes, grey hounds, Irish wolfhounds, etc.). However, some dogs will present with vague, non-specific signs such as anorexia and lethargy. Dogs with oral OS may have dysphagia while dogs with neurologic signs may have spinal OS. Important staging tests include physical examination, complete blood count (CBC), serum chemistries, abdominal ultrasound, and thoracic radiographs. One important parameter to evaluate is serum alkaline phosphatase (SAP). High SAP has been shown in humans, and recently in 2 separate papers in dogs, to be a poor prognostic indicator for dogs with appendicular OSA. (1,2) In the more recent study, dogs treated with amputation and chemotherapy that had normal SAP had a median survival time of 12.5 months whereas dogs with increased SAP had a median survival time of 5.5 months. A second laboratory factor recently shown to correlate with outcome is monocyte count. (3) Dogs with monocyte counts less than 400/uL had disease-free intervals twice as long as those with monocyte counts greater than 400/uL. Dogs with overt monocytosis (>1,000/uL) had extremely poor prognoses. (4) Advanced imaging may include a CT scan or bone scintigraphy (99M-Tc nuclear scintigraphy). This noninvasive and nontoxic screening test for bone metastasis will reveal additional sites of uptake in approximately 7% of dogs, half of which may be areas of bone metastasis from OSA. At least two radiographic views of the affected area of the limb should be obtained. The classic radiographic appearance of OSA is a mixed lytic and proliferative lesion of the metaphysis of the long bone. OSA will typically not cross a joint space. A small percentage of dogs with OSA will present with a pathologic fracture. OSA is most common in the proximal humerus and distal radius of the thoracic limb (away from the elbow), and in the proximal tibia and distal femur of the pelvic limb (toward the knee). Although 90% of dogs with OSA have microscopic pulmonary metastasis at the time of presentation, only approximately 7% have macroscopic evidence of metastasis at diagnosis. Ultimately, the final diagnosis of OSA can be made using large bore needle aspirates of the mass with or without alkaline phosphatase staining, using either the radiographs or ultrasound as a guide, and/or a bone biopsy/histopathology obtained by several means, including: (1) Jamshidi bone core biopsy; (2) Michele trephine; or (3) Open, surgical biopsy. Important differential diagnoses for bony lysis include: (1) Other primary bone tumors (chondrosarcoma, fibrosarcoma, hemangiosarcoma, synovial cell sarcoma); (2) Bone metastasis (most commonly from carcinomas of the lung, mammary gland, or prostate); (3) Bony involvement from systemic neoplasia such as lymphoma or multiple myeloma; (4) Osteomyelitis (fungal or bacterial); (5) Other diseases, such as Legg-Calve-Perthes disease, traumatic osteonecrosis, or aneurysmal bone cyst.

Treatment

Surgery: Local disease is best dealt with by amputation of the affected limb. Amputation of front limb or a hind limb is a simple surgical procedure that is extremely well tolerated in most dogs, although dogs with an amputated HL tend to have a more normal gait. An excellent website for owners dedicated to three-legged dogs is: Tripawds.com. However, amputation alone results in only short-term gains. The median survival time with amputation alone is approximately 4 months, with less than 10% of dogs living for 1 year. (5) Pulmonary metastasis is the eventual cause of death in the majority of patients.

For owners unwilling to perform amputation, other surgical options may be available. Some dogs may be candidates for a limb sparing procedure. (6) In this type of surgery, the diseased portion of bone is resected, and an allograft from a bone bank or a metal spacer is implanted. The adjacent joint is fused, and then additional postoperative chemotherapy is employed. This procedure is most successful with tumors of the distal radius. Limb sparing surgery is performed at a limited number of institutions, is expensive, and has a high rate of complications, including local recurrence, implant failure, and infection. Despite these factors, the overall outcome in patients treated with limb sparing procedures is similar to those treated with amputation. Interestingly, dogs undergoing a limb sparing procedure and developing a postoperative infection will survive twice as long as those not developing an infection. (7)
Stereotactic Radiotherapy (SRT): SRT involves the delivery of one or several large doses of irradiation to an affected body part, using very sophisticated treatment planning and delivery devices to insure avoidance of toxicity to surrounding normal tissues. Multiple US centers are offering as a limb-sparing option for dogs with OS. Recent preliminary data suggest that a combination of SRT and chemotherapy may result in outcomes similar to traditional amputation and chemotherapy, with very good preservation of function. (8) Pathologic fracture is a serious complication following SRT in some dogs. The outcome seems to be improved in dogs with relatively small lesions with minimal lysis.

Chemotherapy: The addition of systemic chemotherapy to amputation significantly prolongs the time from diagnosis to death. The addition of cisplatin increases the median survival time from ~4 months to 10-12 months, with approximately 20% of dogs living longer than 2 years. However, Cisplatin is nephrotoxic and strongly stimulates the emetic response. It must be administered with vigorous intravenous diuresis with sodium chloride to prevent kidney damage, and anti-emetics should be administered to prevent vomiting. The related drug carboplatin is not nephrotoxic, and usually does not cause gastrointestinal signs. Carboplatin does not require diuresis or antiemetic administration. Most studies suggest that carboplatin and cisplatin have approximately equal efficacy. Given the current availability of generic carboplatin, a dose of carboplatin at most practices is actually less than administering cisplatin.

Other investigators have evaluated doxorubicin (Adriamycin) for the adjuvant treatment of OSA. Although 30 mg/m² doxorubicin given every 3 weeks resulted in poor survival times in one study, the same dose given every 2 weeks for 5 treatments resulted in survival times close to those reported for cisplatin.

Doxorubicin/platinum combinations have been evaluated in several studies, either administered at reduced doses at the same time or sequentially. The majority of published studies have revealed no significant improvement in outcome when compared with single-agent platinum protocols.

A recent study of 470 dogs with appendicular OSA treated with chemotherapy compared outcomes in dogs receiving either 4 or 6 doses of carboplatin, doxorubicin q2 or q3 weeks for 5 treatments, or alternating carboplatin and doxorubicin (3 each). There was no statistically significant difference in outcome between any of the chemotherapy protocols, however dogs receiving 6 doses of carboplatin had the numerically longest survival times. (9)

Palliative Irradiation Therapy: A reasonable palliative option available in most parts of the United States is radiation therapy (RT) to the affected bone. A relatively inexpensive and well-tolerated form of RT involving 1 to 4 weekly treatments can provide good pain control in approximately 75% of dogs with OSA, which persists for a median of 3-4 months. It is not clear whether the addition of chemotherapy or bisphosphonates to RT improves duration of response or overall survival.

Other palliative options to control pain: Aminobisphosphonates are bone anti-resorptive agents that are thought to exert their analgesic effects against lytic bony diseases through selective inhibition of osteoclast function. However, multiple studies have also demonstrated that bisphosphonates are also capable of direct antitumor effects, inhibition of angiogenesis, and immunomodulation. It is not clear if bisphosphonate concentrations capable of these antitumor effects are achievable in canine patients. A recent study evaluated the effects of the bisphosphonate drug pamidronate in dogs with OSA. (10) Approximately 30% of dogs experienced meaningful improvement in pain, which persisted for a median of 7.5 months. Early studies with another, more potent bisphosphonate drug, zoledronate, are extremely encouraging, and zoledronate has recently become more affordable in the USA. (11)

Therapy for metastatic disease: Following the completion of post-operative chemotherapy, dogs are rechecked monthly for 3 months, and then every 3 months for one year for evidence of pulmonary metastasis. Although I have seen some great responses to single-agent doxorubicin in a small percentage of dogs, the average survival after the clinical detection of metastatic disease is only 2 months. However, some dogs may benefit
from surgical removal of the pulmonary metastasis. Certain criteria should be met in order for this type of treatment to be useful: (1) A “reasonable: amount of time from primary tumor diagnosis (>10 months?); (2) A “manageable" number of metastatic lesions (3 or less?); (3) A relatively slow rate of growth. Often, when metastasis is first detected, the dog will be sent home and rechecked 2-4 weeks later to determine how quickly the lesions are changing. If rapid progression is detected, then the utility of metastasectomy is minimal. With proper case selection, the median survival time after metastasectomy is approximately 6 months, and most owners are very satisfied with the outcome. It is unknown whether the use of additional chemotherapy after metastasectomy improves prognosis. A recent prospective study of single-agent toceranib in dogs with measurable OSA lung metastasis suggested minimal benefit (3 of 17 dogs experienced stable disease for 8 weeks or longer). (12)

**Keywords:** Canine, Osteosarcoma, Chemotherapy, Endosteal

**References**
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Diagnosis and Treatment of Canine Hemangiosarcoma

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Summary
Canine hemangiosarcoma (HSA), a highly malignant tumor of blood vessel endothelial cells, is a relatively common malignancy in dogs compared with other species. Unfortunately, since it is such a rare tumor in humans, we do not have abundant human literature from which to extrapolate when making treatment decisions. Certain breeds (e.g. German shepherds, golden retrievers) appear to be at increased risk for the development of HSA, suggesting a possible genetic predisposition. With few exceptions (i.e. purely cutaneous tumors with no evidence of subcutaneous infiltration), canine HSA is an aggressive neoplasm that tends to metastasize widely and early in the course of disease. In fact, most dogs with non-cutaneous HSA have micrometastatic disease at diagnosis. Although non-cutaneous HSA can occur in many locations, splenic HSA is by far the most common disease seen in practice.

Clinical presentation
These tumors are extremely well vascularized and very friable, and thus dogs with visceral forms of HSA will often present with a history of acute weakness or collapse as a result of intra-abdominal hemorrhage. Careful history from the owner may reveal similar episodes of weakness having occurred in the past, only to resolve spontaneously within 24 to 48 hours due to “autotransfusion” of red blood cells from the abdominal cavity back into circulation. Occasionally, a more chronic history of weakness, lethargy and inappetence may be reported, or the patient may be asymptomatic and an abdominal mass or effusion detected on routine physical examination.

Diagnosis and Staging
Dogs with HSA may have pale mucous membranes, slow capillary refill, and tachycardia or tachypnea. A cranial abdominal mass or abdominal effusion may be palpable. A complete blood count (CBC) is essential, as many dogs will have evidence of anemia (regenerative or non-regenerative, depending on the duration-called microangiopathic anemia) and/or thrombocytopenia (due to chronic, low-grade DIC). The presence of schistocytes, acanthocytes or target cells is highly suggestive of HAS.

Abdominal radiographs will often reveal loss of detail due to the presence of effusion, and/or a mass effect in the cranial abdomen. Abdominal ultrasound is a superior imaging modality, allowing for the assessment of the internal architecture of the organs (even in the presence of effusion) and evaluation for intra-abdominal metastasis. HSA will often appear as a solitary or multifocal, poorly demarcated nodule of mixed echogenicity, often containing large hypoechoic areas. Thoracic radiographs (3 views) should be obtained to evaluate for evidence of pulmonary metastasis or pericardial effusion (as a result of cardiac involvement). Some practitioners will routinely use echocardiography to screen for occult cardiac metastasis, although this is a relatively infrequent finding at the time of presentation in the author’s experience.

Abdominocentesis will often yield a hemorrhagic effusion that fails to clot. Although tumor cells are probably present in the effusion, they are difficult to detect given the relatively large quantity of blood cells usually present. Hemoabdomen is often considered an indication for immediate exploratory laparotomy,
however euthanasia will be considered by some owners if evidence of gross metastasis is found by the staging methods outlined above. Needle aspiration cytology of suspect lesions is usually unrewarding, and needle-core (Tru-cut) biopsy may precipitate hemorrhage.

Although a definitive diagnosis is rarely reached prior to surgery, the presence of a cavitated splenic mass in an older dog, combined with appropriate historical and hematological findings, is often highly suggestive of HSA. Other important differential diagnoses for splenic masses include benign processes such as hematoma, regenerative hyperplasia and hemangioma, and other malignancies such as leiomyosarcoma and malignant fibrous histiocytoma (a.k.a. fibrohistiocytic nodules). Studies suggest that approximately 45% of canine splenic masses will be HSA, and this number increases to approximately 70% in dogs with a history of nontraumatic hemoabdomen.

Prior to contemplating surgery, a coagulation profile and crossmatch are useful. Platelet count, prothrombin time, partial thromboplastin time, or fibrin degradation products can be abnormal in some patients. In some studies, as many as 90% of dogs with HSA have had one or more hemostatic alterations. If the above tests are not immediately available, evaluation of a peripheral blood smear to estimate platelet number, combined with buccal mucosal bleeding time and/or activated clotting time, can yield important information as to whether whole blood or blood component therapy is necessary prior to surgery.

Treatment

Surgery

Surgical excision is the first line of defense in treating HSA. Exploratory laparotomy and splenectomy can be performed in most practices. Cardiac arrhythmias can be encountered during splenectomy, and thus careful electrocardiographic monitoring is recommended. The abdomen should be thoroughly evaluated for evidence of metastasis at the time of laparotomy, with special attention paid to the liver and omentum. Peritoneal and omental metastasis can occur as a result of mass rupture and tumor cell “seeding”. Prior to closure, the abdomen should be copiously lavaged with warm saline solution, and instruments changed. The entire excised specimen should be submitted for histopathology.

Systemic chemotherapy

With the exception of purely cutaneous HSA, the outcome with surgery alone is very disappointing, with median survival times between 1 and 3 months, and less than 10% of dogs surviving longer than one year. (1) Thus, additional systemic therapy should be offered in all cases of splenic HSA. The most effective treatments are chemotherapy protocols containing doxorubicin (DOX). (2-5) These treatments are generally well tolerated and relatively inexpensive. Following splenectomy with a DOX-containing chemotherapy protocol extends the median survival times to approximately 5-7 months, however 90% of dogs are still likely to succumb to metastasis within 1 year. The prognosis is somewhat better if surgery is performed prior to rupture of the tumor. Treatment of patients with gross metastasis is usually unrewarding, and survival, even with chemotherapy, is usually measured in weeks. A recent study evaluated the outcome of dogs with gross HSA treated with DOX-based chemotherapy. (6) Approximately 40% responded, with a median response duration of 53 days.

There is some recent evidence that dogs with subcutaneous HSA may have a better short- to intermediate-term prognosis than previously thought, although the current recommendation remains to follow surgery with adjuvant chemotherapy for these tumors. (7,8) A recent large case series of dogs treated with surgery +/- chemotherapy for cardiac HSA was recently reported. (9) These dogs had acceptable perioperative morbidity and had survival times roughly equivalent to those reported for other visceral sites.

Novel Treatments and Diagnostics

There is obviously considerable room for improvement in the treatment of dogs with HSA. Investigational therapies that have shown promise include the addition of nonspecific immunotherapy (L-MTP-PE) to standard
chemotherapy, and the addition of inhaled DOX to systemic chemotherapy. (10) Unfortunately, these are not generally available at the current time.

A recent small study evaluated the efficacy of postoperative therapy with low-dose, continuous (metronomic) chemotherapy and piroxicam in dogs with splenic HSA undergoing splenectomy. (11) The well-tolerated protocol resulted in an outcome roughly the same as what has been reported with DOX-based chemotherapy, and may be a reasonable consideration in dogs with HSA where DOX-based chemotherapy is declined or not feasible.

A recently published study evaluated a combination of splenectomy and DOX, followed by “maintenance” therapy with the receptor tyrosine kinase (RTK) inhibitor toceranib (Palladia®, Pfizer). (12) This is based on the observation that canine HSA cells express a number of RTKs that are inhibited by toceranib (KIT, PDGFR, VEGFR2) and that a toceranib-like drug was shown to inhibit canine HSA growth in a nude mouse model. Unfortunately, the outcome in patients treated on this study was not better than historical data using DOX alone. Many other clinicians are offering “maintenance” therapy with metronomic cyclophosphamide plus a NSAID following DOX. Information regarding efficacy of this approach is not currently available.

There remains a need for presurgical tests capable of distinguishing between HSA and benign/other conditions, that may help owners make more informed decisions regarding whether to move forward with surgical therapy. Furthermore, inexpensive screening tests that may lead to earlier diagnosis would likewise be very useful, especially for at-risk populations such as German shepherds and golden retrievers. One recent study demonstrated that MRI may be useful in distinguishing benign versus malignant splenic lesions. (13) 2 recent studies have suggested that cardiac troponin I (cTnI) may be very useful in distinguishing between hemopericardium from HSA and idiopathic hemopericardium; however, this would not be useful for splenic disease. (14,15) A recent publication utilized a flow cytometry-based technique for the detection of circulating HSA cells in blood, which may prove useful. (16) Most recently, the enzymatic activity of thymidine kinase 1 (TK1), an enzyme important in DNA replication, was shown to be elevated in the majority of dogs with HSA versus normal dogs, and may prove useful as a screening or diagnostic test for dogs with HSA in the future. (17)

**Keywords:** Canine, Hemangiosarcoma, Chemotherapy, Anemia

**References**

Debunking Nutritional Myths: Facts and Fallacies
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Summary
Many owners are turning to the internet to find guidance on how to feed their dog or cat. However, much of the information on the internet is not peer-reviewed and can sound very scientific when it is not. This makes it hard for many clients to decide what information to accept and what to reject. Veterinarians and their associates should be aware of what information is out there and how to address questions and concerns when they arise. This lecture will cover some of the popular nutrition discussions topics from blogs and websites to help sort through what is true and what is false or not scientifically supported.

Natural pet foods are better for my pet: It is difficult for veterinarians and consumers alike to know which marketing terms and descriptors such as organic, natural, holistic, human grade, premium, and super premium are regulated terms and which are solely marketing. Currently, only the terms natural and organic have regulatory guidelines associated with their use. The Association of American Feed Control Officials (AAFCO) defines the term natural as: “A feed or ingredient derived solely from plant, animal or mined sources, either in its unprocessed state or having been subject to physical processing, heat processing, rendering, purification, extraction, hydrolysis, enzymolysis or fermentation, but not having been produced by or subject to a chemically synthetic process and not containing any additives or processing aids that are chemically synthetic except in amounts as might occur unavoidably in good manufacturing practices.”(1) Natural pet foods are sometimes marketed with the claim that they contain no artificial ingredients. This may or may not be true depending upon the company. One of the common additives of concern to owners are fat preservatives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ethoxyquin. Products that do not contain a fat preservative will have a decreased shelf life because of problems with rancidity. The definition of “natural” includes ingredients that are subject to traditional processing methods, such as rendering and extraction. Owners are often unaware that “natural preservatives” such as vitamin E or mixed tocopherols, vitamin C, or rosemary extract may also be processed and/or extracted. The term “natural” also permits a disclaimer clause for products containing synthetic components in order to assure nutritional adequacy, such as “with added vitamins and minerals”. “Trace nutrients” may also be added to this disclaimer when purified amino acids such as taurine are added. Finally, veterinarians and owners also must be cautious of products that contain natural additives such as herbs, because the safety of many of these compounds has not been tested. According to AAFCO the term “organic” has been defined as “a formula feed or a specific ingredient within a formula feed that has been produced or handled in compliance with the requirements of the USDA National Organic Program (Title 7, Part 205 of the Code of Federal Regulations)”.(1) The terms “human grade” and “human quality” are used with increasing frequency these days on food labels and marketing materials. Currently there are no official definitions regulating these terms and according to AAFCO these terms are not permitted (1). However, since there is no legal definition for the term “human grade", it is likely to be interpreted and used differently from company to company. Other terms that have no legal definition include premium, super premium, gourmet, and holistic. These labels are used on a variety of
foods with different nutrient profiles, ingredients and quality. They can even appear on foods intended for supplemental feeding only. A client that is relying on these terms to select a diet may unknowingly choose a food that if fed solely could result in a nutritional deficiency long-term. Concern has been expressed about 'holistic', as it implies a therapeutic benefit when none may exist.

**By-products are unhealthy for my pet:** It is not uncommon for many manufacturers, particularly of certain marketing segments to advertise their foods to contain, “no by-products” or to suggest that the inclusion of by-products in a food creates a diet of lower quality. You may find your clients asking your opinion regarding the use of by-products in a diet or feeding regime. The term by-product has unfairly earned a bad reputation as a consequence of marketing and misinformation. Just because some segments of the population find anything other than the flesh of an animal or bird unappealing, doesn’t make it unhealthy. In fact, in many countries parts classified as by-products in this country are highly desirable. Furthermore, they are a rich source of minerals and vitamins. This author finds it particularly ironic that many of the companies that market an absence of by-products in their diets, simultaneously espouse their product to be ones that are more “natural” or closer to the Paleo- lithic diet of the dog and cat. However, observations that in domestic cats that hunt, the first things they consume are the head and entrails of their prey and that wolves and wild cats preferentially eat the abdominal organs from their kill. One should look at products carefully as many manufacturers will avoid using the term by-product by simply listing the organ of inclusion instead (i.e. beef tripe or beef liver). Remember that a product ingredient list tells you nothing about the quality or digestibility of an ingredient.

**Flaxseed is a good source of omega-3 fatty acids:** Emerging evidence suggests that omega-3 fatty acids may provide some health benefits to dogs and cats, especially during growth and development (2). Specifically, benefits may be derived from docosahexaenoic (DHA, 22:5 n-3) and eicosapentaenoic (EPA, 20:5 n-3) acids. While alpha-linolenic acid (18:3 n-3) is often supplied in products by ingredients such as flaxseed or canola oil, dogs and cats cannot efficiently convert alpha-linolenic acid to EPA and DHA, so fish, krill, or algal oil sources are required (3).

**Grain-free diets are better for dogs and cats:** There has been a trend recently amongst some veterinarians, animal professionals and pet owners to malign carbohydrates as an unhealthful food source for dogs and cats. As an obligate carnivore, much of the focus and controversy has centered on the cat. The basis for the argument is that since starch and related carbohydrates were not part of the cat’s natural diet, it is unhealthy for such products to be consumed. The simultaneous increase in the use of carbohydrates in many commercial pet foods and the increasing rates of obesity and diabetes mellitus in cats is frequently cited as evidence for this theory. However, the scientific evidence summarized below counters these claims. Insufficient insulin secretion and impaired insulin sensitivity are the major abnormalities of feline diabetes (4). Three recent population studies further refute the hypothesis that feeding dry-type extruded diets long-term are the cause of diabetes in cats (5,6,7). The association between obesity in cats and the development of diabetes mellitus has been well documented (5). However epidemiological studies report that obesity is associated with high fat foods and not high carbohydrate food (8,9). In fact there are some studies that suggest that high carbohydrate, low fat diets have an obesity-protective effect (10, 11). Exchanging dietary carbohydrate for protein does appear to be helpful for weight loss and managing diabetes in some cats; however, a similar macronutrient exchange does not appear to prevent weight gain in post-ovariohysterectomized cats (4). Current scientific evidence does not support the argument or negative press that carbohydrates in pet foods are currently receiving.

**Raw food pathogens don’t make animals sick:** The veterinarian’s job is not only to care for the health and well being of their animal patients but also those who are the guardians of these pets as well. From this perspective concerns regarding pathogenic bacteria in raw diets and subsequent environmental contamination
are paramount. There is growing evidence to support these concerns. Scientific evidence for transmission of food-borne pathogenic bacteria from dogs to humans exists (12). In Alberta, Canada, 9 of 12 case patients with S. infantis infection had been exposed to pig ear treats and S. infantis was isolated from a pig ear treat collected from one of the case patients. The isolate recovered from the pig ear was indistinguishable from S. infantis isolates recovered from fecal samples obtained from humans with salmonellosis (13). Potential human pathogens have been isolated in both commercial and home-prepared raw diets (14, 15). Animals fed raw diets have been reported to shed the same viable organisms that were isolated in their food (16). Arguably, while many animals never become ill while consuming raw food diets, they still pose a risk to humans and other animals through environmental shedding (16). Individuals preparing raw diets are also at risk by handling contaminated meat and egg products. Those greatest at risk are the very young and old, in addition to the immunocompromised. There is no documented evidence that feeding raw meat has any health or nutritional advantages over cooked foods.

Commercial pet foods contain euthanized dogs and cats: It has been suggested through a variety of resources that euthanized dogs and cats are going into the pet food chain. The rationale provided for this claim is that many dead dogs and cats coming from shelters or other locations may end up at rendering plants and that in some cases the products from those rendering facilities are sold to pet food manufacturers. The FDA has looked into these claims and developed PCR tests to look for dog and cat specific products in dog foodS (17). The findings of these tests support the unlikelihood of dead dogs and cats ending up in commercial pet foods.

Can the carbohydrate content of a diet reliably be determined from the guaranteed analysis? There is no analytical method to determine the carbohydrate concentration of a diet. The amount of carbohydrate in a pet food is determined by a calculated difference. Carbohydrate = 100 – (max protein + max fat + min fiber + min moisture). Remember that the guaranteed analysis provides minimal guarantees for protein and fat and maximum guarantees for water and fiber, so what is provided on the can or bag is not entirely reflective of the product. A typical analysis may give you a closer approximation. Diets vary in the amount and type of dietary fiber they contain. Currently pet foods are required to report crude fiber on the label. However, crude fiber represents only a portion of the total fiber in the product because it only accounts for insoluble fiber and some hemicelluloses. It does not account for any of the soluble fiber in a pet food. The amount of soluble fiber in a food can vary but will likely be significant in canned products as they often used gums/pectins in the canning process. So any fiber not captured in the crude fiber analysis then gets ‘dumped’ into the carbohydrate fraction, yielding an inaccurate result. Research supports that in maintenance dog foods, crude fiber captured 28% of total dietary fiber in dry, extruded diets and only 17% of total dietary fiber in canned products (18). This not only results in an overestimation in carbohydrate content, but subsequent caloric density as well. You get a much closer approximation by using total dietary fiber, although not many companies report that information.

Keywords: Nutrition, Diet, Internet

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Are Raw Food Diets Radical or Reasonable?
A Review of the Evidence

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Summary
Raw food diets are often referred to by the acronym “BARF” which can stand for “bones and raw food” or “biologically appropriate raw food” diet. Proponents of raw food diets proclaim many health benefits associated with this feeding regime, stating that dogs and cats are carnivores and as such they evolved eating raw food. However, there have been no studies to date to support that this feeding approach has any long-term health benefits compared to feeding other types of pet food. Despite the absence of long-term studies, there has been research looking at the nutritional performance as well as the possible risks and benefits of bones and raw food diets. This lecture will exam the scientific studies behind raw food diets.

Nutritional adequacy: Nutritional adequacy should be one of the first concerns of every practitioner regarding their patient’s feeding program. Concerns regarding nutritional adequacy not only apply to home-prepared raw diets but to commercial raw diets as well. One study that looked at the nutritional adequacy of home-prepared raw food, also looked at the nutritional adequacy of several commercial raw food diets (1,2). Two diets were commercial products, the remaining three home-prepared. All five diets had essential nutrients that were analyzed to be below (Association of American Feed Control Officials) AAFCO minimum recommendations (1,2). The home-prepared diets had excessive concentrations of vitamins D and E, as well as inappropriate calcium to phosphorus ratios. A recent survey of dog owners in Germany that were feeding home-prepared bone and raw food diets found that 76% of the 77 rations analyzed had at least one nutritional imbalance (3). Clinical case reports of problems in animals consuming commercial raw food diets are now beginning to appear in the literature as well (4).

The literature contains a variety of papers documenting the palatability, chemical composition, digestibility and bioavailability of many traditional animal and plant-based pet food ingredients. There are also a number of papers examining the effect of animal and plant-based ingredients on fecal quality and quantity. Recently more information with respect to ingredient and diet characteristics and qualities for raw diets are becoming available in the scientific literature. A recent publication reported that cooking raw meat (as one might do for a home-prepared diet) did not alter apparent total track energy or macronutrient digestibility and may also minimize the risk of microbial contamination (5). These researchers concluded that since cooking minimizes the risk of microbial contamination and results of their study demonstrated no difference between cooked and raw beef, that cooking might be an appropriate modification to the raw food strategy.

Based on some of the previous studies, it appears as though raw protein sources are highly digestible, but these studies do not provide information about availability of the nitrogen or the amino acids from these protein sources. With respect to cats, taurine is one amino acid of concern because the consequences of a deficiency are frequently fatal. The taurine content in animal proteins can vary significantly, with muscle generally containing less taurine than organ meats (6). A recent survey of commercial raw diets intended to be fed
to captive exotic cats found that some contained taurine concentrations lower than 0.1% (7). Cooking also influences taurine concentrations, and it can be lost to a significant extent when using cooking methods that expose proteins to water, thereby leaching the taurine from the food (6). These findings imply that if one doesn’t cook the protein source, taurine deficiency is less of a concern; however, the literature does not support this thinking. Taurine deficiency has been recognized in cats consuming home-prepared diets using raw protein. One research update reported dilated cardiomyopathy associated with taurine deficiency in a group of growing cats fed a diet consisting solely of whole ground raw rabbit (8). Cats were fed either whole, ground rabbit or a commercial kibble diet that had passed AAFCO feeding trials for growth. Rabbits were selected over mice for ease of processing and in places where rabbits are abundant, feral cats are known to prefer them as prey. The growth curves of cats on both diets were identical, indicating the raw rabbit diet supported normal growth. Coat quality was better (by subjective assessment) and stool quantity smaller (with less water) in the cats that were consuming the raw rabbit diet. However, the reason(s) for the differences in stool consistency of the respective diets is unknown. The investigators could find no relationship between the type of diet consumed and: 1) the rate of growth, 2) degree of inflammation in the tissue lining the intestinal tract, or 3) the numbers of bacteria in the upper small intestine. After consuming the raw rabbit diet for 10 months one of the cats died from dilated cardiomyopathy and was determined to be taurine deficient. Moreover, 70% of the remaining cats consuming the raw rabbit diet, which appeared outwardly healthy, also had heart muscle changes compatible with taurine deficiency. For the remaining three months of the study, the raw rabbit diet was supplemented with taurine and blood taurine concentrations returned to normal. The investigators concluded there were no other significant benefits to feeding the raw rabbit diet, even when supplemented with taurine compared to a traditional feline kibble. A second study evaluated plasma taurine concentrations in sand cats (Felis margarita) fed either a commercial feline kibble or a raw, horsemeat and meat by-product based diet (9). Despite a 15% increase in digestibility and a 40% increase in taurine content compared to the kibble diet, cats consuming the raw food diet had significantly lower plasma taurine concentrations. Although the plasma taurine concentrations were not below the point at which clinical taurine deficiency would be seen, they were reduced by approximately 25% during the twelve-day study period. Arguably a crude estimate at best, but if one were to project the continued rate of decline, plasma taurine would fall below the concentration where the clinical signs of taurine deficiency are frequently noted at approximately day 20 of raw food consumption. These effects would likely be more pronounced under the conditions of a more demanding life stage than maintenance, such as during growth or reproduction. The exact mechanism of how raw diets can potentiate taurine deficiency is unknown at this time. The amount of taurine available to the cat from its diet is dependent upon a number of factors including the quality and quantity of dietary protein, as well as how that protein is processed (10, 11). These factors in turn influence gastrointestinal microbial numbers and/or species that can cause taurine loss by accelerating turnover of bile acids conjugated with taurine and decrease recycling of taurine by the enterohepatic route. These factors may influence changes in bacterial populations that favor those that degrade taurine. In addition to these factors, low levels of vitamin E in a diet can cause meat to lose taurine when it is processed and ground (12).

**Oral health:** One of the many claims made by those who support a raw meat and bone feeding method is that feeding of raw bones is beneficial to the oral and dental health of the animal. Dental disease, including calculus, gingivitis and periodontitis, is considered to be one of the most common diseases diagnosed in dogs and cats (13). Periodontal disease is of most concern as it can result in tooth loss. Current research supports that dogs and cats consuming commercial diets are at risk for eventually developing periodontal disease. It appears that softer diets (including canned, semi-moist or even home-prepared foods) are even worse than dry diets (14). The supplementation of an oxtail to a commercial diet feeding regime appeared to slow down the development of periodontal disease (15). So one might surmise from these findings that the consumption of a more “natural” diet might be beneficial in the prevention of dental disease, particularly periodontitis. However, current research doesn’t necessarily support that thinking. One study in African wild dogs, whose diet is largely small antelope, found that 41% of the skulls examined had evidence of periodontitis, while only 2 had dental
calculi (16). A study in feral cats from Marion Island, where the main source of food is birds, reported evidence of periodontitis in approximately 62% of cats while only 9% had calculus (17). It has been speculated that the highly specific diet of sea birds favored the development of periodontal disease in these cats secondary to gum trauma induced by the sharp bones in the carcass (17). A smaller study in Australia established that the prevalence of oral disease was no different in cats fed a commercial diet versus those whose diet was mainly small prey (18). While a natural diet of raw meat and bones may reduce dental calculi, it does not appear to protect against periodontal disease.

**Zoonotic concerns:** The veterinarian’s job is not only to care for the health and well-being of their animal patients but also those who are the guardians of these pets as well. From this perspective concerns regarding pathogenic bacteria in raw diets and subsequent environmental contamination are paramount. There are numerous publications documenting pathogenic organisms in raw meat and raw food diets. A few that are particularly relevant to the veterinary practitioner or are very recent are highlighted below. There is growing evidence to support these concerns. Evidence for transmission of food-borne pathogenic bacteria from dogs to humans exists. In Alberta, Canada, 9 of 12 case patients with S. infantis infection had been exposed to pig ear treats and S. infantis was isolated from a pig ear treat collected from one of the case patients. The isolate recovered from the pig ear was indistinguishable from S. infantis isolates recovered from fecal samples obtained from humans with salmonellosis (19, 20). Potential human pathogens have been isolated in both commercial and home-prepared raw diets (1, 21). Animals fed raw diets have been reported to shed the same viable organisms that were isolated in their food. There have been reports of racing greyhounds, sled dogs, guard dogs and cats with Salmonella infections due to consumption of contaminated raw meat (22, 23). A recent publication supports testing fecal samples on more than one occasion if one is suspect of contamination in an animal’s feces (24). Arguably, while many animals never become ill consuming raw food diets, they still pose a risk through environmental shedding. Individuals preparing raw diets are also at risk by handling contaminated meat and egg products. The greatest at risk are the very young and old, in addition to the immunocompromised. In some cases, despite understanding all of the risks, an owner may wish to continue to feed a raw diet. Practitioners should refer their clients to the FDA’s website and go over safe handling of food and cleaning practices. It has been shown that simple routine washing may not be enough to eliminate potential food-borne pathogens in the pet’s food bowl and environment (25). It is also important to document any discussions one has on this subject, as it may have legal ramifications (26).

**Keywords:** Nutrition, Diet, Raw Food

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The New Trend of Small Animal Clinical Nutrition

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Summary
Clinical nutrition is the most ancient medicine, but also a recent science for small animals. All prescription diets for dogs have originally been formulated by vets, and still are nowadays, by vet nutrition specialists. Year after year, the pet food industry increase the offer, and numerous diets are available on the market, corresponding to various conditions, at least the most common ones. But, when none of these diets fits the specific requirements of one dog or cat, either because of a complex condition, or because the pet definitely refuses to eat any adapted prescription diet, but would accept only home-prepare food, then a specialist in clinical nutrition may formulate a special recipe.

Thanks to an intense research, adaptation of nutrition is proven to be helpful to pets suffering of various conditions. But nutrition is not like an infection that the right antibiotic can fix. Nutrition is the way to bring in the body about 40 different nutrients at a time, in an amount and with a form that is compatible with the animal and its condition, and also compatible with the ability of the owner to make it. Even if research is abundant, it is rarely one recipe for one condition. To cover nutritional requirements of a dog or a cat means to consider various individual data: specie, breed in some cases, optimal body weight, sexual status, age, activity/sedentarity, disease.

Here is a non exhaustive list of conditions that can benefit of nutrition adaptation (compare to the requirement of a healthy adult at maintenance, intact and living in a garden the day, indoor at night), with some of the main adaptations to consider:
- growth and reproduction: increased requirement for energy, protein, essential fatty acids, minerals, vitamins, decreased tolerance to or lower appetite so fiber and starch amount must be limited,
- neutering: decreased energy requirement, but not other requirements neither appetite,
- aging: increased demand for anti-oxidants, protein, some fatty acids, decreased mineral requirement especially phosphorus, but energy expenditure and adaptation of fiber variable upon individuals,
- medical treatment: i.e. probiotics to maintain the microflora around the prescription of antibiotics, or adaptations to cover appetite along with corticotherapy without increasing the energy intake,
- food intolerance and food allergy: to diagnose a real allergy by offering an adapted elimination diet and provocation tests to allow a long term coverage of requirements especially protein requirement,
- cardiology: to provide enough energy and protein to delay cachexia, additionally to L-carnitine and taurine,
- dermatology: after a symptomatic treatment usually required at first, the goal is to cover all nutrient requirements due to the huge impact of malnutrition, especially in protein and amino-acids, polyunsaturated fatty acids, vitamins and trace-elements to skin and hair health,
as well as digestive troubles including stomach, pancreas, liver, small intestine and bowel, endocrine disease especially diabetes, nutrition management around surgery to consider convalescence, progressive refeeding after a period of digestive emptiness, obesity treatment, poor appetite, kidney disease, low urinary tract disease, some neurology troubles associated with lipid metabolism...
Cases will be presented and discussed.

Clinical nutrition is the most ancient medicine, as it has already been suggested by the father of modern medicine, Hippocrates (about 400 b.c.), and his famous quote: "Let food be your medicine and medicine be your food". In fact Hippocrates did more: he separated medicine from religion, explaining that a disease was not a punishment inflicted by the gods but rather the product of environmental factors, diet, and living habits. He also proposed to adapt diet to people's humor, and often used lifestyle modifications such as diet and exercise to treat diseases like diabetes mellitus.

In the next centuries, animal nutrition has been grossly oriented to feed animals and, especially since World War II, to provide more and cheaper animal protein to the people. Veterinary medicine and nutrition research has been first used on clinical nutrition linked to metabolism disorders frequently observed in intensive production such as keto-acidosis of dairy cows...

Small animal -and individual- clinical nutrition has developed with the increasing room let to pets in the household, first with dogs, more recently with cats, and nowadays even with ferrets, rabbits and other new companion animals... So clinical nutrition is sometimes considered as a recent science even by veterinarians. The European Society of Comparative and Veterinary Nutrition (ESVCN) has been created in 1991, became independent of ECVIM in 1996, and held its first conference in Munich in 1997, and the European College of Comparative and Veterinary Nutrition (ECVCN), sometimes called by mistake College of Clinical Nutrition, was created with de facto Diplomates in 1998, and the first Diplomates by examination in 2001.

At the same time, clinical nutrition has developed...

The oldest book in English I could find was Nutrition of the Dog, first published in 1943 (1) by Clive McCay, Professor of nutrition from Cornell University. No mention of therapeutics.

The next one I could find "Basic guide to Canine nutrition, with a special section on nutritional requirements of cats, was edited in 1965 (2), by Walter N. Chimel, director of the Gaines Dog Research Center. This first chapter is entitled The veterinarian's role in practical canine nutrition...and the subtitle is Nutrition in therapy. In this book, Nutritional therapeutics last 10 pages, and the Nutritional requirements of cats for 8 pages!

The more recent and well known "Small Animal Clinical Nutrition" was first edited by Mark Morris Jr, a veterinarian, and the 5th edition was published in 2010... it contains 1315 pages(3) !

Today, a lot of situations may be identified as caused by inappropriate nutrition, and even more clinical situations may benefit from a nutritional evaluation and adaptation. In 2010, Global Nutrition Guidelines were proposed by WSAVA (see http://www.wsava.org/nutrition-toolkit) and provided a worldwide focus on the need of a nutrition evaluation as a part of clinical examination at every consultation.

Last but not least, pet owners’ demand about the nutrition of their pets increase, as observed in a German study (4), representing a daily challenge to veterinarians, who miss skills and time for more education. When a dog or a cat is in pain, the owners look for a resolution of the problem. In various occasion, nutrition may be a precious helper, as, as an example, this recent communication about the beneficial distribution of a probiotic and a prebiotic to lower incidence of diarrhea in a dog shelter (5).

Food has a special room in the relationship between a pet parent and the animal. It is given daily. It must be adapted to the animal, not only for convenience or price, but first to cover properly the individual requirements. Main individual factors affecting nutrition requirements, main situations and related nutrition adaptations to consider are summarized in tables 1 and 2. The veterinarian then can consider the overall situation to prescribe an adapted diet, either a ready-to-eat diet or a balanced home-prepared one.
Table 1 Main individual characteristics influencing the nutrition requirements of Dogs and Cats

<table>
<thead>
<tr>
<th>Healthy or not</th>
<th>Main nutrition adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal Body weight</td>
<td>Energy, protein and amino acids, lipids and essential fatty acids minerals, vitamins, trace elements</td>
</tr>
<tr>
<td>Neutering (male&amp;female)</td>
<td>(-20%) Energy</td>
</tr>
<tr>
<td>Haircoat</td>
<td>(+) protein if long hair (+) aromatic amino acids (Phe+Tyr) if black</td>
</tr>
<tr>
<td>Breed</td>
<td>Energy (-) in nördic breeds (+) in nude breeds, Greyhounds and Great Danes</td>
</tr>
<tr>
<td>Activity (if intense exercise)</td>
<td>Energy (+ protein, minerals, vitamins, trace elements)</td>
</tr>
<tr>
<td>Age (growth) Gestation Lactation</td>
<td>(+) Energy, protein and amino acids, lipids and essential fatty acids minerals, vitamins, trace elements (-) Carb.</td>
</tr>
<tr>
<td>Aging</td>
<td>(-) Phosphorus &amp; minerals (+) anti-oxidants</td>
</tr>
<tr>
<td>MAY RESULT IN</td>
<td>Adapted diet to provide all required nutrients in an appropriate volume</td>
</tr>
</tbody>
</table>

Table 2 Main conditions and diseases in which a nutrition adaptation may be recommended in Dogs and Cats

<table>
<thead>
<tr>
<th>Disease</th>
<th>Main nutrition adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic kidney disease</td>
<td>(-) Phosphorus, (+) Ca:P ratio, limit protein to cover minimal requirement (+/-) Energy to go back to normal body condition if appropriate</td>
</tr>
<tr>
<td>Obesity (treatment)</td>
<td>(-) Energy (but not other nutrients)</td>
</tr>
<tr>
<td>Skin disorders (in case of food-intolerance or food-allergy)</td>
<td>Good coverage of requirements with focus on protein and amino acids, lipids and essential fatty acids, minerals, vitamins, trace elements (+) EPA and DHA (+) appropriate choice of ingredients</td>
</tr>
<tr>
<td>Atopy</td>
<td>(+) specific fatty acids EPA and DHA</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>(+) specific fatty acids EPA and DHA (+/-) Energy to go back to normal body condition if appropriate</td>
</tr>
<tr>
<td>Anorexia Digestive emptiness</td>
<td>Tube feeding as soon as possible, specific liquid diet for dogs &amp; cats Progressive refeeding (1/N*requirement on Day 1 of refeeding, 2/N on Day 2... until Day N, with 2&lt;N&lt;10)</td>
</tr>
<tr>
<td>Urinary tract disease</td>
<td>(+) zucchini (+) water +/- salt (-) minerals (but still cover requirements and maintain recommended ratios) to lower urinary saturation</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>(+) protein, (-) carb. to lower insulin required, stability day after day (+/-) Energy to go back to normal body condition if appropriate</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>(+) addition of enough pancreatic extract with each meal or food consumption Diet highly digestible</td>
</tr>
<tr>
<td>Intestinal disorders</td>
<td>Good coverage of requirements with focus on protein and amino acids, essential fatty acids, minerals, vitamins, trace elements (+) split the daily amount into 3 meals per day (+) probiotics (+/-) insoluble fiber -enough to ensure stools texture, not too much to not lower digestibility and dilute the diet excessively- (+/-) soluble fiber (i.e. prebiotics) to stimulate colocytes, but avoid watery stools</td>
</tr>
<tr>
<td>Perineal disease / Pelvis fracture / abscess of the anal sacs...</td>
<td>Very high digestibility and low residue diet (+/-) soluble fiber to soften feces</td>
</tr>
<tr>
<td>Hepatic insufficiency</td>
<td>(+/-) protein and lipids: individual adaptations &amp; tolerance</td>
</tr>
<tr>
<td>Cardiovascular disturbances</td>
<td>Good coverage of requirements with focus on protein and amino acids, lipids and essential fatty acids, minerals, vitamins, trace elements (+) EPA and DHA (+) taurine and L-carnitine in appropriate cases</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>DOG: cover minimal protein and lipid requirements (individual tolerance) CAT: cover minimal protein and lipid requirements, do not increase carb. (+) split the daily amount into 2 to 3 meals per day</td>
</tr>
</tbody>
</table>
Keywords: Clinical Nutrition, Cat, Dog

References
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Is There a Need for Specialists?

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Summary
Most veterinarian have studied nutrition at some point during their veterinary studies, at least nutrition for food animals. And only a few hours of pet nutrition, if any. If the principles of nutrition are similar regardless the specie, the goals are different: feeding food animals is based on finding the best cost effective ratio to produce food. The goal of small animal nutrition is based on feeding the best diet to increase life expectancy, enhance healthy and well-being.

Clinical nutrition for companion animals is a recent science. Additionally, practitioners for companion animals may not feel like doctors when thinking about nutrition. The increasing market for petfood has moved the interest for nutrition to a medical trust, as if a diet could be as unique and active as a drug. It is partly justified in most cases, but not all.
First, all petfoods, even with the same target, are not similar.
Second, petfood does not cover all situations and diversity of pets.
Third, some animals do not accept or cannot afford petfood.
Forth, food sanitary crisis, petfood recalls and social pressure may affect the trust of consumers in the petfood industry. Then, pet owners have started to look for other options. Veterinarians are first on line to answer.
Last but not least, some owners do not want to give only petfood to their pet, but a mix, including petfood and human food like meat or veggies for instance. And this is possible if the owner is properly guided and the overall diet balanced... by a vet nutrition specialist...
A minimal training in nutrition is required for the vet practitioners to be aware of the situations that may be either caused by malnutrition or improved or cured by adapted nutrition. Then, the need for adaptation of the diet may be understood and really prescribed, and not only suggested.
This prescription requires skills and specific nutrition prescription. Vet practitioner, even aware of this demand, and conscious of the role of adapted nutrition, may have no time for a long nutrition course to get enough skills to formulate recipes or safely associate foods. At this point, specialists in nutrition, as for any specialty, may act together with practitioners to provide the best prescription to the patient, considering the individual nutritional requirements due to the global situation of the animal and the request of the owner.
Since a few years the number of vet clinical nutrition specialist opening a specialist consultation has increased in Europe and in the US but is still small.
Since almost 10 years now, together with my team, we have developed an online e-nutrition service which offers to vet practitioners the access to a nutrition specialist, in response to a documented demand from the vet.
Slowly, the vet profession adapt itself to both science and public demand, and can use clinical nutrition as a full vet specialty. But with only a few specialists around, an online service seems to be a complementary
option to the private specialized consultation offer in nutrition.

Nutrition is life. The role of a vet nutrition specialist is to formulate a balanced diet. A balanced diet must be edible by the target animal and givably by the owner. A balanced diet must provide enough energy to maintain optimal body weight, and, in this amount of food, provide all required nutrients to cover the animal requirements. The composition of the daily diet consumed must consider all factors affecting the nutrition requirements.

For instance, a 16kg adult intact dog living in a closed garden requires 1000 kcal ME and 60 grams of protein... Fed with a wet food, it may receive 1kg of a diet containing 6% of protein. Fed with a dryfood, it may receive 250 grams of a diet containing 4 kcal/gram and 24% protein...

If this dog is neutered, his energy requirement decrease by 20%, due to neutering, so to maintain its body weight stable, it must ingest only 800 kcalME, but still requires 60 grams of protein, and the same appetite. With the same fist diet, he must receive only 800 grams of the wet food, or 200 grams of the dry food, to avoid weight gain. But doing this, he will receive 48 grams of protein instead of 60...

Proteins are necessary for all functions, such as optimal growth and reproduction, but also healthy skin and haircoat, dental health, digestion, gastrointestinal function, heart function, muscle mass, immunity (including resistance to all kind of disease and parasites)... Same story for other essential nutrients (fatty acids, minerals, vitamins...). Malnutrition does not kill rapidly, and short term shortage has no visible consequences, but affects long term health.

To cover nutrient requirements and appetite, the best is to change the diet for a diet with a lower energy density and, for all nutrients but energy, a higher nutrient to energy ratio (for instance a wet with 80kcal/100 grams and still 6% protein or a dry with 330 kcal /100 grams and still 24% protein).

Let's now consider this dog becomes inactive, living indoor, with only a one-hour walk per day, or is hospitalized or has to limit activity due to surgery or to some debilitating disease. This sedentariness decreases the energy spent, and so energy requirement. To avoid any weight gain, it requires a reduction of energy intake by 20%. With the same first diet, he must receive only 1000 * 0.8 * 0.8 = 640 grams of the wet food, or 160 grams of the dry food only... But these amounts may not cover appetite and certainly any of the other nutrition requirements. The best option is to provide a diet with a lower energy density and an increased protein to energy ratio (for instance a wet food with 64kcal/100 grams and still 6% protein, or a dry food with 250 kcal /100 grams and still 24% protein)...

Additionally to this, some breeds, like nordic dogs Husky or Retriever, require less energy than others, due to selection of the breed to the climate adaptation, and this characteristic comes on top of lifestyle and neutering previous adaptations. Then it may become really tricky to cover all requirements, and appetite, but not exceed energy provided with the diets on the market. A mix with human food may be a good option, but has to be calculated to cover requirements.

Additional constraints may be added, like the climate (hot or cold) which modify the energy requirement, some diseases which modify the requirements for some nutrients (protein, lipid, carbohydrate, calcium, phosphorus, copper,...).... This may require the formulation of an individualised diet but a vet nutrition specialist. The goal is to prescribe a balanced diet, considering all requirements, and the composition of foods, and to use petfoods, but also human foods, and supplements when required to provide a global and acceptable recipe.

There are a lot of options to cover properly nutrition requirements of dogs and cats.

* petfood only: dry and/or wet complete foods may be chosen on their composition to cover requirements, and the amount calculated to cover but not exceed energy requirement.

If treats are provided, they must be considered as a part of the diet prior to calculating the amount of food and not exceed 10% of the energy requirement.

* petfood (dry and/or wet) +/- veggies +/- fruits +/- dairy product +/- treats : the total must be considered
to make sure the requirements for essential nutrients are covered. Veggies and fruits and dairy product may be added, without problem if well tolerated and if the amount is reasonnable (10g of veggies like carrots or zucchini per kg of body weight for instance, and 10% of energy requirement for dairy products)  
* petfood (dry and/or wet) and/or lean meat or fish... Meat of Fish filet os not a complet food, so for more than a bite per day, adding meat of fish may require the addition of a mineral vitamin supplement (with calcium but no phosphorus) and, depending of the amount the addition of essential fatty acid source (canola oil and possibly fish oil, depending on the amount of meat). Addition of veggies, fruits and treats is also possible.  
* home-prepared diet: meat/fish + mineral vitamin supplement (with calcium to phosphorus ratio of 2 to 3 or no phosphorus added) + essential fatty acid source (canola oil and sometimes fish oil) + veggies (cooked) +/- cooked carbohydrate source +/- fruits +/- dairy product +/- treats  
* barf or raw diet: usually based on a concept of meat given raw (risk of bacteria, that are killed only by 60°C heat for a few minutes and of parasites killed also by heat or by freezing), offals (with a risk of deficiency of excess of vitamins A and D depending on the liver provided, not offals are nutritionally equivalents), and bones (with the main risk of excess of minerals, which may lead to digestive occlusion, excess of calcium and phosphorus and zinc and copper secondary deficiency due to the excess of calcium), brewers yeast, risk of not enough essential fatty acids, and not enough fiber with often raw veggies and fruits brought in very small amount... These regiment can be balanced and safe with appropriate advice.

There is also lot of situations that require to adapt or revise nutrition: see other lecture: New trend of small animal clinical nutrition.

It often appears difficult to veterinarians to prescribe nutritional change to the pet owner. Mainly because they do not feel confortable providing any advice apart about petfood. But most people do not want to give only petfood to their pets, mostly because they are companion animals. A lot of pet owners give treats to their dogs (89% in France) (1) and give meat or fish almost daily to their cat (28% in France) (2). And this is not a problem. It is the opposite of a problem. It is a way for veterinarians to play their roles of professional adviser to pet owners.

The service of a vet nutrition specialist may be a huge help. Veterinarians who experienced our online service found it very easy to offer a nutrition service to the owner, who indeed are looking for independant service and advice. In the clinics who have adopted a nutrition service, the number of clinical nutrition prescription sold has increased from 0 to 8 per month in the first 9 months of use of the online service... A few considerations later... the number have doubled to 18 prescriptions per month... We have identified a few situations in which the service is communly used by practitionners, but the list is not exhaustive:  
*Any situation where the diet includes petfood and meat or fish, or petfood at one meal, and human food at the other meal (the classi mix of meat and veggies and rice), as the petfood will not compensate what the mix do not provide, it may request a balancer (i.e. canola or soybean oil and a mineral vitamin supplement must be added).  
*Consultations for vaccination, dental care, annual check, geriatric consultation, reproduction (gestation and lactation), growth, obesity, weight loss, digestive troubles lasting for more than a few days, any skin disease and hair coat troubles, corticotherapy, diagnosis of any chronic disease either organic or metabolic or endocrinologic, outbreake of any disease, surgery, refeeding after more than one day of digestive emptiness, convalescence and recovery after hospitalisation.

Here are a few suggestions to make the change: move from suggestion to prescription...
1-Feel comfortable with prescription of nutrition instead of suggesting a nutrition change. When a nutrition adaptation is interesting because of the actual diet is not adapted to the present pet situation, it has to be presented as a medical recommendation by the vet. People are free to refuse.

2-Realise a nutrition evaluation at each consultation for each pet, using the nutritional assessment checklist for instance (see http://www.wsava.org/sites/default/files/Extended%20diet%20history%20form.pdf) to identify objectively the situation requiring nutrition adaptation.

3-If the situation requires nutrition adaptation, make it clear to the owner and propose an option: the situation requires a nutrition adaptation, and we work with a vet nutrition specialist (inside the clinics or referring to a private referral center or to a university service or using an online service)...  

4-It is not for free... set a cost line in the list of rates, per recipe, in case of healthy situation / pathology / ... The service may be included in some package (for instance a recommendation for the first few weeks after surgery for instance), but may be listed as included or offered, but not free, as it is valuable.

5-Always take into consideration the kind of food the owner wants to give (dry/wet/home-prepared or barf/mix... treats), an why, to stick to it if it is compatible with the situation of the pet (and if not, explain why).

**Keywords:** Clinical Nutrition Service, Vet Nutrition Specialist

**References**


Nutritional Management of Kidney Disease in Dogs and Cats

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Summary
Dietary therapy has remained the cornerstone of management of chronic kidney failure for decades. The goals of dietary modification are to 1.) Meet the patient’s nutrient and energy requirement, 2.) Alleviate clinical signs and consequences of the uremia intoxications, 3.) Minimize disturbances in fluid, electrolyte, vitamin, mineral and acid base balance and 4.) Slow the progression of kidney failure. Chronic kidney failure is progressive and dynamic, hence serial clinical and laboratory assessment and modification of therapy in response to changes in the patient’s condition is integral to successful patient management.

Energy: Sufficient energy needs to be provided to prevent endogenous protein catabolism that results in malnutrition and exacerbation of azotemia. Although the energy requirements of dogs and cats with chronic kidney failure are unknown, they are presumed to be similar to healthy dogs and cats. Determination of caloric requirements may vary by as much as 25%, therefore should be individualized to patients needs based on serial determinations of body weight and body condition score. Diets designed for the management of kidney failure are usually formulated with a relatively high fat content. Fat provides approximately twice the energy per gram than carbohydrates, increases the palatability and energy density of the diet, which allows the patient to obtain its nutritional requirements from a smaller volume of food.

Water: Animals with chronic kidney disease have a compensatory polydipsia secondary to the osmotically driven polyuria of their disease. As a consequence, fresh water should be available at all times for these patients to encourage water intake and prevent dehydration. Regardless in some cases, animals will need supplementation. Maintenance fluids can be administered subcutaneously

Protein: Dietary restriction of protein has been advocated in patients with chronic kidney disease for two reasons. The first reason is to limit the progression of renal disease. Studies in rodents have demonstrated a beneficial effect when protein is restricted in remnant kidney models (1). Similar studies in dogs and cats have not yielded comparable results (2-4). Although the impact of protein restriction on the progression of renal failure remains controversial, it does have other potentially positive effects on clinical signs. Lower protein diets moderate the magnitude of polyuria and polydipsia through the delivery of less solute to the kidneys as proteinaceous waste products. The magnitude of anemia may also be reduced, as nitrogenous waste products have been incriminated in hemolysis, shortened red blood cell survival, and blood loss through gastrointestinal ulcerations and impaired platelet function. The second reason to restrict dietary protein is to alleviate the clinical signs of uremia that are magnified by protein catabolism. There is general agreement among veterinary nephrologists that the presence of clinical signs secondary to uremia is a valid reason to restrict dietary protein (5). Azotemia and uremia are due to the accumulation of protein metabolites derived from excessive dietary protein and degradation of endogenous protein. High protein intake exacerbates the azotemia and morbidity of chronic kidney failure, while protein malnutrition is strongly correlated with morbidity and mortality. The rationale for formulating a diet that contains a reduced quantity of high quality
protein and adequate non-protein calories is based on the premise that controlled reduction of non-essential protein results in decreased production of nitrogenous wastes, with consequent amelioration or elimination of clinical signs even though renal function may remain essentially unchanged. Although urea is not a major uremic toxin it is regarded as an index for all nitrogenous wastes, hence therapies designed to reduce urea concentration are presumed to reduce other uremia toxins and usually correlate with clinical improvement. However, it is important to remember that BUN is influenced not only by dietary intake but dehydration, catabolism, gastrointestinal bleeding and sepsis. Research has shown that modifying dietary protein intake can reduce blood urea nitrogen and provide clinical benefits to dogs and cats with chronic kidney disease (8,9). Patients symptomatic for International Renal Interest Society (IRIS) Stage III/IV chronic kidney disease should benefit from a protein-restricted diet (11). This degree of restriction is necessary only in animals with profound renal failure, and more protein can be fed to dogs and cats with greater renal function. However, it is more difficult to provide graded protein restriction in cats because their dietary requirements for protein are considerably greater than dogs. The goal of dietary protein restriction is to reduce the BUN as much as possible while avoiding protein calorie malnutrition.

Proteinuria: Proteinuria is a significant independent risk factor for reduced survival in canine and feline patients with naturally occurring chronic kidney disease. Therapeutic strategies should be undertaken to minimize proteinuria in those animals (10). IRIS recommends implementation of a restricted protein diet in conjunction with ACE- inhibitors for dogs in Stages I-IV kidney disease that have a UPC > 0.5 (11). Dietary protein restriction and ACE-inhibitors are recommend for cats with Stages I-IV disease that have a UPC > 0.4 (11).

Phosphorus: Phosphate retention and hyperphosphatemia occur early in the course of renal disease and play a primary role in the genesis and progression of renal secondary hyperparathyroidism, renal osteodystrophy, relative or absolute deficiency of 1,25-dihydroxyvitamin D, and soft tissue calcification. By minimizing hyperphosphatemia, secondary hyperparathyroidism and its sequelae can be prevented. The goal of therapy is to normalize serum phosphate concentration. Restriction of dietary phosphorus has been advocated in healthy older dogs to prevent renal damage (presumably by preventing kidney mineralization). Studies in nephrectomized geriatric dogs failed to demonstrate a positive effect with phosphorus restriction (6,7). The effects of protein, versus phosphorus restriction have also been examined in dogs with reduced kidney function. Studies demonstrated that phosphorus restriction in dogs with induced kidney failure, and moderate to severe azotemia, reduced mortality and prolonged life (7, 12). Less work has been conducted in cats, however one study showed phosphorus restriction in cats with chronic kidney disease had a beneficial effect (8). Controlling blood phosphate concentrations has been reported to reduce mortality in cats with chronic kidney disease (13). How phosphate restriction slows the progression of renal disease is not known but it may be related to decreased phosphate retention, decreased soft tissue mineralization or prevention of secondary hyperparathyroidism. The importance of dietary phosphate was demonstrated in a retrospective study of 211 cats in which there was a 11.8% increased risk of death for each one unit increase in the phosphorus concentration (mg/dL) (14). Diets that are low in protein are generally low in phosphorus, therefore protein restriction and a degree of phosphorus restriction can be achieved by using commercial foods designed for renal disease, but many patients will still require phosphate binders. IRIS recommends that the phosphate concentration should be maintained at 2.7-4.6 mg/dl (or between 0.9 – 1.5 mmol/l) for stages II, III and IV (11). Realistic post-treatment goals for stages III and IV are < 5 mg/dl and < 6 mg/dl (or 1.6 and 1.9 mmol/l respectively (11).

Potassium: Potassium concentrations are of particular concern in feline patients with chronic kidney disease. It has been well established that hypokalemia and renal dysfunction may be related in cats, but the nature of that relationship remains unknown. One study reported an association of hypokalemia with an increased risk of systemic hypertension in cats with chronic kidney disease (15). Fourteen percent of dogs with chronic
kidney disease in a retrospective study were hypokalemic (16). Potassium supplementation is indicated when the serum potassium concentration is less than 4 mEq/L using oral potassium gluconate or potassium citrate supplementation. Potassium chloride can be acidifying and should be avoided. Remember that not all animals will be hypokalemic. One study reported 13% of 116 cats with renal disease were hyperkalemic (17). This finding underscores the importance of monitoring potassium in cats and adjusting intake on an individual basis. Seventy one of 152 dogs with chronic kidney disease had at least one episode of hyperkalemia (16). Possible causes were considered to be concurrent use of angiotensin converting inhibitors, dietary potassium intake and progression of renal disease (16). Many of the dogs in this study were consuming a therapeutic diet designed to manage kidney disease and responded to a home-prepared diet with lower potassium concentrations.

**Sodium:** Sodium restriction has been recommended to alleviate hypertension associated with failure to excrete sodium. Hypertension has also been implicated in the progression of kidney failure. However, severe sodium restriction should be avoided as this may result in volume depletion, pre-renal azotemia, and the inability to reabsorb bicarbonate, exacerbating metabolic acidosis. The capacity to adjust sodium excretion rapidly in response to changes in intake becomes severely impaired as renal failure progresses, therefore a gradual change from the animal’s previous diet to the salt restricted diet is recommended.

**Other nutrients:** Studies in dogs with renal disease suggest that supplementation with \( \cdot \cdot \cdot 3 \) fatty acids reduces inflammation, alters plasma lipid concentrations, lowers systemic blood pressure and aids in the preservation of renal function (18). Omega 3 fatty acids alter eicosanoid production through competition with arachadonic acid. In contrast, \( \cdot \cdot \cdot 6 \) fatty acids appear to be detrimental (19). The ideal \( \cdot \cdot \cdot 6 : \cdot \cdot \cdot 3 \) ratio is unknown, however many commercial diets have an adjusted ratio of 5:1. One retrospective study reports that cats consuming high concentrations of EPA may have longer survival times (20).

**Feeding Management:** Different strategies to encourage food include; warming the diet, offering odorous foods, adding water or salt-free bullions to the diet and using positive reinforcement such as petting or stroking the animal. Appetite stimulants such as benzodiazepam derivatives or serotonin antagonists can be useful in some cases, but often more aggressive therapy in the form of feeding tubes are required. Sick animals are more likely to eat familiar foods than novel foods when ill. If an animal is sick enough to require hospitalization and rehydration therapy, it is best to withhold a dietary change until the animal feels better. A higher rate of acceptance to a new, protein-restricted diet will be achieved if the new diet is introduced in the home environment after the patient is feeling better. Owner dietary compliance can be checked by calculating the BUN:Creatinine ratio. On a normal diet, it will be \( \sim \)25, whereas on a low protein diet it will be \( \sim 10 \).

**Keywords:** Kidney, Diet, Nutrition

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Nutritional Management of the Oncology Patient

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Summary
Differences in tumor biology and the patient's nutritional status on presentation means that no one diet is appropriate for every patient. This lecture will review the literature regarding some of the proposed risk factors for the development of cancer in dogs and cats. It will also examine the scientific evidence behind some of the nutrients and diets recommended for dogs and cats with cancer. Practical recommendations regarding nutritional goals for managing all patients with cancer will also be reviewed.

Prevention: Some risk factors often cited in humans have also been investigated in dogs or cats. At this point, most of the research has been done in dogs. One epidemiological study examined the effect of dietary vegetable consumption on reducing the risk of transitional cell carcinoma (TCC) in the urinary bladder of Scottish Terriers (1). This case controlled study had 92 cases and 83 control dogs. Owners of both the case dogs and the control dogs were asked to complete a questionnaire reporting diet and vitamin intake 1 year prior to a diagnosis of TCC. The study authors reported an association between high vegetable consumption and a lower risk for developing TCC. The authors concluded that the consumption of certain vegetables may prevent or slow the progression of TCC in Scottish Terriers. This study had several limitations including a small sample size (when compared to human studies). Perhaps most important however, is that owners were asked to recall what they fed 1 year prior to the time their dog was diagnosed with TCC. Research strongly supports that diet recall and even prospective reporting is fraught with problems and that what ends up getting reported is often very different than what is consumed (3). Obesity is also frequently cited as a predisposing or risk factor for the development of cancer. But what does the scientific literature tell us about this connection? Sonnenschein and colleagues examined body conformation, diet and risk if breast cancer in pet dogs using a case controlled approach (4). There were 150 case dogs and 300 controls. They reported the risk of mammary cancer among spayed dogs was lower in animals that had been thin at 9-12 months of age. They found no significance in intact dogs. This study has several limitations, perhaps most importantly that owners were asked to recall not only diet history but also body condition 1 year prior to diagnosis and body condition at 9-12 months of age. While the focus of this study is obesity, we also know that many dogs gain weight once they are neutered, often around 9-12 months of age. Given the absence of any findings in intact dogs, one has to wonder what role castration may play as well. Another study by Alenza and colleagues several years later reported that obesity at one year of age in dogs was a risk factor for developing mammary cancer (5). This study shared similar limitations to the one by Sonnenschein and colleagues. A more recent study by Weeth and colleagues examined the prevalence of obesity in dogs with cancer (6). The study looked at 1,777 cases and 12,893 controls. Dogs were categorized based on tumor type. Overall they found a lower incidence of obesity in dogs with cancer, raising the question can obesity possibly have a protective effect? This study encompassed a much larger number of dogs, looked at multiple cancer types and used a standardized, validated body condition scoring system implemented by veterinarians to score the participants. A notable limitation of this study was the absence of historical body weights in the dogs. Alternatively, we know cachexia is a common
problem in certain types of human cancers. What about its prevalence in dogs and cats with cancer? At this point, data on this question is only available from one small study in dogs. Michel and collaborators evaluated body condition and weight loss in 100 dogs with cancer visiting a university oncology service (7). Fifteen percent of the dogs in the study had some evidence of muscle. Only 7% were classified as overweight or obese (body condition score – BCS > 7/9), whereas only 4% were classified as cachectic (BCS < 3). Overall the number of dogs with a declining body weight or loss of lean body mass was less than reported in the human literature.

**Cachexia:** While the currently literature does not support a high prevalence of cachexia among dogs with cancer (we lack data in cats at present), it is important to understand what cachexia is, the underlying pathology and how to assess for it. It is very different than weight loss in that you cannot correct with feeding alone. It was once described as a state where “all you can eat is yourself.” (8). In patients with cancer, cachexia is the result of a reduction in protein synthesis. Not only does the patient lose muscle mass, but also a reduction in vital organ size is noted. While a final common pathway has yet to be elucidated, current evidence points to activation of neuroendocrine and inflammatory systems, increased lipolysis, reduced appetite and malabsorption. This can have significant clinical implications as in starvation: weight loss exceeding 40% of lean body mass is not compatible with life. How do we diagnose cachexia in our canine and feline patients? While the diagnostic criteria used in humans may be difficult to apply in dogs and cats, it is worthwhile considering. One veterinary nutritionist proposed a 5 point grading scale for cachexia (9). No muscle loss is scored as 0, mild muscle is 1, moderate muscle loss is 2, marked muscle is 3, and severe muscle is 4. One might also wish to consider using additional criteria often considered in humans when trying to diagnose cachexia. This includes accounting for the presence of a chronic disease, loss of weight > 5% in the past 12 months, and the presence of at least 3 of the following: lethargy, reduced strength, and biochemical abnormalities such as inflammation, anemia or low albumin.

**Nutrition in the cancer patient:** Two nutrients of interest that have been incorporated into diets for dogs and cats with cancer are the omega 3 fatty acids docosahexaenoic acid and eicosapentaenoic acid (DHA and EPA, respectively). Both fatty acids have been shown to have anti-cancer effects on tumor growth, prevention of cachexia and normalizing lactate concentrations in both human and animal models. The exact mechanism of action is unknown but hypothesized to be anti-inflammatory or anti/prooxidant effects. Arginine is a required amino acid for both dogs and cats (but not adult humans). It is considered to have an important role in the immune system. Currently studies show conflicting results regarding the role of this nutrient in cancer. One publication reported on the effect of a diet supplemented with omega 3 fatty acid and arginine on remission time, survival time, and metabolic aspects of dogs with lymphoma (10). In the study, 32 dogs with either stage IIIa (n=28) or IVa (n=4) lymphoma were randomized and fed one of two diets before and after remission was achieved using up to five doses of doxorubicin. The diets were isocaloric and isonitrogenous. One diet was supplemented with menhaden oil and arginine; the other with soybean oil. Dogs with stage IIIa lymphoma on the experimental diet had a longer disease free interval and survival time compared with the stage IV a group. There were no effects on quality of life or toxicity scores. Based on the study design, it is impossible to know if any derived benefits were from the fatty acids (fish oil) or the arginine. The ratio of n-3 to n-6 fatty acids was also different between the experimental and the control diet, making it difficult to know what role the fat in the diet played. It was also not clear how the dose of fatty acids was selected. Limitations of this study include the small number of dogs in the stage IVa group as well as how the dogs were staged upon entering the study. Dogs were staged by radiographs rather than ultrasound making it harder to know the extent of the cancer in both body cavities. A second study the same group, using a diet similar to the previous study but without arginine, yielded similar limited results (11). Study limitations were similar to the study in canine lymphoma as well. This diet is low in carbohydrates, based on the hypothesis that a reduction in carbohydrate intake will reduce lactate production by cancer cells. It is important to note that this hypothesis has yet to be tested. This diet is very high
in fat and thus may not be well tolerated by some patients. Caution is warranted in feeding this diet to patients at risk for pancreatitis.

**Goals of Nutritional Support:** The beneficial effects derived from the nutritional support of diseased human patients and experimental animal models include enhanced immune function, wound repair, response to therapy, recovery time, and survival. Despite these benefits, the nutritional needs of hospitalized patients are often ignored. In addition, the nutritional needs of critical patients are largely forgotten due to the intense focus on life-threatening medical and surgical problems. The goal of nutritional support is to provide a formula of fuels and nutrients in proportions that can be utilized by the patient with maximal efficiency. Malnutrition is probably more common in veterinary patients than is recognized. Malnutrition is an unbalanced intake of protein and/or calories to support tissue metabolism and has the potential to undermine proper medical or surgical therapeutic management of a hospital case (12). Most veterinary patients are likely to be deficient in both protein and calories due to a decrease in food intake. At first glance patients resting in a cage may appear to require little or no nutrition, when in fact their needs are significant. The benefits of nutritional support are many and include; increased immune function, increased wound healing, increased response to therapy, shortened recovery times, increased survival rates and lower hospitalization costs. One publication estimated the proportion of hospitalized canine patients in a negative energy balance (12). The study related calories consumed in the hospital to appetite at home following discharge, in order to determine why dogs were in a negative-energy balance and the assess the relationship between body condition score (BCS), physical status score (PSS), diagnosis and caloric intake with patient outcome. The study was conducted at three veterinary referral hospitals across the country. Overall, daily feeding data and outcomes for 276 dogs over 821 days in the hospital were evaluated. In only 27% of those days was a positive energy balance obtained (> 95% RER [resting energy requirement]). Eighty-three percent of the dogs were considered to have returned to a normal appetite within 2 days of being discharged. Of the 821 dogs-days analyzed, 601 were determined to be in a negative energy balance. This was attributed to several factors; 22% due to poorly written orders, 34% due to orders to withhold food and 44% of the dogs refused to eat. Overall, caloric intake had a significant, positive effect on patient outcome.

**Indications for Nutritional Support:** As a starting point, animals that have been anorectic for > 5 days or that have lost > 10% of their body weight should be considered for nutritional support. In addition, patients that are hypoalbuminemic, have a condition with increased energy demands or one with excessive nutrient losses also warrant nutritional support. Obtain a complete diet history (including supplement use, diet consumed and caloric intake), a physical examination, and appropriate laboratory work. A body weight and body condition score should always be obtained for every patient, but it is important to realize its limitations. Remember that body weight does not differentiate fat, lean tissue and water.

**Determine caloric needs and select a diet:** There is a paucity of data on the energy requirements of sick animals. It is likely that the true energy needs of most hospitalized patients fall somewhere between RER and MER. However, energy requirements for various disease states in dogs and cats are unknown and extrapolation from other species may have detrimental effects, as overfeeding can be as harmful as inadequate nutrition. Close observation of the patient with respect to body weight changes, ongoing losses, and exam findings will help determine whether to increase the patient’s caloric intake towards MER or to remain at RER respectively. Importantly, it should be standard protocol to calculate a patient’s energy requirements and record food intake on a daily basis to determine the adequacy of caloric intake and the need for nutritional support. There is, at best, limited evidence to support a particular dietary strategy to improve or prolong the life of cancer patients. This nutritionist recommends feeding a diet appropriate to the animal’s life stage and that is palatable to the pet. In cases where the patient has a concurrent condition that is responsive to dietary therapy, a diet appropriate to manage that condition should be fed (i.e. chronic kidney disease, pancreatitis). Assisted enteral feeding may be indicated in patients unable or unwilling to eat enough to meet their energy needs.
Keywords: Nutrition, Cancer, Diet

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For Canine Patients:  
Nutritional Management and Homemade Recipe

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Summary
Homemade recipes for canine patients are highly individually adaptable, and can be formulated in any case, whenever the pet owner ask for it. It may require a specialist to adapt the diet to the unique condition of the dog, and make the advice easy to be followed. It is also the responsibility of the veterinarian to explain the daily importance of each ingredient, their dosage, and their long-term respect.

The basics of nutrition have to be followed: 1-to provide enough energy to reach and maintain optimal body weight and condition; 2- to provide an adapted volume of food to cover appetite and avoid begging, and 3-to balance the diet to cover about 40 nutrients in appropriate amount to maintain and enhance health or compensate the effects of diseases if any... Energy makes the weight, but all other nutrients make health. Homemade recipes allow to take into account at once the all individual characteristics of the dog (age, breed, actual and optimal body weight, neutering, activity, lifestyle, climate, appetite, and individual taste), and of course the health condition.

The goal is to offer various choices of meat of fish, vegetables and carbohydrate source, the dispensable canola oil and sometimes fish oil, to provide essential fatty acids, and, together with meat or fish, an adapted mineral vitamin supplement to bring nutrients that the other basic ingredients do not contain.

In any case, on request of the pet owners and as a choice of the veterinarian, a dairy product, and a treat such as a fruit, a delicatessen, or a ready-to-eat treat can be added, if the amount is properly calculated. On the final recipe, the pet owner must find not only the amount of each ingredient, but also the mode of preparation, preservation and distribution of ingredients and meals.

A few cases will be presented as examples:
1- Recipes for adult healthy dogs and the changes with breed, neutering and lifestyle.
2- Recipe for adult dog with for Kidney disease IRIS Stage 1-2 or protein losing nephropathy
3- recipe for adult dog with chronic pancreatic insufficiency
4- diet for a dog with unknown dermatology or gastroenterology trouble, when the diet is not balanced, as balancing the diet is a first step to make sure the problem is not simply of nutrition origin.
5- Diabetes mellitus in which the dosage of insulin may depend on the composition of the diet in macronutrients, especially in carbohydrates.
6- diet for an obese dog with diabetes mellitus and osteoarthritis and request of weight loss...

A lot of situations are not easy to manage with a single diet, but can be managed with homemade recipes...

In France, about 14% of dog owners prepare a homemade diet to their dogs, as almost exclusive food, and about 48% received homeprepared food as a part of their daily diet (Colliard et al., 2006). Homemade recipes are highly digestible (Blanchard et al., 2011), allowing the use of Atwater energy coefficient as in humans.
Homemade diets are often considered risky, and they are is they are not properly formulated and the recipes followed (with dogs fed meat only (Taylor et al. 2009), or mostly pasta with very few meat and no mineral vitamin supplement (de Fornel-Thibaud et al., 2007). Homemade recipes for canine patients are highly individually adaptable, and can be formulated in any case, whenever the pet owner ask for it. But they are also at risk if they are not balanced or if the recipe is not properly followed (Remillard R 2008). Bacterial and parasites risks are not considered here, but of course must be considered especially with raw meat. Freezing meat may limit the risk of parasites, but of course not risk of bacteria.

The most common nutritional risks encountered may be summarized as follows:

*not enough or too much energy, due to an inappropriate amount of food or inappropriate ingredients
*the lack of mineral vitamin supplement, considered optional when in fact, it covers 40 to 50% of total nutrients, but as the amount is small, and does not impact body weight, it is not recognised as vital (Nisa et al., 2003). Minerals and vitamins must be brought daily when the other ingredients are meat, vegetables and rice, and oil. And the supplement can not be replaced by a dairy product and brewers yeast.
*mineral vitamin supplement and canola oil may be forgotten when people feed a dog kibble in the morning and hemoprepare diet in the evening, and think that kibble will compensate for the lacking part: this is wrong. If Kibble is a complete diet, it is complete for the part of daily food provided, half in this case.
*the last common error encountered is a mistake in the amount of meat, sometimes too low, sometimes too high. To cover protein and amino acids requirements, one can consider about 20 grams of meat per kg of body weight of dog. Of course this amount is not a recommendation but more an average.
*the lack of oil, or a mistake in the oil provided: with 2 different mistakes
**dogs require essential fatty acids (EFA) on their diet, omega 6 and omega 3 fatty acids. Some vegetable oils, such as canola and soybean oil contain both. Olive oil, which is very popular in western countries contains almost no EFA, but mainly oleic acid, an omega 9, not essential.
**dogs require essential fatty acids (EFA) on their diet, omega 6 and omega 3 fatty acids, and not only omega 3. One can think fish oil is rich in omega3, and it is enough. But indeed, fish oil may contain long chain omega 3 FA such as EPA and DHA, but not or very few linolenic acid, another omega 3 EFA, and linoleic acid, an omega 6.  
** people may provide fish oil as cod liver oil to provide EPA and DHA. Cod liver oil is rich in these two FA, but it is tremendously rich in vitamins A and D, essentials but also toxic while in excess.  
** people may provide oil that appears to be poorly palatable, due to the high sensibility of polyunsaturated fatty acids to oxidation. The best is to change the bottle of canola or soybean oil on a regular basis, to maintain the oil in a fresh room, and to bring fich oil in capsules rather than from pump bottles.

As formulating an appropriate recipe may be challenging for veterinarians and due to the small number of specialists in nutrition, an online specialized nutrition service may allow a wider access individualised balanced nutrition options including homemade reciped (see the web site cuisine-a-crocs.com).

Some situations have been modalized, but not all and complex situtaion may still require the assistance of a specialist in nutrition to formulate recipes manually.
Factors of variation of requirements include breed, optimal body weight, neutering, life stage, activity, disease, for energy, lean mass & weight, life stage, hair coat and stress for protein and amino-acids, age, weight, growth and reproduction status, disease for minerals...

The calculation of a recipe requires to consider all individual characteristics of the dog to offer a list of possible choices of meat ot fish, veggie and carbohydrate source.

In any case, as a choice of the veterinarian, one can also add a dairy product, and up to two different treats, including fruit, delicatessen, and ready-to-eat treat. For each of these treats, the amount has to be chosen among a list of possible quantities, depending on the dog.

On the final recipe, the amount of each ingredient, but also the amount of canola oil and of the appropriate vitamin-mineral supplement are also indicated together with the meat or fich, veggies and carbs.
The information about the preparation, preservation and distribution of food are provided, as well as an advice on the follow up, and specific critical information in case of a disease. Here are a few recipe examples (ingredients weight indicated raw, meat and fish should be cooked at least 5 minutes at 65°C, vegetables and white rice cooked enough to be mashed easily, oil and mineral vitamin supplement must not be cooked).

1- Recipes for adult healthy dogs

A- BabaDog is a German Shepherd, male intact, 32Kg, body condition score optimal, no special activity, living in a garden, outdoor, during the day, and in house, indoor, during the night, tempered climate.

RECIPE WITH DUCK & CARROTS:
Duck (meat, skinless, boneless): 410 g, Vit'i5 Canine Ca:P=3: 2 doses (16 g), Rapeseed Oil: 25 ml, Carrot: 270 g, Rice: 160 g

B- KokoDog is a Labrador Retriever, male intact, 32Kg, body condition score optimal, no special activity, living in a garden, outdoor, during the day, and in house, indoor, during the night, tempered climate.

RECIPE WITH CHICKEN & CARROTS:
Chicken or Turkey (lean meat, boneless skinless): 410 g, Vit'i5 Canine Ca:P=3 : 1 dose 3/4 (14 g), Rapeseed Oil: 17.5 ml, Carrot: 370 g, Rice: 100 g, Cheddar Cheese: 10 g, Apple fruit raw : 60 g Change of recipes when energy requirement is lowered by neutering and/or lifestyle is recommended...

=> KokoDog has now been neutered, and lives indoor with one hour walk on leach per day.

RECIPE WITH CHICKEN & CARROTS:
Chicken breast boneless skinless): 410 g, Vit'i5 Canine Ca:P=3 : 1 dose 3/4 (14 g), Rapeseed Oil: 12.5 ml, Carrot : 370 g, Rice: 30 g, Apple raw : 40 g

RECIPE WITH FISH & ZUCCHINI:
Fish white fillet (pollack, coalfish, rattail) fresh or frozen: 460 g, Vit'i5 Canine Ca:P=3 : 2 doses 1/2 (20 g), Rapeseed Oil: 12.5 ml, Zucchini: 650 g, Sweet potato (peeled, to be given cooked): 160 g Cottage cheese (20%Fat): 20 g, Papaya (skinless): 65 g

2- Recipe for adult dog with for Kidney disease IRIS Stage 1-2 or protein losing nephropathy

This situation requires a protein intake limited to cover the requirements, with no excess, to limit the phosphorus intake (so the mineral-vitamin supplement is different) and the addition of long-chain omega 3 fatty acids EPA and DHA. Homemade recipes may also allow to manage kaliemia when required (Segev et al., 2010).

=> For KokoDOG: RECIPE WITH CHICKEN & CARROTS:
Chicken breast (lean meat, boneless skinless): 380 g, Vit'i5 Canine Ca: 2 doses (16 g), Rapeseed Oil: 20 ml, Carrot: 370 g, Rice: 20 g, Apple: 80 g

3- Recipe for adult dog with chronic pancreatic insufficiency

This situation requires to cover all requirements with no fiber excess, to not lower digestibility, but requires also to bring exogenous digestive enzymes with each meal or food intake.

=> For BabaDOG: RECIPE WITH DUCK & CARROTS:
Duck meat (skinless, boneless): 410 g, Vit'i5 Canine Ca:P=3: 2 doses (16 g), Rapeseed Oil: 17.5 ml, Carrot: 210 g, Rice: 160 g, Yogurt plain: 100 g, Apple: 70 g Exogenous enzymes must be added with each meal, each time food is ingested (German, 2012).

4-diet for a dog with unknown gastroenterology trouble (balanced diet wanted)

Facing a situation where the balance of the diet may be critical (Zoran, 2003), to provide a balanced diet if a first step. If this is not enough, specific diet adaptation can be made by a nutrition specialist, considering the overall medical situation.

=> For BabaDOG: RECIPE WITH DUCK & CARROT:
Duck (meat, skinless, boneless): 510, Vit'i5 Canine Ca:P=3: 2 doses 1/2 (20 g), Rapeseed Oil: 17.5
ml, Carrot: 210 g, Potatoe: 560 g, Papaya (skinless, seedless): 55 g

5- Diabetes mellitus
In case of diabetes mellitus, stability of diet day after day is recommended to make easier and repeatable day after day the dosage of insulin. The composition of the diet must cover requirements, but providing less energy as carbohydrates to lower the glucose absorbed (Hewson-Hughes et al., 2011).

=> PooDog is a Poodle, female neutered, 5Kg, body condition score under-optimal with optimal body weight of 6kg, no special activity, living indoor with one hour walk on leach per day, tempered climate (average 10 to 25°C), diabetes mellitus treated with insulin injection twice a day, not balanced, weight loss and very high appetite. Choice of a lower amount of rice.

RECIPE WITH PORK & PUMPKIN
Pork filet: 150 g, Vit'i5 Little Ca: 1 dose 1/2 (6 g), Rapeseed Oil: 7.5 ml, Pumpkin, skinless (raw to be cooked): 140 g, Rice (weigh dry, to be cooked): 10 g, Cheese Camembert 45%fat: 10 g

6- diet for an obese dog with diabetes mellitus and oestoarthritis and request of weight loss
To reach the optimal body weight by restricting energy, but not other nutrient requirements (Blanchard et al., 2004), to cover appetite, adapt insulin to the new diet, and add EPA and DHA (Mehler et al., 2016) WeeDog is a Westy, 12 yrs-old, female, neutered, 12Kg, with optimal body weight of 9kg5 (2.5/9.5=26% overweight = obese), no special activity, osteoarthritis, living indoor with one hour walk on leach per day, tempered climate (average 10 to 25°C). Choice of the less amount of rice.

RECIPE WITH CHICKEN OR FISH & ZUCCHINI:
Chicken breast: 150 g OR Fish white fillet (pollack, coalfish, rattail) fresh or frozen: 200 g, Vit'i5 Little Ca: 1 dose 1/2 (6 g), Rapeseed Oil: 5 ml, Zucchini: 250 g, Rice or Noodle or Oat Flakes: 15 g OR Banana (skinless): 70 grams (half with each meal), 125 g of plain no fat Yogurt OR 1 portion of "Vache qui rit"

Keywords: Canine, Homemade Recipe, Clinical Nutrition

References: upon request
For Feline Patients:
Nutritional Management and Homemade Recipe

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Summary
Homemade recipes for feline patients are highly individually adaptable, and can be formulated in any case, whenever the pet owner ask for it. It may require a specialist to adapt the diet to the unique condition of the cat, and make the advice easy to be followed. It is also the responsability of the veterinarian to explain the daily importance of each ingredient, their dosage, and their long-term respect.

In any case, as for any change of diet in cats, the transition also must be extensively and carefully explained, and understood by the owner, in order to allow a safe and successful change, and avoid anorexia. All ingredients of the food must be provided at once, not the meat only for instance.
The basics of nutrition have to be followed: 1-to provide enough energy to reach and maintain optimal body weight and condition; 2- to provide an adapted volume of food to cover appetite; 3- to recommend to split the daily food in at least 3 meals per day; 4-to balance the diet to cover about 40 nutrients in appropriate amount to maintain and enhance health or compensate the effects of diseases if any... Energy makes the weight, but all other nutrients make health.
Homemade recipes allow to take into account at once the all individual characteristics of the cat (age, breed, actual and optimal body weight, neutering, activity, lifestyle, climate, appetite, and individual taste), and of course the health condition.
The goal is to offer various choices of meat of fish, fiber source such as vegetable or wheat bran, and possibly carbohydrate source, the dispensable canola oil and sometimes fish oil, to provide esssential fatty acids, and, together with meat or fish, an adapted mineral vitamin supplement to bring nutrients that the other basic ingredients do not contain. The mineral vitamin supplement may contain taurine as well, in case the meat of fish provided would not be rich enough, if overcooked for instance.
In any case, on request of the pet owners and as a choice of the veterinarian, a dairy product, and a treat such as a seedless olive, a delicasessen, or a ready-to-eat treat can be added, if the amount is properly calculated.
On the final recipe, the pet owner must find not only the amount of each ingredient, but also the mode of transition, preparation, preservation and distribution of ingredients and meals.
A few cases will be presented as examples :
1- Recipes for adult healthy cats and the changes with breed, neutering and lifestyle.
2- Recipe for adult cat with for Kidney disease IRIS Stage 3
3- Recipe for adult cat with urinary crystals
4- Recipes for a cat with diabetes mellitus, depending on the body condition score.
...
In France, very few cats (1.5%) are fed a homemade diet only, as almost exclusive food, but about 48% received homeprepard food as a part of their daily diet (Colliard et al., 2009). Homemade recipes are highly
digestible (Blanchard et al., 2011), allowing the use of Atwater energy coefficient as in humans. Homemade diets are often considered risky, and they are is they are not properly formulated and the recipes followed (with dogs fed meat only (Taylor et al. 2009), or mostly pasta with very few meat and no mineral vitamin supplement (de Formel-Thibaud et al., 2007).

Homemade recipes for canine patients are highly individually adaptable, and can be formulated in any case, whenever the pet owner ask for it. But they are also at risk if they are not balanced or if the recipe is not properly followed (Remillard R 2008). Bacterial and parasites risks are not considered here, but of course must be considered especially with raw meat. Freezing meat may limit the risk of parasites, but of course not rick of bacteria.

The most common nutritional risks encountered may be summarized as follows:
- the meat of fish additionally to kibble, forgetting that meat of fish is not a complete food, and requires to be balanced at least with a mineral vitamin supplement, if possible with rapeseed oil and a source of fiber.
- the lack of mineral vitamin supplement with the homemade diet, to bring 40 to 50% of micronutrients, and which can not be replaced by a dairy product and brewers yeast,
- mineral vitamin supplement and canola oil may be forgotten when feeding a mixed diet,
- the lack of oil, or a mistake in the oil provided:

**cats require essential fatty acids (EFA) on their diet, omega 6 (arachidonic and linoleic acids) and omega 3 fatty acids (linolenic acid, and at some stages EPA and DHA). Some vegetable oils, such as canola and soybean oil contain both linoleic and linolenis acids. Olive oil contains none. Meat contains arachidonic acid, and some fish oil such as menhadden and wild salmon EPA and DHA. Bringing only fish oil is not enough, and in case of a lake of antioxidant may even be at risk (Niza et al., 2003).

Cod liver oil is also at risk because apart being rich in EPA and DHA, it is tremendously rich in vitamins A and D, essentials but also toxic while in excess.

In order to stick to the state-of-art in clinical nutrition, the formulation of appropriate recipes may require a specialist in nutrition. If no specialised consultation is available around, an online service, as cuisine-a-crocs.com may be used, to consider all factors of variation of requirements, for instance breed, optimal body weight, neutering, life stage, activity and disease, for energy, lean mass & weight, life stage, hair coat and stress for protein and amino-acids, age, weight, growth and reproduction status, and disease for minerals...

Some situations can modalized, and recipes obtained in a few minutes. They must consider all individual characteristics of the cat (age, breed, actual and optimal body weight, neutering, activity, lifestyle, climate, appetite, and individual taste) to offer a list of possible choices of ingredients. In any case, a dairy product, and up to two different treats may be included as a choice of the veterinarian.

On the final recipe, the amount of each ingredient, but also the amount of canola oil and of the appropriate vitamin-mineral supplement are indicated together with the meat or fish, veggies and carbs. The food transition is also described, as well as the information about the preparation, preservation and distribution of food, an advice on the follow up, and specific critical information in case of a disease.

A few cases will be presented as examples (ingredients weight indicated raw):
(all weights indicated raw, meat and fish filet should be cooked 5 minutes at 60°C, vegetables cut in 5mm dice and white rice should to be cooked enough to be mashed, oil and mineral vitamin supplement Vit'i5 or equivalent must be given raw)

1- Recipes for adult healthy cats

A- SiKatKo is a Sphinx, intact female, 2 yrs-old, 4kg, body condition score optimal, no special activity, living outdoor during the day, and indoor during the night, tempered climate (average 10 to 25°C).

**RECIPE WITH FAT BEEF & CARROTS**:

Beef (mince, pure beef 15%fat) : 110 g, Vit'i5 Little Ca:P=3 : 1 dose (4 g), Rapeseed Oil : 2.5 ml, Carrot, canned (weight strained) : 40 g, Rice : 5 g
**B- PeKatKo is a Persan, intact female, 2 yrs-old, 4kg, body condition score optimal, no special activity, living outdoor during the day, and indoor during the night, tempered climate (average 10 to 25°C).**

**RECIPE WITH LEAN BEEF & CARROTS:**
Beef (lean meat 5%fat, shoulder) : 110 g, Vit’i5 Little Ca:P=3 : 1 dose (4 g), Rapeseed Oil : 7.5 ml, Zucchini, (raw to be cooked) : 40 g, White Rice : 5 g

**NEUTERING** leads to changes in the recipes when energy requirement is lowered by neutering is recommended...

**RECIPE WITH CHICKEN & ZUCCHINI:** Chicken breast (raw weight) : 100 g, Vit’i5 Little Ca:P=3 : 1 dose (4 g), Rapeseed Oil : 5 ml, Zucchini : 40 g, Rice : 5 g

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2- **Recipe for PeKatKo with for Kidney disease IRIS Stage 3**
This situation requires a protein intake limited to cover the requirements, with no excess, to limit the phosphorus intake (so the mineral-vitamin supplement is different) and the addition of long-chain omega 3 fatty acids EPA and DHA.

**RECIPE WITH CHICKEN & NO VEGETABLE** (poor appetite, does not like veggies):
Chicken breast: 90 g, Vit’i5 Little Ca: 1 dose 1/2 (6 g), Rapeseed Oil: 7.5 ml, Wheat bran: 1 dose of 5ml, Rice: 5 g

**CRF/CKD stage 3** : ADD a caps of Fish Oil (salmon, menhaden...), in order to bring 500mg of EPA + DHA, long chain omega-3 fatty acids, beneficial to cats with chronic renal disease and chronic inflammatory state. Open caps and mix the oil with a small meal just prior to consumption to avoid oxidation.

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3- **Recipe for PeKatKo but living indoor with Urinary crystals**
This situation requires to cover all requirements, and adding zucchini has been demonstrated to lower urinary saturation in calcium oxalate (Amatto et al., 2016).

**RECIPE WITH CHICKEN & ZUCCHINI**:
Chicken breast: 100 g, Vit’i5 Little Ca: 1/2 dose (2 g), Rapeseed Oil: 2.5 ml, Zucchini: 40 g, Rice: 5 g

**TRANSITION explained** - for instance from kibble to homemade diet...
For a few days, you can: - Prepare a balanced homemade diet, as described for one day, but distribute a small portion (1 tablespoon) with or adjacent to the kibble, once a day, until this amount is accepted and consumed (it may take 5 to 10 days). The prepared balanced homemade diet made be kept 24 hours in refrigerator, or frozen in bites, and defrost overnight in refrigerator. When accepted, give twice daily (2 teaspoons) and decrease a bit the amount of kibble... Continue this method ... until the homemade diet is completely accepted, and provide the daily amount in 3 or 4 meals per day: 1 or 2 meals in the morning, 1 at noon or when back home and 1 or 2 evening meals. The transition may take several weeks. ***MIXED DIET***: If you want to distribute a combined homemade / dry food (morning kibble + evening homemade, for example), you can keep half of the diet as kibble (give half the amount normally expected for a day) and half of the homemade diet (prepared as a complete recipe for 1 day, given in 2 days, keeping half refrigerated). However, it is strongly recommended to not give a homemade diet for a few days and then a petfood for a few days, to avoid digestive disorders. Avoid however giving a homemade diet and leaving kibble free service because they can be over consumed leading to obesity, or get oxidized by the air which can cause digestive troubles. Watering: The cat drinks less with homemade diet then with dry food, but it must nevertheless have access to water permanently.

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4- **Diabetes mellitus**
Once diabetes is balanced and euglycemia obtained with insulin, the goal is to reach optimal body weight. Remission of diabetes may be reached in cats fed low carbohydrate-diet and added fiber (Bjornvad et Jessen, 2015 ; Hewson-Hughes et al., 2011).

**Recipe for PeKatKo but living indoor, with normal weight or underweight, when diabetes diagnosed**
**RECIPE WITH CHICKEN & ZUCCHINI,** to reach and maintain optimal body weight
Chicken or Turkey (lean meat, boneless skinless) : 110 g, Vit’i5 Little Ca:P=3 : 1 dose (4 g).
Rapeseed Oil: 5 ml, Zucchini, (raw to be cooked): 40 g

Recipe for PeKatKo but living indoor, with obese weight when diabetes diagnosed
Obesity can be treated by energy restriction at once with glycemia is managed with insulin twice a day for instance. Once optimal weight (4kg) achieved, diet can be changed to the previous recipe, in order to maintain the optimal body weight.

RECIPE WITH CHICKEN & ZUCCHINI, to reach optimal body weight
Chicken or Turkey (lean meat, boneless skinless): 100 g, Vit'i5 Little Ca:P=3:1 dose (4 g), Rapeseed Oil: 2.5 ml, Zucchini, (raw to be cooked): 60 g

Diabetes mellitus: Scale weight every 2 weeks, and plane a glycemia curve once the diet has been stable day after day for one entire week. The required insulin dosage depends on the composition of the diet, especially the quantity of carbohydrate, protein and fat ingested. Each time the diet is modified, it is necessary to check that glycemia is steady and the diabetes stable and compensated, and to adjust the insulin dosage if necessary. Thereafter, scale weight once a month. A stable optimal body weight is a good indirect evidence of a stable diabetes.

Keywords: Feline, Homemade Recipe, Clinical Nutrition

References
Ophthalmic Imaging: Seeing the Unseen

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Summary

Due to the normal optical clarity of the ocular media, direct visualization of many intra-ocular structures is possible during routine ophthalmic examination. However, when opacity of ocular media precludes visualization of intra-ocular structures, or if evaluation of the orbital and retro-bulbar tissues is indicated, cross-sectional imaging using modalities such as ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI) are generally more informative than plain film radiography. A review of these different imaging modalities, including their indications, practical application, strengths, limitations, and pertinent imaging findings in common ocular and orbital diseases of dogs and cats is presented.

Indications for ophthalmic imaging:

Opacification of ocular media due to corneal disease, or to intraocular disease associated with the presence of hemorrhage, infiltrates, exudates, and/or cataract can preclude direct visualization of ocular structures. Under these circumstances, cross-sectional imaging techniques, in particular ultrasonography can provide valuable information to facilitate the diagnosis and management of intraocular disease. As the orbital contents are surrounded, at least in part, by a bony wall and obscured from view by the globe itself, ultrasonography, conventional and contrast radiography and advanced cross-sectional imaging techniques including CT and MRI provide invaluable, and often complementary information on the location and character of disease processes affecting the orbit, as well as globe.

Choice of imaging technique:

Clinical Presentation, availability and accessibility and cost of imaging technology together determine the most appropriate choice of imaging modality in individual patients. In all instances, imaging of both eyes and orbits is advised, enabling comparison of the “diseased” eye to the “normal” eye whenever possible.

Table 1 A Common Indications for Ophthalmic Imaging

| Severe eyelid and periocular swelling that preclude examination of eye |
| Opacity of ocular media (corneal opacity, intraocular hemorrhage, cataract, exudates/ infiltrates) |
| Suspected globe rupture |
| Suspected intraocular mass (neoplasm) |
| Suspected intraocular foreign body |
| Suspected retinal detachment |
Table 1B Common Indications for orbital imaging

<table>
<thead>
<tr>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exophthalmos</td>
</tr>
<tr>
<td>Strabismus</td>
</tr>
<tr>
<td>Enophthalmos</td>
</tr>
<tr>
<td>Third eyelid protrusion</td>
</tr>
<tr>
<td>Orbital Trauma</td>
</tr>
<tr>
<td>Pain on opening the mouth</td>
</tr>
<tr>
<td>Suspected orbital foreign body</td>
</tr>
<tr>
<td>Suspected optic nerve disease</td>
</tr>
<tr>
<td>Chronic or recurrent nerve disease</td>
</tr>
<tr>
<td>Nasal, dental, or sinus disease</td>
</tr>
<tr>
<td>Facial deformity, e.g. swelling, involving the peri-ocular region</td>
</tr>
</tbody>
</table>

Ultrasonography:
Ultrasound is the most widely available, accessible and cost-effective ophthalmic imaging modality employed in clinical veterinary practice and has the advantage of being generally well-tolerated by conscious, un-sedated dogs and cats. Ocular and orbital ultrasonography is non-invasive, and diagnostic quality images can be acquired without the use of ionizing radiation. Although acquisition of good quality orbital images requires some practice and skill, focal lesions in particular can be readily appreciated by those who do not have extensive training. In general, the probe is placed directly on the topically anaesthetized cornea, although a stand-off may be used if the anterior structures of the globe are of greatest interest. Probes with frequencies of 7.5 – 12MHz are most frequently used for routine ocular and orbital ultrasonography, with lower frequencies providing lower resolution but greater depth of penetration. High resolution ultrasonography (HRUS) / ultrasound biomicroscopy (UBM) uses higher frequencies of 20-60MHz to generate images of exquisite detail and resolution of intraocular structures. However, depth of penetration is limited to the structures of the anterior segment of the eye and equipment is more costly and therefore less accessible to the veterinary practitioner. Use of HRUS/UBM is not considered within the scope of this presentation. While most veterinary practitioners are familiar with B-mode ultrasonography, A-mode ultrasonography may be used to quantify axial length of the globe, relative depth of the ocular chambers and lens position.

Ocular ultrasonography can relatively quickly and cheaply identify such abnormalities as retinal detachment; intraocular mass lesions; lens displacement or rupture, and cataract, as well as identifying features suggestive of globe rupture. (See table 2) However, veterinary clinicians should be aware of the possibility that non-neoplastic lesions, such as blood clots, may mimic intraocular tumors and that extensive exudate or aemorrhage within the vitreous may obscure the presence of retinal detachment or scleral rupture. Important features to note are the location and nature of any increase or decrease in echogenicity; whether these changes are diffuse or focal and heterogeneous or homogeneous; whether a mass effect (with displacement of adjacent tissues and / or indentation of the globe) can be observed; and whether the phenomenon of “after movement” can be appreciated.
### Table 2 Ultrasonographic (US) Findings in Ocular and Orbital Disease:

<table>
<thead>
<tr>
<th>Condition</th>
<th>US features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size changes ↑ or ↓</td>
<td>Best determined by A-mode biometry</td>
</tr>
<tr>
<td>Ruptured globe</td>
<td>Reduced globe size, lack of continuity of outer sclera</td>
</tr>
<tr>
<td>Panophthalmitis</td>
<td>Multiple small echogenic points within otherwise hypoechoic anterior and vitreous chambers</td>
</tr>
<tr>
<td>Cataract</td>
<td>Hyperechoic, may be increase or decrease in size of lens</td>
</tr>
<tr>
<td>Ruptured lens</td>
<td>Altered lens size and contour</td>
</tr>
<tr>
<td>(Sub)luxated lens</td>
<td>Altered lens position</td>
</tr>
<tr>
<td>Ocular Foreign Body</td>
<td>Appearance will depend on nature of foreign body, e.g. metallic FB will have acoustic shadowing, plant material may not be visible.</td>
</tr>
<tr>
<td>Ocular hemorrhage</td>
<td>May be diffusely hyperechoic and fill anterior or vitreous chamber; or focal areas are hyperechoic or have mixed echogenicity, may demonstrate &quot;after-movement&quot;</td>
</tr>
<tr>
<td>Vitreal degeneration, asteroid hyalosis, synchisis scintillans</td>
<td>Small echogenic foci within vitreous cavity; often demonstrating &quot;after-movement&quot;</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>Curvilinear echogenic structures that can be traced to the optic nerve head. A complete detachment will have a &quot;gull-wing&quot; appearance.</td>
</tr>
<tr>
<td>Vitreal detachment</td>
<td>Thin echogenic linear structure, typically at posterior pole of eye</td>
</tr>
<tr>
<td>Optic nerve head changes</td>
<td>Focal swelling and increase echogenicity may be seen in the region of the optic nerve head.</td>
</tr>
<tr>
<td>Intra-ocular neoplasia</td>
<td>Mass effect may distort adjacent intra-ocular structures, less &quot;after-movement&quot; than blood-clots</td>
</tr>
<tr>
<td>Retrobulbar abscess</td>
<td>May be diffuse increase in echogenicity without mass effect (cellulitis) or may have hypoechoic center (cavity lesion) if abscess</td>
</tr>
<tr>
<td>Orbital neoplasia</td>
<td>Mass effect may be seen, generally hyperechoic, although hypo-echoic in some instances. May be irregularity of medial bony orbital wall.</td>
</tr>
</tbody>
</table>

### Radiography:
Radiography, including both plain film and contrast techniques, such as dacrocystorhinography, is the imaging modality most widely available to veterinary practitioners. However radiography of this region is technically demanding and due to the complex anatomy of bones of the skull, including the orbit, both accurate positioning and interpretation of resulting films requires considerable expertise. Radiography provides very little information on soft tissues, or on the “true” extent of bone lesions. Heavy sedation or general anesthesia are typically required for immobilization and accurate positioning in veterinary patients, and to limit exposure of personnel and patient to harmful ionizing radiation.

### Computed tomography:
CT is the imaging modality of choice for lesions involving, or suspected to involve, bones of the skull. CT does not allow accurate discrimination between inflammatory and neoplastic lesions but allows guided biopsy of identifiable lesions. CT exposes the patient to a large amount of ionizing radiation and requires general anaesthesia for positioning and immobilization. CT is more costly than ultrasonography, and less accessible to the general veterinary practitioner.

### Magnetic resonance imaging (MRI):
MRI offers superior definition of soft tissue lesions including brain and optic nerves and is considered by many to represent the diagnostic imaging modality of choice in the investigation of orbital disease and for surgical treatment planning. A number of different pulse-sequences are commonly used for orbital MRI but detailed review is out-with the scope of this presentation. As with CT, MRI is increasingly accessible by referral but is relatively costly to perform, and some imaging routines can be very time consuming. While general anaesthesia is required to limit movement, patient positioning is less critical than for CT and radiography.

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Keywords: Diagnostic Imaging, Eye, Orbit, Ophthalmology, Ultrasound

References
The Future of Phacoemulsification and Cataract Surgery

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Summary
Cataract surgery has changed dramatically in recent years with regards to surgical technique, ocular pharmacology, the availability of anti-inflammatory agents, viscoelastic agents, phacoemulsification, and the most recent advancement, intraocular lens implantation for dogs. Despite these dramatic changes, cataract surgery remains a procedure whose successful outcome depends on meticulous attention to detail, surgeon skill and experience, and appropriate patient selection. The goals of canine cataract and lens instability surgery should be to ensure a small incision, minimal tissue trauma, minimize astigmatism, efficient surgical time, maintenance of the anterior chamber and restoration of emmetropia through the use of a stable intraocular lens specifically designed for the canine eye.

Cataract surgery is indicated in animals with a decrease in vision associated with the cataract or in animals with a progressive cataract for which vision loss is imminent (Figure 1). The accepted standard of care for cataract surgery in veterinary ophthalmology is phacoemulsification. With the introduction of intraocular lens implants, cataract surgery can also be offered in cases of unilateral cataract to restore binocular emmetropia (1).

Over the past decade, cataract surgery in veterinary ophthalmology has seen numerous advances in surgical techniques, equipment and intraocular lens (IOL) design. Intraocular lens implantation has become the standard of care for most canine cataract patients (1). Surgeons utilize the techniques of one-handed and two-handed phacoemulsification and the various manipulations involved in the techniques of lens sculpting, divide and conquer, phaco chop, chip and flip and other techniques designed to remove the lens in an efficient and atraumatic manner (Figure 2). Canine IOL’s are constructed from polymethylmethacrylate (PMMA) and acrylic materials (Figure 3), the latter allowing implantation through a small incision using a shooter or folding forceps. In instances of lens or lens capsule instability it may necessary to find an alternative method for IOL implantation if pseudophakic vision restoration is to be achieved (1).
Figure 1 An immature anterior and posterior cortical cataract with equatorials vacuoles. The vacuoles indicate a high likelihood of rapid progression.

Figure 2 One handed phacoemulsification with sculpting of the cataract using a flared 45 degree Kelman tip

Figure 3 Appearance of an acrylic intraocular lens one year postoperatively. There is mild posterior capsule opacification at the edge of the optic.

With the current advances in veterinary cataract surgery and an expected successful outcome of +/- 90% for long-term vision restoration the remaining significant issues we face as cataract surgeons are post-operative hypertension (POH), post-operative glaucoma, management of concurrent or post-operative retinal detachment, posterior capsule opacification (PCO), the ability to more successfully manage lens instability with restoration of emmetropia and the integration of new technologies and pharmacologic agents into our
While we do not fully understand POH, we do have evidence that the Labrador retriever appears to be both at increased risk for POH and more likely to suffer irreversible vision loss associated with POH (2). Numerous studies looking at the iridocorneal angle by gonioscopy and ultrasound biomicroscopy, pre and post-operative medications including mydriatics, NSAID’s, antiglaucoma drugs and others have all failed to reach consensus on how to both predict the occurrence of POH and to prevent its occurrence. This remains an area for ongoing study.

The area of retinal reattachment surgery is an emerging one and may be associated with or required following cataract surgery. Controversy still remains over breed predisposition to retinal detachment associated with cataract surgery with the Bichon Frise and Shih Tzu most often cited. Various prophylactic procedures for the prevention of retinal detachment, transsceral, transpupillary and endolaser retinopexy, exist. Should retinal detachment occur treatment ranges from barrier retinopexy to vitrectomy and retinal reattachment surgery. The techniques of retinal reattachment surgery in veterinary ophthalmology still require refinement and while successes are described, significant and numerous long-term complications remain (3).

The prevalence of PCO in veterinary cataract surgery is 100%. Fortunately the severity is often mild and with the level of visual acuity in our veterinary patients PCO is often less functionally significant than in humans. Never the less, prevention of PCO is desirable and has cross species significance. We continue to evaluate both surgical and pharmacologic means to control PCO. This includes capsule polishing, IOL design, capsular tension rings, distilled water, capsule washing techniques, NSAID’s, COX-2 inhibitors and other efforts to remove residual lens epithelial cells and to prevent them from undergoing epithelial-mesenchymal transformation.

The management of canine glaucoma at the time of or following cataract surgery benefits from the newer techniques of endocyclophotocoagulation (ECP) and filtration surgery. If elevated IOP is present at the time of cataract surgery and the eye is visual, ECP can easily be combined with phacoemulsification adding only a few minutes to the surgical time.

Finally, the newer emerging technologies in human cataract surgery will eventually transfer to veterinary ophthalmology in a manner similar to how phacoemulsification did. The use of techniques such as the femtosecond laser are revolutionizing human cataract surgery with lower fluid volumes, reduction in intraocular time and more precise incisions and capsulorhexis.

Keywords: Phacoemulsification, Cataract, Intraocular lens, IOL, Cataract Surgery, Luxation

References
Lens Luxation, Risks, Surgical Techniques and Sutured Lenses

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Summary
The goals of canine cataract and lens instability surgery should be to ensure a small incision, minimal tissue trauma, shortened surgical time, maintenance of the anterior chamber and restoration of emmetropia through the use of a stable intraocular lens specifically designed for the canine eye. While this is usually the case with routine phacoemulsification and in-the-bag intraocular lens implantation it is often not the case with lens instability, lens luxation or large posterior capsular ruptures. In such cases the incisions are often larger, surgical time and tissue trauma are excessive and the patient is often left aphakic. In instances of lens or lens capsule instability it may necessary to find an alternative method for IOL implantation if pseudophakic vision restoration is to be achieved. This would include eyes with zonular dehiscence, lens subluxation, luxation or a lens capsular rupture secondary to trauma, diabetes mellitus or surgeon trauma.

Over the past decade, cataract surgery in veterinary ophthalmology has seen numerous advances in surgical techniques, equipment and intraocular lens (IOL) design. Intraocular lens implantation has become the standard of care for most canine cataract patients (1). Surgeons utilize the techniques of one-handed and two-handed phacoemulsification and the various manipulations involved in the techniques of lens sculpting, divide and conquer, phaco chop, chip and flip and other techniques designed to remove the lens in an efficient and atraumatic manner. Canine IOL's are constructed from polymethylmethacrylate (PMMA) and acrylic materials, the latter allowing implantation through a small incision using a shooter or folding forceps. The goals of canine cataract and lens instability surgery should be to ensure a small incision, minimal tissue trauma, minimize astigmatism, efficient surgical time, maintenance of the anterior chamber and restoration of emmetropia through the use of a stable intraocular lens specifically designed for the canine eye (1). While this is usually the case with routine phacoemulsification and in-the-bag intraocular lens implantation it is often not the case with lens instability, lens luxation or large posterior capsular ruptures. In such cases the incisions are often larger, surgical time and tissue trauma are excessive and the patient is often left aphakic. In instances of lens or lens capsule instability it may necessary to find an alternative method for IOL implantation if pseudophakic vision restoration is to be achieved. This would include eyes with zonular dehiscence, lens subluxation, luxation or a lens capsular rupture secondary to trauma, diabetes mellitus or surgeon trauma (1,2).

Lens instability is the result of weakness and breaking of the ciliary zonules. Zonular damage can be primary (breed-related) or secondary to chronic uveitis, glaucoma, trauma and cataract hypermaturity. Lens instability complicates all aspects of cataract surgery from capsulorhexis to IOL implantation. As a result, there is considerable diversity of opinion amongst veterinary ophthalmologists regarding when unstable lenses should
be removed, with some individuals avoiding surgical intervention for as long as possible. However, it has been demonstrated with respect to lens instability that complications such as retinal detachment and glaucoma are often associated with long-term surgical failure of intracapsular lens extraction (ICLE) once the lens has luxated. Lens instability presumably allows excessive movement of the vitreous base with associated peripheral retinal traction, tears and detachment. The mechanisms by which unstable lenses cause glaucoma have not been identified, but some believe this degenerative process begins early in the course of lens instability and then rapidly becomes irreversible. As a result, it has been advocated that unstable lenses should be removed as soon as the instability is detected to avoid associated secondary complications. Early surgical intervention will often allow the lens to be removed by phacoemulsification which may be preferred over ICLE due to several advantages such as a small corneal incision, less damage to the corneal endothelium, less disruption of the vitreous face and the potential for salvaging the lens capsule for IOL implantation.

The technique for removal of an unstable lens will depend on the degree of instability, equipment availability and surgeon preference. In general, if the lens is stable enough to permit phacoemulsification then this is always the preferred method of extraction. This can be facilitated using Vannas scissors for the capsulorhexis and a two-handed technique with or without the use of a capsule tension ring (3). A high-viscosity, cohesive viscoelastic and if possible, endo-cyclophotocoagulation, will aid in stabilizing the vitreous and lens. Zonular dehiscence of 120° or less should be amenable to phacoemulsification, but some surgeons will perform phacoemulsification with even larger areas of dialysis (up to complete luxation) if the vitreous is stable. Use of a 2-handed technique is advised, with the second instrument used to stabilize the lens and aid in fragmentation (Figure 1). If the lens has less than 120 degrees of zonular instability the use of a capsular tension ring implanted immediately following hydrodissection or following completion of phacoemulsification may allow an IOL to be placed in the lens capsule (Figure 2). If capsular instability is severe, capsulorhexis and phacoemulsification may be compromised and conversion to an open sky extracapsular or ICLE may be indicated. If the lens instability exceeds 120 degrees, or in the opinion of the surgeon the lens capsule will not support an IOL the then entire lens capsule may be removed and a sulcus IOL implanted (Figure 3,4,5).

Lens capsule instability can also occur iatrogenically as a result of inadvertent rupture of the posterior lens capsule during phacoemulsification or spontaneously as seen with spontaneous lens capsule rupture in diabetic dogs (4). Often these eyes may be left aphakic as the surgeon may prefer to avoid enlarging the incision to perform an ab-interno IOL implantation and further complicate an eye compromised by lens-induced uveitis. When there is lens instability, a capsular tear or spontaneous lens capsular rupture as seen with diabetes mellitus, the lens capsule may no longer be capable of supporting a stable, axially-located IOL. In such cases, the surgeon may choose to leave the patient aphakic or to implant an IOL in the ciliary sulcus, stabilizing it with non-absorbable sutures (Figure 3,4,5).

To use small incision surgery to restore emmetropia, a modified ab externo technique using a PMMA IOL (2) (Figure 3,4,5) or now, a newly designed acrylic IOL (Figure 6) will allow the use of small incision surgery and phacoemulsification to decrease the risk of retinal detachment while restoring emmetropia. If combined with endocyclophotocoagulation, the long-term risk for post-operative glaucoma can also be managed.
Figure 1 Two-hand phacoemulsification of a complete anterior lens luxation

Figure 2 Loading of a capsular tension ring to be placed prior to cataractous, subluxated canine lens

Figure 3 A Modified Ab-Externo approach for suture fixation of a PMMA intraocular lens implant

Figure 4 A Modified Ab-Externo approach for suture fixation of a PMMA intraocular lens implant
Figure 5 Post-operative appearance following suture fixation of a PMMA intraocular lens implant using a modified Ab-Externo approach

Figure 6 A newly design acrylic intraocular lens implant for suture fixation in the canine eye

Keywords: Phacoemulsification, Cataract, Intraocular lens, Cataract Surgery, Luxation, Sutured lens

References
Tools and Tricks in Glaucoma Diagnosis:  
From Clinical Signs to Tonometry  

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Summary
Glaucoma is an important cause of ocular pain and loss of vision in dogs, with damage to the retina and optic nerve caused by elevated intraocular pressure (IOP). Although less frequently diagnosed in cats, glaucoma is not uncommon, and may in fact be under-diagnosed due to the insidious onset of clinical signs in this species. Glaucoma is often acute in onset in dogs and is an important consideration in the differential diagnosis of a “red eye”. Early recognition and prompt, appropriate therapy are essential if vision is to be retained, as damage sustained by the optic nerve and retina in glaucoma rapidly becomes irreversible. This lecture aims to provide the general veterinary practitioner with a basic framework for their clinical approach to the diagnosis of glaucoma in dogs and cats.

Recognizing the signs:
Clinical signs of glaucoma (outlined in Table 1) will vary depending on the speed of onset, duration and degree of IOP elevation, and age of the animal. Clinical signs in individual animals will also vary according to the underlying cause (e.g. signs of uveitis, systemic disease, or an intraocular mass). Clinical signs are generally more subtle in glaucomatous cats.

Table 1 Clinical Signs of Glaucoma in Dogs

<table>
<thead>
<tr>
<th></th>
<th>ACUTE</th>
<th>CHRONIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pain</strong></td>
<td>may be severe (squinting, tearing, rubbing, lethargy, innappetance)</td>
<td>Signs of discomfort variable less frequent / overt signs in cats</td>
</tr>
<tr>
<td><strong>Episcleral injection</strong></td>
<td>often accompanied by conjunctival injection</td>
<td>Episcleral injection may also see scleral thinning / staphyloma</td>
</tr>
<tr>
<td><strong>Corneal vascularization</strong></td>
<td>deep 360° limbal “brush border”</td>
<td>Corneal vascularization may be exposure keratitis, with ulceration, branching vascularization and pigment</td>
</tr>
<tr>
<td><strong>Corneal edema</strong></td>
<td></td>
<td>Corneal edema &amp; striae</td>
</tr>
<tr>
<td><strong>Mydriasis</strong></td>
<td></td>
<td>Lens subluxation aphakic crescent differentiate from primary lens luxation</td>
</tr>
<tr>
<td><strong>Optic disc changes</strong></td>
<td>may be “cupped” or more often swollen in acute cases</td>
<td>Optic disc cupping &amp; atrophy</td>
</tr>
<tr>
<td><strong>Loss of vision</strong></td>
<td>absent direct &amp; consensual pupillary light reflex absent menace response absent dazzle reflex</td>
<td>Loss of vision usually complete, irreversible, with mydriasis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buphthalmos or phthisis bulbi Enlarged, or end stage, shrunken globe</td>
</tr>
</tbody>
</table>
Diagnostic considerations:

**Tonometry** provides a quantitative estimate of IOP. Commonly used tonometers in veterinary practice include the Tono-pen XL (an applanation tonometer) and the TonoVet (a rebound, or induction-impact tonometer). Clinicians should be familiar with the normal range of IOP for the tonometer type available to them as these differ between tonometers. It is important to bear in mind that each measurement represents only a snapshot of a dynamic process and fluctuations in IOP can be accentuated in animals with glaucoma. Caution should be exercised in diagnosing glaucoma based solely on a single, elevated IOP (e.g. ≥25mmHg).

For example, IOP readings can be influenced by a number of other factors including age, positioning and restraint of the patient (Table 2). Furthermore, IOP may be normal, or even low, in animals with glaucoma, particularly in patients with chronic glaucoma, or animals with concurrent uveitis. All methods of tonometry are imperfect and factors that influence IOP and its estimation should be avoided if possible or must be taken into consideration when evaluating IOP in the context of other clinical signs.

**Applanation tonometry:** These tonometers measure the force required to flatten the central cornea. Care should be taken to avoid indenting the eye. These tend to underestimate high IOPs in veterinary species. Variants of the Tonopen applanation tonometer (-Vet, -XL and Avia-vet) should always be used with a tip cover – which should not be too loose or too tight – and topical anesthetic should be applied to the ocular surface before use. With exception of Avia-vet these tonometers need to be calibrated by the user before use.

**Rebound tonometry:** A small lightweight probe contacts the corneal surface very briefly and topical anesthesia is not necessary. It is essential that the probe is directed horizontally, a consistent distance from the eye (4-8mm from and perpendicular to the cornea), although the handle of the device can be rotated. The original TonoVet tonometer is calibrated for dogs ("d"-setting) and horses ("h"-setting). For all species with the exception of horses, it should be used in the “d”-setting. The “p” setting has not been calibrated at all and is not intended for use in animal subjects. A new TonoVet tonometer will have separate specifically calibrated settings for dogs, cats, rabbits and horses.

<table>
<thead>
<tr>
<th>Factors affecting tonometry values</th>
<th>Effect on IOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure applied by fingers to globe or eyelids</td>
<td>Increase</td>
</tr>
<tr>
<td>Squeezing of eyelids, eye movements</td>
<td>Can increase (usual effect) or decrease</td>
</tr>
<tr>
<td>Corneal pathology (e.g. scarring / edema)</td>
<td>Can affect IOP readings – try to avoid area (if focal)</td>
</tr>
<tr>
<td>Tear film viscosity (e.g. viscous drop or ointment; dry eye)</td>
<td>Affects IOP readings</td>
</tr>
<tr>
<td>Firm restraint / pressure on neck/ tight collar</td>
<td>Can increase</td>
</tr>
<tr>
<td>Posture / head position</td>
<td>Head-down position can increase</td>
</tr>
<tr>
<td>Drug administration (including sedatives)</td>
<td>Can increase (e.g. ketamine) or decrease</td>
</tr>
<tr>
<td>Repeated measurements</td>
<td>Decrease due to pressure on eye</td>
</tr>
<tr>
<td>Time of Day</td>
<td>Circadian rhythm to IOP varies by species</td>
</tr>
<tr>
<td>Tonometer characteristics</td>
<td>Accuracy depends on tonometer type and species so be consistent and specify tonometer used!</td>
</tr>
<tr>
<td>Operator obtaining measurements</td>
<td>Use the tonometer properly!</td>
</tr>
<tr>
<td>Age of subject</td>
<td>Often low in very young animals, increases with maturity then decreases in older dogs and cats</td>
</tr>
<tr>
<td>Activity level / “White coat effect”</td>
<td>Increase with exertion and stress</td>
</tr>
</tbody>
</table>

**Gonioscopy** enables identification of abnormalities of the pectinate ligament and anterior-most portion of the trabecular meshwork in dogs with primary glaucoma, but is technically demanding and interpretation of findings requires considerable expertise. A goniolens is generally required for optimal visualization of the pectinate ligament in dogs, to overcome total internal reflection of light within the canine drainage angle. However, when a goniolens
is not available, a convex lens (as used for indirect ophthalmoscopy) can also be used to touch against the anesthetized corneal surface to very slightly indent the ocular surface, while viewing obliquely through this point of contact between the lens and cornea to visualize the pectinate ligament and iridocorneal angle. In unilateral glaucoma, gonioscopy should always be performed in the “normal” eye as extreme IOP elevation will lead to appositional closure of the drainage angle and corneal edema.

**Causes of Glaucoma**

Glaucoma may be considered primary when it occurs in the absence of other underlying ocular disease. Primary glaucoma is relatively common in purebred dogs including Cocker and Springer spaniels, Basset hounds, Welsh Terriers, Bouviers, Huskies and Samoyeds and is occasionally seen in mixed breed dogs. Secondary glaucoma is associated with antecedent disease, most commonly uveitis or intraocular neoplasia (particularly in cats); intraocular haemorrhage, or lens disease (particularly lens luxation in terriers). However, it is important, when selecting treatment for glaucoma, that different mechanisms and sites of aqueous humor outflow obstruction are identified and addressed. Normal IOP is dependent on the equilibrium between production of aqueous humor by the ciliary body processes, and its unimpeded outflow, first through the pupil to anterior chamber of the eye, and subsequently via the trabecular meshwork located within the irido-corneal angle and ciliary cleft and then via an aqueous plexus and collector channels in the episclera and sclera, which in turn are continuous with the episcleral and scleral veins. Obstruction occurring at any location in this path results in elevated IOP and glaucoma. Potential causes of outflow obstruction include pupil block (e.g. by posterior synechiae or in lens luxation); obstruction of the angle opening (e.g. by extensive, peripheral anterior synechia or iris neovascularization; obliteration or absence of normal trabecular meshwork, and collapse of the ciliary cleft (e.g. by inflammatory infiltrates, ocular neoplasia or in primary glaucoma).

**When to Refer?**

As the etiopathogenesis of glaucoma can be complex, and treatment challenging, early referral for specialist evaluation is strongly recommended. In acute cases of glaucoma in dogs, this should be considered an emergency or at least a matter of urgency, whereas in cats the disease process tends to be more chronic and insidious. In animals with unilateral glaucoma and chronically blind eyes that are buphthalmic, non-emergent referral is also strongly recommended in order to ensure that both the underlying cause and the risk of glaucoma in the opposite eye are determined. Unfortunately, IOP monitoring alone generally fails to identify “early” glaucoma in predisposed eyes. Thus client education and vigilance of owners of dogs with primary glaucoma is paramount. While enucleation may be unavoidable in some patients with chronically blind, painful eyes, globes should always be submitted for histopathology to ensure that the underlying cause of glaucoma is definitively established where possible.

**Keywords:** Glaucoma, Diagnosis, Tonometry, Clinical Signs

**References**


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Advances in Glaucoma Genetics and Pathology
- What Do They Mean?

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Summary
Glaucoma is not a single disease entity. “The glaucomas” represent a large, diverse group of disorders, unified by a final common pathway of characteristic optic nerve and retinal damage that results in loss of vision. Currently, treatment for glaucoma in veterinary and human patients targets intraocular pressure (IOP) – which is the single most consistent risk factor for loss of vision, as well as a potential source of pain and discomfort. However, the development of more effective treatment strategies that target either the root causes of IOP increase in individual patients, or the complex processes that lead to subsequent irreversible damage to the optic nerve and retina, is currently hampered by lack of understanding of these genetic and pathological processes. Fortunately, though progress has been slow, new insights have been gained in recent years. The current status of knowledge of glaucoma genetics in dogs and cats will be reviewed in this presentation, as well as recent advances in our understanding of glaucoma pathology in dogs. Primary angle closure glaucoma (PACG) is the most common form of glaucoma in dogs, but evidence points to this being a complex genetic trait with contributions likely also made by age, sex and environmental, vascular and immunologic factors. Open angle forms of glaucoma in dogs are less common than PACG but, together with glaucoma secondary to lens luxation, have the advantage of being monogenic traits for which disease causing mutations have been identified for many of the affected breeds.

Key Features of Glaucoma Pathophysiology in Dogs
Glaucoma is a common, painful and blinding disorder that frequently results in enucleation in veterinary patients. Glaucoma, however, is not a single disease: it represents a group of diseases that share a final common pathway leading to vision loss due to irreversible and progressive damage to the retina and optic nerve. In dogs and cats with glaucoma, elevated intraocular pressure (IOP) is a consistent, unifying risk factor. Pathology of disease is complex and involves both the anterior segment (perturbing regulation of IOP) and posterior segment of the eye (retina and optic nerve). Though canine glaucoma pathogenesis hinges on processes that decrease aqueous outflow, thereby increasing IOP, the real picture is considerably more complex. Factors that contribute to the development of glaucoma are outlined in Figure 1.
Figure 1 Processes involved in vision loss in glaucoma are complex, and include:

1) **Genetics**: strong breed predisposition (see below) to both glaucoma and goniodysgenesis / pectinate ligament dysplasia
2) **Age**: the prevalence of glaucoma increases with age
3) **Sex**: prevalence of primary glaucoma is about 2 x greater in female than in male dogs
4) **Environmental factors**: (including lifestyle and light levels)
5) **Immunologic Factors**: molecular and cellular inflammation might represent cause or effect in glaucoma patients
6) **Vascular Factors**: high IOP and low systemic blood pressure can contribute to poor blood flow to retina and optic nerve tissues (ocular perfusion pressure).

**Genetics of Canine and Feline Primary Glaucoma:**
Over 30 breeds of dog are considered predisposed to glaucoma. The most common form of primary glaucoma in dogs is PACG, which has a prevalence of up to ~8% in some breeds. An important goal of research in glaucoma is to develop genetic tests to facilitate early diagnosis (to improve treatment outcomes) and to limit breeding of affected dogs to reduce the incidence of this debilitating disease. Earlier, mostly unsuccessful, efforts focused on a candidate gene approach but with ongoing refinement of canine genomic resources, genome wide association studies (GWAS) have been successful in identifying causal mutations for glaucoma in several breeds [2]. At least 6 genes, mutations or susceptibility loci have been identified as contributing to or causal for glaucoma in dogs (see Table 1), and the LTBP2 gene has been identified as causal for congenital glaucoma in the Siamese cat.

PACG represents the greatest challenge to current genomic approaches. Unlike many forms of retinal degeneration, as well as canine primary open angle glaucoma (POAG) and congenital glaucoma in the Siamese (inherited as autosomal recessive traits), identifying genetic causes of PACG is hampered by the complexity of its genetics and the many other factors that contribute to PACG development (Fig.1). Success in GWAS approaches relies on accurate identification of cases and controls for comparison. Unfortunately, this can be very challenging for PACG, which is not a fully penetrant, autosomal trait and affected animals may not develop clinical signs of disease until late in life, so it can be very difficult to confidently recruit suitable control subjects. While Pectinate Ligament Dysplasia (PLD) is a quantifiable and heritable trait, it does not represent a good predictor for subsequent glaucoma development, and cannot be relied upon as the only means of establishing “glaucoma” phenotype in subjects for genomic study. Only a small proportion of dogs with significant PLD go on to develop glaucoma, in which subsequent collapse of the ciliary cleft is a unifying feature.
Table 1 Known Molecular Genetic Associations with Glaucoma in Canine Breeds:

<table>
<thead>
<tr>
<th>Form of Glaucoma / Breed</th>
<th>Gene/ locus / susceptibility allele</th>
<th>Genetic Test?</th>
</tr>
</thead>
<tbody>
<tr>
<td>PACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basset Hound</td>
<td>3 susceptibility genes: COL1A2, RAB22A, NEB</td>
<td>No</td>
</tr>
<tr>
<td>Dandie Dinmont</td>
<td>Locus on canine chromosome 8</td>
<td>No</td>
</tr>
<tr>
<td>Shiba Inu</td>
<td>Susceptibility gene: SRBD1</td>
<td>No</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>Susceptibility gene: SRBD1</td>
<td>No</td>
</tr>
<tr>
<td>Open Angle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beagle</td>
<td>ADAMTS 10</td>
<td>Yes</td>
</tr>
<tr>
<td>Norwegian Elkhound</td>
<td>ADAMTS 10</td>
<td>No</td>
</tr>
<tr>
<td>PBGV</td>
<td>ADAMTS 17</td>
<td>Yes</td>
</tr>
<tr>
<td>(Basset hound)*</td>
<td>ADAMTS 17</td>
<td>Yes</td>
</tr>
<tr>
<td>Shar Pei (POAG/ Lens luxation)</td>
<td>** ????</td>
<td>Yes</td>
</tr>
<tr>
<td>Primary lens luxation</td>
<td>26 breeds including Terriers,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chinese Crested dog and ADAMTS17</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Australian Cattle dog***</td>
<td></td>
</tr>
</tbody>
</table>

*POAG is not a common form of glaucoma in the Basset hound, in which PACG is most prevalent; **Genetic mutation not published at time of writing.*** This mutation is NOT causal for lens luxation in the Border Collie, Brittany Spaniel or Shar Pei. (Table adapted from Komáromy and Petersen-Jones (2015) [2])

Relating Genetics and Ocular Pathology: where might this lead us?
The following pathologic features of canine glaucoma show great promise as potential therapeutic targets or biomarkers of disease:

Extracellular matrix genes and abnormalities: The extracellular matrix (ECM) forms a prominent structural component of the aqueous outflow pathways. This is not a "passive" scaffold between cells, but rather conveys signals between cells and their environment, contributes to aqueous outflow resistance, and changes dynamically throughout life. Progressive ECM deposition (e.g. increased collagen) and remodeling may be responsible for progressive age-related changes in degree of severity of gonioscopic findings identified in some dogs in large screening studies, that prompted the ECVO to recommend gonioscopic exam every 3 years.

Veterinary ophthalmologists have long recognized that differences exist between breeds in their susceptibility to high IOP, and it is becoming apparent that relative stiffness or compliance of ocular tissues might play a role in these differences between breeds in their ability to maintain vision despite chronic glaucoma and effectively “tolerate” high IOP. Mutations identified in extracellular matrix (ECM) genes involved in microfibril assembly, e.g. ADAMTS10, ADAMTS17, LTBP2, have provided powerful evidence of the importance of ECM biology in the development of aqueous humor outflow pathways; properties of the sclera and optic nerve head, and the stability of the lens. The relationship between primary lens luxation and glaucoma has traditionally been very difficult to untangle clinically. However genetic studies have revealed that these two clinical presentations may simply represent different points on a spectrum of phenotype severity caused by the same genetic mutation that affects ECM assembly or turnover in the eye tissues.

Retinal and Optic Nerve Circulation: Vascular Compromise and ischemia are key features of canine PACG due to acute onset of extreme IOP elevation. Thus, in addition to inner retinal and optic nerve damage seen in other species with glaucoma, segmental ischemia and necrosis of outer retina (photoreceptors and RPE) are also frequently observed. Clinically, this is recognized soon after glaucoma onset as wedge-shaped areas of altered tapetal reflectivity. Compared to other species, the dog is uniquely susceptible to this ischemic damage because the short posterior ciliary arteries that supply the retina and optic nerve head are poorly supported by adjacent sclera.

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Pigment Dispersion: Free pigment (melanin) granules and pigment within “melanophages” are commonly identified in dogs with a clinical history of glaucoma [3]. This may contribute to glaucoma development, both by clogging aqueous outflow channels, and by inciting oxidative stress and / or inflammation. Pigment dispersion increases with age (often seen with iris atrophy) and may be associated with other evidence of iris-lens contact. The latter may be intensified by age-related increase in lens size and hardness.

A Role for the Immune System in Glaucoma: A common feature between species, and forms of glaucoma, is the activation of the immune system and inflammatory responses in the retina and optic nerve head. Within these tissues, activation of resident microglia cells, and recruitment or macrophages, shifts the balance from the normal immuno-tolerant system to a pro-inflammatory system. Increase in production and release of pro-inflammatory mediators (Interleukin-1β, TNF-α and many others) contributes to development of chronic, low-grade, sub-clinical inflammation. This is likely a mechanism for progressive loss of vision despite control of IOP in many patients.

The role of the veterinary general practitioner in glaucoma research:
Clearly, there remains much to learn about the genetics and pathology of glaucoma. Veterinarians can make a real contribution to enhancing understanding of this disease by remaining vigilant for the signs of glaucoma in their veterinary patients and seeking advice from veterinary ophthalmologists in establishing underlying cause. This is both a necessary step in ensuring appropriate medical and / or surgical therapy for patients with glaucoma, and a means to gather valuable information about the prevalence of different forms of glaucoma in the animal population. Pedigree history should also be obtained if possible…occasionally owners will volunteer that they are aware that a related dog also presented with glaucoma previously. Screening of related dogs is recommended, whenever possible.

Glaucoma is generally a bilateral condition but often only one eye appears affected at the time of initial presentation. Irreversibly blind, uncomfortable, end-stage glaucomatous eyes should always be submitted to a veterinary pathologist with training and expertise in interpretation of ophthalmic pathology. This ensures that accurate diagnosis is made, and that the risk for development of primary glaucoma in the opposite eye is identified and appropriately addressed. Plans for prophylactic therapy and monitoring should be made in consultation with a veterinary ophthalmologist.

Keywords: Ophthalmology, Glaucoma, Pathology, Genetics

References
Glaucoma Surgery: Endolaser, Filtration Surgery Update

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Summary
Glaucoma is an increase in intraocular pressure (IOP) incompatible with the health of the eye and is a common cause of blindness in dogs. Once diagnosed, glaucoma is divided into primary and secondary and acute and chronic groups. Of these, acute primary glaucoma is the most likely to be treated and retain vision. Treatment will include both medical and surgical management. The most current surgical recommendations include endocyclophotocoagulation, placement of a drainage device or a combination of the two.

Glaucoma is an increase in intraocular pressure (IOP) incompatible with the health of the eye and is a common cause of blindness in dogs. In general, when performing applanation tonometry (Tonopen) or indentation tonometry (Tonovet) most dogs have an IOP <20 mmHg. The Tonovet will tend to read slightly higher than the Tonopen. For predisposed canine breeds with an IOP >20 mmHg treatment and possible referral should be considered. While it is debated, I personally advise annual determination of the IOP in all predisposed breeds after the age of 3-4 years. It is also essential to determine the IOP in all eyes with anisocoria, fixed and dilated pupils, uveitis, all red eyes, cloudy eyes, painful eyes, blind eyes, enlarged eyes, all eyes with a diagnosis of glaucoma on therapy and the contralateral eye in all dogs with primary glaucoma in the affected eye. It is therefore essential that all veterinary practices have access to a working, reliable tonometer and know how to use it effectively.

Once diagnosed, glaucoma is divided into primary and secondary etiologies and acute and chronic duration. Of these, acute primary glaucoma is the most likely to be treated and also retain vision. Treatment of acute glaucoma should include both medical and surgical management. Of the medical treatment, topical prostaglandins, topical and oral carbonic anhydrase inhibitors are the medications most commonly used that have significant clinical efficacy in the canine glaucoma. While medical therapy is the immediate and initial treatment for acute primary glaucoma, if used alone, treatment will fail and blindness is the expected outcome. To succeed in vision preservation, medical therapy must be combined with surgical therapy. Surgical options for a visual eye will include procedures to decrease aqueous production or increase outflow.

Use of the diode laser (810nm) for transscleral cyclophotoblation (TSCP) (Figure 1,2) or endocyclophotocoagulation (ECP) (Figure 3,4) will result in coagulation necrosis of the ciliary body and decreased aqueous humor production. The 810nm laser energy is absorbed by the melanin of the ciliary processes and transformed into heat, resulting in cyclodestruction. By comparison ECP (80% at 1yr) has a significantly greater success at controlling IOP and preserving vision when compared to TSCP (50% at 1yr). However the cost, equipment and skill required for ECP make it unavailable for many. In addition, with ECP it is advisable to first perform phacoemulsification and IOL implantation to provide better access...
to the heads and tails of the ciliary processes and also to avoid secondary cataract formation. The amount of ciliary process to treat with ECP will depend on the preoperative IOP and whether the surgeon can access only the heads or both the heads and tails of the ciliary processes. Treatment area will range from 200-360 degrees. To be able to treat >230 degrees with ECP will require a two-handed approach. It is also advisable to use a high viscosity viscoelastic to open the ciliary sulcus when performing ECP. Personally, following ECP I remove the viscoelastic from the anterior chamber, but leave it in the ciliary sulcus to tamponade the ciliary processes. With either TSCP or ECP the immediate stability of the postoperative IOP and control of inflammation are the greatest concerns. Frequent monitoring of the postoperative IOP and intervention with medical therapy and/or aqueocentesis may be required. To assist with the management of postoperative IOP instability some surgeons will combine ECP with implantation of a drainage device. Additional complications of TSCP or ECP include hypema, hypotony, corneal ulceration, uveitis, cataract, retinal detachment and a failure to control the IOP.

Implantation of a drainage or filtering device is designed to re-direct aqueous outflow to the subconjunctival space, frontal sinus, suprachoroidal or other location (Figure 5,6). They can be valved or non-valved with valved devices being unidirectional and requiring a specific IOP (8-12mmHg) to start the flow of aqueous. The immediate postoperative concern with drainage devices is obstruction with intraluminal fibrin which can be managed with intracameral tissue plasminogen activator. Long-term, fibrosis and encapsulation of the filtration bleb result in failure of aqueous resorption and elevation of IOP. This can be managed surgical with revision of the filtration bleb and at the time of surgery with the use of anti-fibrotics such as mitomycin C or 5-fluorouracil.

Chronic glaucoma results in blindness and pain requiring enucleation, intrascleral prosthesis or pharmacologic ablation.
Keywords: Glaucoma, Endocyclophotocoagulation, ECP, Diode Laser, Filtration Surgery, Drainage Device

References
Updates in Ocular Therapeutics and Drug Therapy

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Summary
The most common route of administration for treatment of ocular disease is topical drug delivery, usually by ocular instillation or subconjunctival injection. Topical administration can be used to treat diseases such as keratitis, conjunctivitis, blepharitis, but is also used for other intraocular diseases, such as uveitis and glaucoma. Upon administration, the preocular factors and anatomical barriers contribute to the lower bioavailability of topical formulations. Often only 5-10% of topicaly administered drugs reach the site of action, in opposition to the other 90-95% which is eliminated by several factors. Preocular factors, which are natural and mechanical barriers for the entrance of foreign substances, are responsible for this low bioavailability. These include: (i) solution drainage; (ii) blinking; (iii) tear film; (iv) tear turn over, and (v) induced lacration. Another important factor is the preocular mucin present in the tear film that acts as a protective barrier responsible for the elimination of pathogens, and also contributes for the impedance of drug’s entry. One additional factor is related to the anatomic volume of cul-de-sac and the tear volume, which contributes to the elimination of eye drops.

Ocular drug delivery is a very challenging route of administration of pharmaceuticals, complicated by the eye’s anatomy, physiology, and biochemistry, which presents resistance to the entry of drugs. The ocular globe can be divided in two major segments, namely, the anterior and posterior segments. The most common route of administration for treatment of ocular disease is topical drug delivery, usually by ocular instillation or subconjunctival injection. Additional routes of administration include intracameral, intravitreal, suprachoroidal, periocular, subretinal and systemic (Figure 1). When choosing the route of drug delivery the clinician must consider the intended target tissue, the ability of the owner to apply the medication, the compliance of the animal and the duration of delivery required. As clinicians we must choose between noninvasive (topical, oral) vs. invasive (intracameral, intravitreal, suprachoroidal) routes of administration.

Topical administration can be used to treat diseases such as keratitis, conjunctivitis, blepharitis, but is also used for other intraocular diseases, such as uveitis and glaucoma. Only 5-10% of topically administered drugs reach the site of action, in opposition to the other 90-95% which is eliminated by several factors (1,2). The ability of the eye to remove these medications by processes of lacrimation, tear dilution and turnover leads to a poor bioavailability of many drugs, and moreover limit the drug permeation to the anterior segment of the eye and render it impossible to reach the posterior segment. To improve the efficacy of noninvasive, topical medications we must work to increase the residence time of the drug and/or enhance its permeation through tissue. This done through the use of gel technology, nanoparticle technology, permeation enhanced pro-drug development, solubilization agents, microdroplets and drug-eluting contact lenses (1-4). The advantage of these delivery systems includes (i) improvement of the bioavailability of drugs by increasing the drug residence time on the ocular tissues; (ii) reduction of the administration frequency of drugs due the controlled release of drugs; (iii) increase of patient compliance; (iv) absence of toxicity; (v) more adjusted drug doses,
since conventional systems use very concentrated solutions, which could induce toxic side effects and cellular damage at the ocular surface as also could be dangerous for drugs with a narrow therapeutic indices and (vi) biocompatibility of more efficient systems.

Other strategies involve invasive and often controlled, sustained-release drug delivery systems (5-8). These devices can be biodegradable or non-biodegradable (2). They may be placed subconjunctival or episcleral for treatment of anterior segment disease (6,7,8) or intrascleral, suprachoroidal, intravitreal or subretinal for posterior segment disease (5). Additionally, the coating of an intraocular lens or capsular tension device may also provide ways to deliver drugs to the target tissue during procedures such as cataract surgery.

**Figure 1** Routes of administration for ocular drug delivery

**Keywords:** Ocular Pharmacology, Drug Delivery, Sustained Release, Topical, Intracameral, Intravitreal, Suprachoroidal

**References**
Understanding the Fundus: Is That Normal or Abnormal?!

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Summary
The ocular fundus is the part of the posterior segment of the eye that is viewed with the ophthalmoscope and shouldn’t be confused with the “retina”, as the latter structure is not solely responsible for what you see. In fact, the neurosensory retina is mostly translucent and under normal circumstances should hardly be visible. In this session, the anatomy of the ocular fundus will be reviewed and a systematic approach to fundus evaluation will be presented. Normal variation in fundus appearance within and between species will be highlighted. These variations, as well as ophthalmoscopically visible hallmarks of ocular pathology, will be presented in the context of underlying anatomy and disease processes. Ophthalmoscopy can be particularly valuable in patients with suspected systemic infections or neurologic disease, as we are able to directly observe components of the CNS and vascular systems directly. It is imperative that the clinician is familiar with the wide range of normal variation if incorrect diagnosis is to be avoided. Few, if any, fundus lesions may be considered pathognomonic for a single disease and caution should be exercised in making etiological diagnoses based solely on fundus appearance. Nevertheless, ophthalmoscopy remains a powerful tool for clinicians armed with knowledge of the basic anatomy of the structures they are examining, and the range of pathologic processes that can affect these structures.

The contribution made by different anatomic structures to the fundus appearance is presented in Table 1:

Table 1 Structures normally seen are listed from innermost (closest to vitreous …and hence closer to the observer with an ophthalmoscope) to outermost (closest to sclera):

<table>
<thead>
<tr>
<th>Anatomical structure / layer</th>
<th>Ophthalmoscopic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner limiting membrane</td>
<td>Imparts a “sheen” to inner surface of retina</td>
</tr>
<tr>
<td>Neurosensory retina</td>
<td>Translucent — like a thin layer of “dust” on “mirror” (over tapetum) or “chalkboard” (pigmented fundus)</td>
</tr>
<tr>
<td>Retinal vasculature (within neurosensory retina)</td>
<td>Venules darker red and larger than arterioles. Number, branching and tortuosity can vary but typically 3-5 veins converge at the disc, and 10-12 main arterioles are seen (fewer in cats).</td>
</tr>
<tr>
<td>Retinal Pigment Epithelium (RPE)</td>
<td>Degree of pigmentation variable and often relates to iris / coat color. Degrees of pigment dilution in the RPE and choroid lead to variations ranging from brown-black to red (common in dogs with pale, yellow irises) to the light red, “tiger striped” appearance characteristic of blue-eyed, dogs. RPE is transparent where overlies tapetum.</td>
</tr>
<tr>
<td>Tapetum (within the inner choroid)</td>
<td>Bright, shiny sweep of color over a variable extent of superior (upper) fundus. May be small “islands” of pigment in the tapetum, or “islands” of tapetum within the non-tapetal fundus</td>
</tr>
</tbody>
</table>
| Choroid (medium & large)    | Degree of pigmentation variable and often relates to iris /coat color (as for
Anatomical structure / layer | Ophthalmoscopic appearance
--- | ---
Vessel layers of pigmented, vascular structure, continuous with anterior uvea | Blood vessels are large, approximately radial (spoke-like) in orientation and are lighter, orange-red than overlying retinal blood vessels and may appear as “tiger stripes”. Where tapetum is present or pigment in choroid or RPE pigment is dense, these obscure blood vessels in choroid.

Sclera (collagenous, outer coat of the eye) | May be seen as a white or pale pink background to choroidal blood vessels, where the tapetum is absent in sub-albinotic animals (blue eyed or merle) that lack pigmentation in their overlying choroid and RPE.

Optic Nerve head (disc) | Round to triangular or irregular shape & white to pink in dogs; round & pink to gray in cats. Small grey spot at the center of disc is “physiologic pit” (where hyaloid artery attached). May be surrounding hyper-reflective ring or crescent (“conus”) or pigment.

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**Figure 1** Understanding fundus anatomy

**Ophthalmoscopic lesions / pathological variants are summarized in table 2 below:**

**Table 2** Summary of ophthalmoscopic abnormalities as they relate to underlying pathology

<table>
<thead>
<tr>
<th>Ophthalmoscopic abnormality</th>
<th>Underlying pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyper-reflectivity of tapetum</td>
<td>Thinning of overlying neurosensory retina (less “dust on mirror”), e.g. in PRA; complete detachment with tearing of neurosensory retina</td>
</tr>
<tr>
<td>Reduced tapetal reflectivity</td>
<td>Thickening of neurosensory retina by edema or infiltrates (more “dust on mirror”). Retinal detachment with subretinal exudate/infiltrate/transudate</td>
</tr>
<tr>
<td>Altered tapetal color</td>
<td>May indicate choroidal damage (e.g. trauma, infarction, inflammation), with disruption of tapetal structure, or disease of overlying RPE</td>
</tr>
<tr>
<td>Altered non-tapetal fundus pigmentation / color</td>
<td>Migration, loss or clumping of RPE cells, which is often patchy (non-specific response to degeneration, inflammation, detachment). Thickenig of overlying neurosensory retina by edema or infiltrates (i.e. “more dust”) will appear as pale areas against the dark “chalkboard” background of the underlying RPE and choroid.</td>
</tr>
<tr>
<td>Vascular abnormalities</td>
<td>Changes in blood vessel caliber e.g. narrowing/ attenuation: smaller arterioles and branches “lost” first – indicates retinal degeneration. Pallor (anemia, lipemia); distension or aneurysms or increase in tortuosity (hypertension, hyperviscosity). Perivascular “cuffing” by infiltrates (inflammation).</td>
</tr>
</tbody>
</table>

Distinct, bright to dark red: Hemorrhages may be:
<table>
<thead>
<tr>
<th>Ophthalmoscopic abnormality</th>
<th>Underlying pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>keel shape</td>
<td>pre-retinal (trapped between retinal surface and posterior vitreous face) within nerve fiber layer</td>
</tr>
<tr>
<td>flame or brush-like</td>
<td>within the retina</td>
</tr>
<tr>
<td>Dot-blot hemorrhages</td>
<td>sub-retinal</td>
</tr>
<tr>
<td>Darker, diffuse hemorrhage</td>
<td>Hemorrhage may occur as a result of local or systemic vascular disease, e.g. hypertension, vasculitis, or may reflect a systemic coagulopathy</td>
</tr>
<tr>
<td>Altered optic nerve head appearance</td>
<td>May be smaller or larger than normal, elevated or depressed, darker or paler in color, blurred outline</td>
</tr>
</tbody>
</table>

The neurosensory retina is only firmly attached at the optic nerve head and, peripherally / anteriorly where it merges with the ciliary body. Between these points, the attachment is weak - relying on the close apposition of RPE and the support of the vitreous.

*Retinal detachment* almost always reflects separation of the neurosensory retina from the RPE, as the RPE remains attached to the underlying choroid. Areas of detachment/exudates may appear as focal gray areas within the tapetal fundus (with local reduction in tapetal reflectivity) or non-tapetal fundus (where they appear paler than the surrounding area). Evaluate the course of the retinal blood vessels carefully, retinal detachment will cause them to deviate from their original course. More extensive retinal detachments will no longer be within the normal plane of focus of the retina and will appear as gray sheets and folds, with surface blood vessels, that are progressively out of focus as they billow towards the observer. Detachment may be associated with vitreous degeneration or hemorrhage.

**Optic Nerve Head (ONH) Abnormalities**

*Small size* may be indicative of atrophy or hypoplasia...or a “normal” variant (“micropapilla”). Pathologic variants are distinguished from normal variants by subjective assessment of visual function and pupillary light reflexes (PLR). Optic nerve degeneration is seldom observed in dogs and cats in the absence of vascular attenuation or altered pigmentation and/ or hyper-reflectivity. The margins of the degenerate ONH may appear shrunken and “crenated” as myelin is also lost.

*Glaucomatous Cupping* In chronic glaucoma, loss of myelin reveals the lamina cribrosa structure (“laminar dot sign”) and a dark, excavated ONH. In acute glaucoma in dogs, the optic nerve head often appears congested and swollen (see below).

*Colobomatous defect.* As these lesions are depressed relative to the rest of the fundus, they appear out-of-focus, but generally have sharply demarcated margins and may appear blue / gray in contrast to more normal regions of the ONH. They contribute to an “enlarged” ONH if they involve the adjacent choroid and sclera.

*Enlargement:* may reflect infiltration (e.g. inflammation, neoplasia) or papilledema (increased intracranial pressure). Swelling and enlargement of the ONH is also seen early in canine acute glaucoma. True swelling should be distinguished from so-called “pseudo-papilledema” due to normal pronounced myelination by considering the following questions: Are margins distinct and sharply demarcated or ill-defined and poorly focused? Is the physiologic pit visible? Is there hemorrhage evident? (particularly overlying the ONH). Is there involvement of the retina, choroid and or adjacent vitreous? Are there any other clinical / neurologic signs?

**General principles of funduscopic interpretation**

*Document* the appearance by a detailed description and accurate, labeled drawings. It is advisable to always describe lesions in detail, AT THE TIME of your examination, rather than relying on your memory to make
notes or drawings later. Allowing even a few minutes to elapse before recording your findings can cloud your recollection of the fundus appearance!
If possible take fundus photographs: a smart phone can often be used for this if the pupil is dilated. Multiple layers of translucent tape over the flash may be necessary to reduce its intensity.

_Distinguishing between normal and abnormal can be challenging._ The following are some tips that can help:
Try to explain the funduscopic appearance in terms of underlying histopathology. If you cannot do this, it is more likely that the appearance represents a normal variant. _Use higher magnification and/or change your angle of viewing._ Use of a red-free (i.e. green) filter can be helpful in delineating smaller blood vessels and can help to distinguish between hemorrhage (which will look completely black with a red-free filter) and pigmentation. _Consider other clinical features that one might expect to see_ in association with the appearance you are confronted with. Similarly, there may be _involvement of other ocular structures or signs of systemic disease._ If you cannot identify any other features of disease or demonstrate signs of compromised vision, it is more likely that the appearance represents a normal variant. _Seek a second opinion from an expert._ Photos can be extremely helpful and referral is not a sign of weakness! If inherited disease is suspected, e.g. “PRA”, is there a genetic test available? Many pathological processes are progressive. The only way to _determine progression_ is to evaluate on more than one occasion.

**Keywords:** Ophthalmology, Fundus, Retina, Ophthalmoscopy

**References**
Technological Advances in Fundus Imaging: New Horizons

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Summary
Recent technological advances in fundus imaging are becoming increasingly available to comparative ophthalmologists for both clinical and research applications in animal subjects. As these exciting technologies filter into clinical practice from the research domain, they bring the promise of enhanced understanding of pathological processes that underlie “mysterious” fundus lesions that may be seen with an ophthalmoscope. The objective of this “state of the art” presentation is to provide an overview of the theoretical basis, clinical indications, practical applications and limitations of Optical Coherence Tomography (OCT) and confocal Laser Scanning Ophthalmoscopy (cLSO). OCT and cLSO are non-invasive, non-contact optical imaging techniques capable of producing high resolution images of the retina and optic nerve head. Fundus lesions identified on ophthalmoscopy and/or fundus photography can be better characterized with OCT, which more clearly identifies which retinal layers are involved and allows the location, progression and/or resolution of retinal lesions to be documented and followed longitudinally. Use of cLSO and adaptive optics provide high contrast images that provide complementary information, though use of the latter remains restricted to a research setting.

Introduction:
Optical imaging enables the retina and optic nerve head to be seen in greater detail than by conventional ophthalmoscopy and photography. However, the optical properties of the eye impose their own limitations and clarity of the eye can be compromised by disease. Each fundus imaging modality also has limitations and a multi-modal approach is often necessary and helpful, to utilize several different complementary techniques. Biomedical optics leverages complex tissue-light interactions: effects of photons on tissue and tissue on photons: i.e. absorption, reflection, scattering and fluorescence.

OCT Technology:
First developed in the 1990s, OCT now allows the acquisition of 3D data sets that provide exquisitely detailed cross-sectional images of the fundus, approaching in vivo histopathology. Minor modifications in OCT technology allow high resolution imaging of anterior segment structures as well, including the cornea. The principle of OCT, “low coherence interferometry”, is broadly comparable to ultrasonography, except that it uses light rather than sound. OCT uses the reflectivity of light waves from sub-surface features (e.g. retina layers and choroid) to generate “A-scans” that are then combined to produce detailed cross-sectional images of ocular tissues. Different layers within tissues (e.g. cellular layers in the retina) have different refractive indices. Thus, layers of different reflectivity are visible as alternating bright-dark signals in cross-sectional images of the retina, and these correlate with histologically identifiable retinal layers. The number of “layers” that are routinely detectable on OCT images varies with species and instrument (Fig.1).
Figure 1 OCT image of the retina in a cat (right) with corresponding histology (left). Notice that layers containing cell nuclei appear dark, whereas areas with “horizontal structural alignment” e.g. the retinal nerve fiber layer (RNFL), outer plexiform layer (OPL) and external limiting membrane (ELM) appear relatively bright. In this image, 3 distinct reflective lines in the outer retina can be seen, that are thought to represent the external / outer limiting membrane (ELM); the ellipsoid zone (EZ) where there is a dense concentration of mitochondria in the outermost part of photoreceptor inner segments; and the interdigitation zone (IZ) that represents the tips of the photoreceptor segments and apices of retinal pigment epithelium (RPE) microvilli that surround them. The IZ line may blend with a line formed by RPE / Bruch’s membrane.

Unlike ultrasonography, OCT does not require any contact with the eye (thus avoiding the potential for tissue compression and/or distortion) and it allows measurement of structures and distances <10µm in scale, versus the 100µm scale of all but the highest-resolution ultrasonography. Modern Spectral Domain (SD)-OCT systems generally capture many A-scans in a fraction of a second (a rate of 20,000 - >70, 000/ sec) and have an axial resolution of ~ 2-5µm (transverse resolution is “only” ~ 10-15µm). It is possible to acquire high quality scans in many species but the range of normal variation within differing species needs to be defined. In recent years, a number of different commercially available instruments have entered the market (reviewed elsewhere) [1]. However, rapid developments in OCT technology mean that the list of available instruments changes continuously - which may actually work in favor of veterinary clinicians! Unfortunately, measurements are generally not comparable between instruments. Deriving quantitative measurements from OCT instruments is hindered by software algorithm errors in detecting the edges of the distinct retinal layers (segmentation) in animals. Fortunately, most instruments have software features that allow these manual corrections to be made and several also have 3-D and en face visualization features to aid in qualitative assessments.

Reproducibility and reliability are central to the use of OCT in quantitative and longitudinal studies eg. to monitor disease progression or lesion resolution. In clinical practice, many of the animals that present with retinal or optic nerve disease are middle aged or older, so age-matched “control” scans are helpful. Potential effects of aging on thickness values should be considered when reassessing animals in longitudinal studies. Some instruments’ software packages offer an “alignment tool” that detects blood vessel orientation and helps ensure follow up scans are obtained in a consistent region of fundus. Various artifacts can affect quantitative and qualitative assessment of OCT scans and so every effort should be made during imaging to minimize these artifacts.

Image Acquisition and Scan evaluation:

OCT scanning can be accomplished in a wide variety of animal species. A “portable” instrument with a hand-held scanner facilitates scan acquisition – even in standing, sedated horses.[2] However, each species presents its own challenges and large or small globe sizes may prevent acquisition of high quality images.
with some instruments [1].

_Globe immobilization, eye axis alignment and pupil dilation:_ General anesthesia or heavy sedation may be needed to maintain a steady eye position to limit motion artifact. Application of a mydriatic, such as 0.5-1% tropicamide, is generally recommended (and is necessary for rodents), and aids in obtaining properly aligned (i.e. “flat” rather than tilted) scans in the regions of interest. When evaluating the optic nerve head in glaucoma subjects, IOP should be within the normal range when scans are acquired to limit corneal edema and reversible “cupping” of the nerve.

_Clear ocular Media & Corneal Hydration:_ As OCT is an optical imaging technique, even minimal cataract, corneal opacity (including drying!), inflammatory cells, flare or hemorrhage within aqueous or vitreous can compromise the image quality. Frequent wetting of the cornea (every few seconds) is needed in anesthetized subjects to maintain adequate corneal clarity.

_Scan quality:_ Each OCT instrument has a unique method of assessing scan quality. Most use a combination of intensity level as well as uniformity within a scan. Scans should be evaluated by the operator during the scanning session, to ensure that scans meet pre-determined requirements for alignment, signal strength and absence of areas of signal drop out.

_Segmentation:_ Recognition and segmentation of retinal layers is an ongoing issue in OCT image analysis and segmentation errors can have a huge impact on thickness “calculations”. Each instrument comes with a proprietary segmentation algorithm that delineates retinal layers in a slightly different way and uses different layers to calculate thickness values. Thus, thickness values generated by the software cannot often be compared between different instruments. Manual segmentation, although more subjective and laborious, can be carried out using freely available image analysis software. Unfortunately, significant inter-observer variability generally mandates evaluation of scans by a single, consistent observer.

_Clinical applications of OCT:_
The most common clinical applications for posterior segment OCT with companion animal species are to assist in the diagnosis and documentation of treatment effects in research studies, or assessment of pathology or progression of spontaneous or genetic retinal disease, as well as glaucoma. Qualitative evaluation of ocular lesions may be valuable and all that is needed in a clinical setting. For example retinal scars can be distinguished from retinal folds or dysplasia. For all applications, scan quality is paramount and should be assessed first. Scans should be relatively flat (not tilted). Assess the location of the scan: e.g. if evaluating the optic nerve, or inner retina (retinal nerve fiber layer thickness) scans need to centered on the optic nerve head; retinal layers may be thinner or less defined in the peripheral versus the central retina. Ideally, scans should be available from the same location in approximately age matched normal subjects of the same species and breed for comparison. Compare the number of reflective “layers” that can be seen in the retina, and the relative organization and thickness of layers and tissues (using manual caliper functions if necessary). Do not assume that automated software measurements are accurate and always consider OCT results in conjunction with other clinical findings!

_Confocal laser scanning ophthalmoscopy:_
First described in the 1980s, cLSO provides detailed, high resolution images of the fundus. The laser is projected into the eye as a narrow beam, typically occupying just 1 mm or less of the pupil. As this leaves a large area of the pupil available for the reflected light from the fundus to pass through, imaging low light intensities is possible. Capturing images through a confocal aperture, essentially a “pinhole effect”, greatly enhances contrast. Such a narrow illumination beam can also be positioned to minimize the detrimental, light-scattering effect of opacities in the ocular media. The laser is typically directed in a raster pattern
(i.e. an x-y matrix of parallel lines), providing 2D images though some specialized instruments (e.g. Heidelberg Retinal Tomograph) provides a 3D “z-stack” of parallel images. By varying the wavelength of the laser used, imaging can be carried out at those wavelengths that give most information on structures of interest, for example, by using an infrared laser to image structures deep in the fundus.

Various modifications of cLSO technology have led to instruments (some combining multiple imaging modalities) that can provide complementary information about structure or physiologic properties of the fundus. When cLSO lasers of specific wavelengths (e.g. blue to excite fluorescein) are combined with barrier filters, it becomes possible to document fundus auto-fluorescence in vivo (e.g. lipofuscin within the RPE cells) and visualize fluorescent labeled cells in the retina in vivo; and angiographic studies are enhanced. This modality may be incorporated in commercially available fundus imaging platforms, e.g. the Heidelberg Spectralis HRA + OCT.

**Adaptive Optics**

Adaptive optics systems, first applied to retinal imaging in the late 1990s, provide amazing highest resolution images of fundus structures. These systems use wavefront sensing techniques, similar to that used in astronomy, to measure optical aberrations of the eye tissues. These aberrations can then be corrected using a complex system of lenses and deformable mirrors. With <2µm lateral resolution, these systems make it possible to visualize the living photoreceptor “mosaic”, nerve fibers and flow of blood cells within retinal capillaries. High resolution systems are currently restricted to research applications, are sensitive to movement artifact and are limited by narrow field of view. In the future, expect adaptive optics systems to become available as an “upgrade” to other modalities, like OCT. The future of ophthalmic imaging is bright!

**Keywords:** OCT, Confocal Laser Scanning Ophthalmoscopy, Adaptive Optics, Retina

**References**

The Eye and Systemic Diseases. What We Can Learn from the Eye

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Summary
The ocular examination and ocular abnormalities are able to provide significant information as related to systemic disease. It may be that the patient presents for an ophthalmic abnormality which is in fact a symptom of a larger, systemic disease. Alternately, it may be that the ophthalmic examination assists in the diagnosis of a patient presenting with systemic abnormalities. It must be understood that the eye is the only organ that allows the clinician to evaluate arteries, veins and the central nervous system by direct visualization in a live, awake animal. It should also be understood that the eye has the largest blood flow by volume of any organ in the body making it both susceptible to blood-borne diseases and also a wealth of systemic information. Finally the eye is innervated by 6 different cranial nerves as well as the sympathetic and parasympathetic systems all of which can be affected by numerous systemic diseases.

Ocular involvement in systemic disease may include all aspects of the eye anterior from eyelid to posterior and the retina/optic nerve (Table 1). Clinical signs many vary to include neurologic, inflammatory, cataract, blindness, dry eye, hemorrhagic and a variety of other symptoms. It may be that the patient presents for an ophthalmic abnormality which is in fact a symptom of a larger, systemic disease. Alternately, it may be that the ophthalmic examination assists in the diagnosis of a patient presenting with systemic abnormalities. Many systemic diseases such an endocrinopathies, vascular disorders, or hematogenously disseminated infectious/neoplastic diseases to name just a few are commonly associated with ocular abnormalities. It must be understood that the eye is the only organ that allows the clinician to evaluate arteries, veins and the central nervous system by direct visualization in a live, awake animal. It should also be understood that the eye has the largest blood flow by volume of any organ in the body making it both susceptible to blood-borne diseases and also a wealth of systemic information. Finally the eye is innervated by 6 different cranial nerves as well as the sympathetic and parasympathetic systems all of which can be affected by numerous systemic diseases. Some of the most common ocular manifestation of systemic disease include uveitis, retinal detachment, cataract and blindness.

Uveitis
The clinical signs of anterior uveitis include miosis, aqueous flare, hypotony, keratic precipitates, redness, photophobia and deep corneal vascularization while posterior uveitis may result in retinal detachment and blindness when severe. When diagnosed, it is essential to then decide if the uveitis is from a primary ocular etiology or an ocular manifestation of a systemic disease. There are 4 primary ocular reasons for anterior uveitis. They are corneal ulceration, cataract with lens-induced uveitis, ocular trauma or a primary intraocular neoplasia. If one of these is not the etiology then the clinician must consider a systemic etiology. Posterior uveitis is almost always a manifestation of a systemic disease. Systemic etiologies include idiopathic, immune-mediated,
metastatic neoplasia (lymphosarcoma most common) and numerous infectious causes. In general approximately 50% of anterior uveitis cases will fall into the idiopathic/immune group, 25% into the neoplastic group and 25% into the infectious group. The variability is in the infectious group where the type and likelihood of an infectious etiology will vary by geographic location and time of the year. In addition the infectious etiologies differ between dog and cat. In the canine – tick associated disease (erlichia, RMSF, Lyme), mycotic infections, prothecosis, bacteremia and septicemias are most common while in the cat FeLV, FIV, Toxoplasmosis, FIP, cryptococcosis and Bartonella are most common. Keep in mind, in the cat these are not mutually exclusive diseases and it is common for a cat to be infected with multiple etiologies.

When presented with a case of anterior uveitis of a non-ocular etiology the clinician must obtain a detailed history, perform a complete physical examination and consider blood work for a complete blood count, serum chemistry and serologic testing. In addition a urinalysis, chest radiographs, abdominal ultrasound, fine-needle aspirate, cytology and histology may also be indicated.

**Cataract**
First cataract must be differentiated from the normal ageing change, lenticular (nuclear) sclerosis. Sclerosis occurs in all animals at middle age and is seen first in the dogs and cat at approximately 6 years of age. It is a change in central density and does not prevent a fundic examination or vision.

Cataract is defined as any opacity of the lens or its capsule. Cataracts are then divided by severity (incipient, immature, mature and hypermature), by location (capsular, cortical-anterior, posterior, equatorial, nuclear), by etiology (inherited, metabolic, traumatic, inflammatory, electric, nutritional, radiation, toxic) and by age of onset (congenital, juvenile, adult, senile). The most common systemic reasons for cataracts in dogs is diabetes mellitus and in dogs and cats, anterior uveitis. It is important to know that all dogs with diabetes mellitus will get cataracts with >60% cataractous within a year of onset of diabetes. Of those dogs requiring surgery, diabetes may account for 30-50% of surgical cataracts.

**Retina**
It is essential to realize that a fundic examination is the only location that allows the clinician to evaluate arteries, veins and the central nervous system by direct visualization in a live, awake animal. There is a tremendous blood flow to the posterior segment making it susceptible to blood-borne pathogens, metastatic neoplasia, hypertension, vasculitis, vascular disorders and hyperviscosity to name just a few diseases. There are also systemic immune-mediated diseases that have ocular manifestations, such as uveo-dermatologic syndrome. Since the retina and optic nerve are in fact the anterior most extension of the central nervous system (CNS) and the CSF extends up to the optic nerve head, they eye may be involved in or assist in the diagnosis of CNS diseases. Posterior segment abnormalities associated with systemic diseases may include chorioretinitis, retinal detachment, vasculitis, optic neuritis, blindness and others.

**Keywords:** Ocular Manifestations of Systemic Disease, Chorioretinitis, Optic Neuritis, Anterior Uveitis, Diabetes Mellitus, Cataract, Blindness, Retinal Detachment, Hypertension, Hyperviscosity, Neoplasia

**References**
Table 1 Presenting Ophthalmic Clinical Signs and Potential Associated Systemic Diseases

<table>
<thead>
<tr>
<th>Ophthalmic Clinical Sign</th>
<th>Associated Systemic Disease</th>
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<tbody>
<tr>
<td>Keratitis Sicca</td>
<td>Hypothyroidism, Diabetes mellitus, Autoimmune</td>
</tr>
<tr>
<td></td>
<td>Drug-induced – sulfonamides, Facial nerve paralysis, Otitis media/interna, Canine distemper</td>
</tr>
<tr>
<td></td>
<td>Infectious: bacterial, rickettsial, mycotic, viral, protozoal</td>
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<tr>
<td></td>
<td>Traumatic</td>
</tr>
<tr>
<td></td>
<td>Neoplastic – primary vs metastatic, Autoimmune</td>
</tr>
<tr>
<td>Anterior Uveitis/Chorioretinitis</td>
<td>Inflammatory - see Anterior Uveitis, Neoplasia – primary vs metastatic, Autoimmune</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>Trauma, Diabetes mellitus, Nutritional</td>
</tr>
<tr>
<td></td>
<td>Radiation, Inflammatory - see Anterior Uveitis, Electrical shock, Toxic/drug (ketoconazole)</td>
</tr>
<tr>
<td>Cataract</td>
<td>Trauma, Infectious - bacterial, rickettsial, mycotic, viral, protozoal</td>
</tr>
<tr>
<td></td>
<td>Neoplasia – primary vs metastatic, Chorioretinitis – see above, Autoimmune, Hypertension</td>
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<td></td>
<td>Hyperviscosity</td>
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<tr>
<td>Retinal Detachment</td>
<td>Trauma, Infectious - bacterial, rickettsial, mycotic, viral, protozoal</td>
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<tr>
<td></td>
<td>Neoplasia – primary vs metastatic, Autoimmune, Hypertension</td>
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<tr>
<td></td>
<td>Hyperviscosity</td>
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<tr>
<td>Blindness</td>
<td>Trauma, Neoplasia, Retinal detachment – see above, Reticulosis/CNS disease, Autoimmune,</td>
</tr>
<tr>
<td></td>
<td>Toxic/drug (enrofloxacin in cats)</td>
</tr>
<tr>
<td>Hyphema</td>
<td>Thoracic mass, Diabetes mellitus, Hypothyroidism</td>
</tr>
<tr>
<td></td>
<td>Spinal cord lesion C1-T2, Neck trauma, Otitis media/interna, Orbital mass lesion</td>
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<td></td>
<td>Coagulopathy</td>
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<tr>
<td></td>
<td>Trauma, Hypertension - renal, pheochromocytoma, hyperthyroidism, idiopathic</td>
</tr>
<tr>
<td>Hyphema</td>
<td>Infectious – see anterior uveitis, Neoplasia – primary vs metastatic</td>
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A Basic Toolkit for the Diagnosis and Treatment of Equine Eye Disease

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Summary

Only patience and a limited amount of “specialist” equipment are required to perform an adequate examination of the equine eye. The objective of this presentation is to provide an understanding of how to effectively conduct a thorough evaluation of the eye equine eye, even in an ambulatory setting, using limited, affordable and portable equipment. A basic “toolkit” for equine eye examination should include: a bright focal light source; a magnifying lens, and a direct ophthalmoscope which has a slit beam; blue and green (“red” free) filters. In addition to fluorescein strips, topical anesthetic, tropicamide and sterile saline or eye-wash that are commonly used in examination of small animal patients, the following supplies are frequently needed when assessing horses: syringes, needles, appropriate sedation and injectable local anesthetic e.g. 1% lidocaine for nerve blocks. Supplies for microbial culture, cytology and biopsy should also be accessible. In contrast to the small gauge lacrimal cannulae used dogs, a 6-10 FG urinary catheter is typically used to cannulate the distal nasolacrimal duct of horses. Although not always available, a hand-held tonometer is helpful to have on hand if glaucoma is suspected. Additional supplies used in the management of equine ophthalmic patients include commercially available subpalpebral lavage systems that can be placed in standing horses at the time of initial sedated eye exam, to facilitate frequent medication of painful eyes. Often overlooked, but perhaps most important of all, is the need for a quiet, darkened area in which to conduct the exam.

Eye Exam “Toolkit”:

Important Equipment includes: a bright focal light source (eg. Focusing pen-light; transilluminator); a magnifying lens (eg. otoscope, or preferably 20D condensing lens), and a direct ophthalmoscope (with functioning, charged batteries!). The PanOptic ophthalmoscope (Welch Allyn) is also worthy of consideration as it offers a compromise between wider field of view of indirect ophthalmoscopy and magnification and relatively short working distance of a direct ophthalmoscope. Many direct ophthalmoscopes have a slit beam function - shine into the eye from an angle and observed from the front, this beam is a useful “substitute” for a slit-lamp. Your smart phone (with micropore tape to decrease flash intensity; or e.g. “magnifying glass with light” i-phone app.) provides a digital camera to document findings. Most importantly - a darkened stall/building in which to conduct the exam will limit troublesome external reflections that can obscure ocular lesions!

Important Consumable Supplies include: fluorescein strips; eye wash or sterile saline for rinsing / flushing; and mydriatic (1% tropicamide which takes 30 mins and lasts 8-12 hrs, NOT atropine- which can last for >10 days!). Topical anesthetic (e.g. 0.5% tetracaine or proparacaine) is generally effective in less than 1 minute and, in practice, lasts for about 10 minutes but timeframe is variable (if in doubt, apply again). Topical anesthetic solutions should be stored in the refrigerator when not in use and should be discarded
if the solution appears brown or turbid. In addition to local anesthetic (eg. 2% mepivacaine; 1% lidocaine) for nerve blocks/local infiltration, 1-2ml syringes with 25g needles for instilling topical drugs and 3-5ml syringes and 5/8", 23g needles for nerve blocks should be included in supplies for equine eye exam. For clinical pathology, culturettes (calcium alginate with transport medium) for microbiology; sterile scalpel blades (an economical alternative to the Kimura spatula for obtaining scrape samples for cytology) and clean glass microscope slides with plastic slide holders to transport to the clinic for Diff-Quik staining and microscopy. Additional supplies include sterile cotton swabs, gauze, sterile gloves, dilute betadine solution NOT scrub (1:20-1:50 dilution in sterile saline), serum tubes, small jars of 10% formalin for biopsies and supplies for nasolacrimal duct cannulation, e.g. 6-10 FG urinary catheters and lidocaine gel.

Sedatives and analgesics: Sedation facilitates equine ophthalmic examination and should be considered for safety of examiner, patient and handlers when examining fractious animals. Options of either xylazine, or detomidine with butorphanol (latter best choice if eye is painful) generally work well at our institution.

Other equipment that is useful but not essential for routine exam in practice includes: A headband light-source that directs bright illumination to the field of interest for minor diagnostic sampling and surgical procedures and economical headband loupes (e.g. Optivisor) that can provide hands-free magnification for foreign body removal or minor surgical procedures, and for which an LED attachment can be purchased. A tonometer will be necessary for diagnosis of glaucoma but is expensive. Commonly used tonometers include the Tono-pen applanation tonometer (Avia, Vet or XL models) and TonoVet (rebound tonometer). The TonoVet has the advantage of not requiring topical anesthetic. For the enthusiast, a slit lamp biomicroscope, will revolutionize your exam of the anterior segment and lens and enable very tiny foreign bodies to be located but is also an expensive tool, with a steep learning curve.

Important points to note:
In horses, the Pupillary light reflex is slow and incomplete (compared with cats and dogs)
Vision may be difficult to assess (assess ability to negotiate obstacles/ slopes)
Mydriatics have a prolonged effect in the equine eye (e.g. atropine lasts days-weeks!)
Bear in mind that the equine optic nerve exits the globe ventrally – in order to examine the optic nerve head, one usually has to look down into the eye. (Short examiners may need to stand on a box to achieve this in a large patient, although sedation facilitates the examination by providing a lower head position).
Equine eyelid muscles are powerful. Thus, when examining a painful eye a palpebral (motor) nerve block may also be required to relieve blepharospasm. A palpebral nerve block is essential if there is a possibility of globe rupture / penetrating injury or severe ulceration!

Auriculo-palpebral and Palpebral nerve blocks (A, B):- the palpebral nerve (a branch of the facial nerve) is generally palpable as it crosses the bone just anterior to the highest point of the zygomatic arch. Inject 2-3ml of local anesthetic (e.g. lidocaine) subcutaneously at this site (B). A successful block leads to drooping of the upper lid within a few minutes. Remember that this is a motor nerve block, analgesia will still be required. You will still need topical and/or local anesthesia!
Supra-orbital nerve block (C ) :- the supraorbital or frontal nerve is a branch of the trigeminal nerve which is responsible for sensory innervation of the middle 2/3 of the upper lid. Insert needle approx. ½" into the supraorbital foramen (palpable on dorsal aspect of bony orbital rim) and inject 1-2ml through the foramen then 2-3ml as the needle is withdrawn.

Line Blocks: Additional sensory blocks include the lacrimal nerve
Exam protocol:

- **Obtain history** of performance, onset, duration and rate of progression of problem, prior therapy, including tetanus prophylaxis?
- **Observe** globe symmetry, eyelid / eyelash position, discharges, blepharospasm
- **Palpate** globes (assuming globe integrity is not suspect), orbital bones and supra-orbital fossae.
- Assessment of reflexes and menace responses. Palpebral and pupillary light reflexes (direct and indirect) should be evaluated and/or the latter it can help to have an assistant. Dazzle reflexes. (*Note that pupil size and menace response can be affected by sedation, so do these before sedating if possible*)
  - Sedate +/- nerve block +/- apply topical anesthetic if necessary (fractious, or painful)
- **Evaluate** adnexa: examine eyelids for masses, irregularity, obvious trauma, ulceration, conformation; evaluate third eyelid surface appearance, position, motility, regularity and assess conjunctiva to determine if it is thickened, irregular, or injected. Use bright illumination and magnification
- **Examine Cornea** for opacities, irregularity, neovascularization (and fluorescein uptake) see below.
  - **Examine anterior chamber and iris** using smallest circular light beam on ophthalmoscope to look for “aqueous flare” and slit-beam setting to assess contours of surfaces (iris, cornea, lens capsule). Assess iris color, surface contour, presence and appearance of corpora nigra, pupil size and margins.
  - Dilate pupils (tropicamide) - if suspect lesions of lens, vitreous or fundus.
- **Assess lens and vitreous for opacities** by looking through direct ophthalmoscope (set on zero) from a distance
- **Evaluate fundus** by direct ophthalmoscopy or PanOptic examination. To help highlight the small blood vessels that surround the optic disc, try using a green (“red-free”) filter on your ophthalmoscope. Although it is more challenging and typically requires pupil dilation, indirect ophthalmoscopy (light source, hand-held lens) is strongly recommended.

Ancillary diagnostic tests & their indications:

- **Schirmer tear test** – indicated if ocular surface appears dull and dry. Note that horses with ocular surface disease usually exhibit profuse increased tearing, and dry eye is relatively uncommon.
- **Fluorescein Stain**: corneal abrasions and ulcers are so common in horses that fluorescein staining should be done as a matter of course in all horses that present with eye problems. Do this AFTER the anterior chamber and iris have been evaluated.
- **Swabs for microbial culture /sensitivity & scrapings for corneal cytology** should be obtained (see below) for progressive corneal ulcers and ulcers or abscesses with yellow-white infiltrate
- **Nasolacrimal flush** – if chronic ocular discharge, conjunctivitis
- **Biopsy of conjunctival, third eyelid or eyelid lesions** (standing sedation / topical anesthesia is generally sufficient). Note that cytology is unrewarding when squamous cell carcinoma is suspected and histomorphological diagnosis is necessary.
- **Tonometry** is not necessary for every case but suspect glaucoma (corneal ‘striae’ or diffuse edema). Refer to a specialist if possible! IOP can fluctuate widely in horses with glaucoma (so a single “normal” IOP doesn’t exclude glaucoma) and be affected by eyelid closure, external pressure on globe, sedation and head position.
- **Evaluation of visual performance** – e.g. obstacles, ridden (caution!)
- **Ocular Ultrasonography** can be informative if can’t see back of eye or suspect orbital disease. For horses, 7.5-12.5MHz transducer affords adequate depth of penetration and acceptable resolution.

**Cytology & Culture**

Where microbial keratitis is suspected, corneal scrapes should be obtained and submitted to cytological examination after application of topical anesthetic to the eye. Swabs for bacterial and fungal culture and susceptibility testing can be obtained after topical anesthetic if the eye is painful (if bacteria are the problem,
they'll still grow!). A corneal scraping can be obtained using a Kimura spatula, or the blunt, handle-end of a scalpel blade and transferred to clean microscope slides. Even simple “Diff-Quik” staining of smears can provide useful information, however Gram-staining will facilitate characterization of microbial organisms.

**Topical medication of the equine ophthalmic patient.**
Particularly where frequent application of topical medication to a painful eye is necessary, placement of sub-palpebral lavage (SPL) system (or, less desirable, an indwelling nasolacrimal catheter) is highly recommended.
A commercially available, single-entry SPL system produced by MILA International, Inc. is a good, widely used option (www.milainternational.com). If placed inferio-medially, through the lower eyelid, risk of corneal abrasion is substantially reduced. This form of SPL system is relatively easy to install in standing, sedated horses.

**Useful References:**

**Keywords:** Equine, Ophthalmology, Examination, Eye, Practical Techniques
Advances in Equine Intraocular Disease

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Summary
With respect to equine intraocular disease, the immune-mediated syndrome of Equine Recurrent Uveitis is the most common and potential blinding disease with reported prevalence of 10-25%. With the availability of specific T-helper suppressing medications and surgical implantation of sustained-release delivery devices, the treatment of this disease has been revolutionized. Additional intraocular diseases such as cataracts, glaucoma and corpora nigra cysts have seen significant improvements in surgical techniques and outcomes. Finally, our understanding of several equine intraocular diseases, including those of the posterior segment continue to advance.

Uvea
Cysts of the anterior uvea have been reported in the horse. They are usually heavily pigmented and may not transilluminate making a uveal melanoma the most likely differential. Ocular ultrasonography will reveal these as cystic structures and confirm the diagnosis. Treatment is not required unless the cysts are numerous or visually impairing. The cyst can be collapsed and/or aspirated by paracentesis or if available, disrupted using laser energy delivered transcorneal which is the preferred treatment of choice (1).

Uveitis is inflammation of the iris and ciliary body (anterior) and choroid (posterior). Uveitis is a common manifestation of many infectious and noninfectious diseases, both ocular and systemic, and as such it is essential to attempt to ascertain the etiology. It is also essential to be able to differentiate uveitis from other ophthalmic diseases resulting in a red and painful eye such as glaucoma, corneal ulceration, and conjunctivitis. Uveitis is reported as the leading cause of blindness in horses throughout the world with equine recurrent uveitis (ERU) (moon blindness, periodic ophthalmia) the most common etiology. The Appaloosa has been shown to be a predisposed breed for ERU with one report suggesting an 8-fold increase in risk in the Appaloosa as compared with other breeds. In addition, ERU affected Appaloosa’s are 4-times more likely to loose vision than ERU affected non-Appaloosa’s. ERU is a T-cell mediated immune-mediated disease with numerous etiologies proposed as initiating or exacerbating factors. The clinical signs of ERU are similar to other causes of uveitis with the distinguishing feature being its recurrent nature. The frequency and severity of each recurrence is extremely variable. It is believed that even in times of clinical quiescence inflammation continues at a sub-clinical level resulting in further intraocular damage. Diagnosis is based on history and clinical examination. Although serologic evaluation for *Leptospira* spp., *Brucella* spp., or Toxoplasma, and conjunctival biopsies to evaluate for the presence of *Onchocerca cervicalis* have been advocated, it is rare that the results of these tests will alter individual therapy. This is in part due to the chronic nature of the disease and that the inciting cause may have begun years prior to development of uveitis and clinical presentation may definitive diagnosis difficult. It has been demonstrated that horses seropositive for *Leptospira interrogans* serovar *pomona* are 13-times more likely to develop ERU than
non-seropositive horses and that seropositive horses are 4.5-times more likely to have ERU result in blindness than non-seropositive horses. Treatment of an individual horse is directed towards suppression of inflammation, control of pain, and prevention of sequelae. In situations where the prevalence of ERU in a barn exceeds that normally expected then serologic testing and examination to attempt to determine an etiology is indicated. Non-specific therapy includes topical and systemic corticosteroids and non-steroidal antiinflammatory drugs to decrease inflammation and atropine to dilate the pupil and decrease the pain of ciliary muscle spasm. Which medication to select, the frequency of treatment, and route of administration are dependent on the etiology and severity of the uveitis and whether the uveitis is anterior, posterior, or both. A sustained-release, suprachoroidal cyclosporin A implant has been shown to preserve vision in 85% of horses over a 5-yr period (2). In addition, others advocate performing a complete vitrectomy for affected horses with the claim of a decrease in severity and frequency following surgery (1). Failure to control intraocular inflammation may lead to severe secondary ophthalmic complications such as glaucoma, synechiae, cataract, retinal detachment, phthisis bulbi, and blindness (1).

Glaucoma, an elevation of intraocular pressure (IOP) to a level incompatible with the health of the eye, is rare in the horse. The reported normal intraocular pressure in the horse (20-30 mmHg) varies according to the technique used and whether sedation is employed, with sedation resulting in a decrease in IOP. An auriculopalpebral nerve block may be performed prior to determination of IOP in the horse. Applanation and indentation tonometers are best suited for determination of equine IOP. As the use of such devices in practice increases, the true incidence of equine glaucoma will become apparent. Although congenital glaucoma has been reported in the horse, secondary glaucoma resulting from anterior uveitis, especially ERU, is the most common cause of equine glaucoma. The clinical signs of glaucoma in the horse are often more subtle than in other species. Unfortunately most glaucomatous horse eyes are presented late in the disease. Medical management using 0.5% timolol maleate, 2% dorzolamide or the combination of timolol and dorzolamide is the topical treatment of choice in equine glaucoma. Surgery for the equine glaucoma patient includes transcleral cyclophotocoagulation (TSCP) using a diode laser with a published success of 80% at 1 year (1,3).

**Lens**

A cataract is any opacity, regardless of any size, involving the lens or its capsule. Although many etiologies for cataract exist in other species, most have not been documented in the horse. Potential causes of cataract formation include inherited, inflammatory, traumatic, metabolic, toxic, and nutritional abnormalities. Suspected inherited cataracts have been reported in several breeds of horses (Belgian, Morgan) and are thought to occur in other breeds, but are difficult to prove due to the small number of offspring produced. It is important to remember that inherited cataracts are not necessarily congenital, nor are all congenital cataracts inherited. In general, if an etiology for a cataract cannot be determined, and there is no evidence of intraocular inflammation breeding of this animal should be discouraged. The most common etiology for cataract formation in the horse is ERU. Treatment of cataracts, if required, is surgical. The decision on whether to treat depends on the severity and cause of the cataract and the presence or absence of concomitant ocular disease. Cataracts that are unilateral, or not severe enough to significantly interfere with vision generally do not require treatment. Cataracts resulting from intraocular inflammation, such as those seen in association with ERU are often not amenable to surgery. Treatment of cataracts seen in association with abnormalities of the retina, such as retinal detachment or degeneration, is not indicated. If the cataracts are bilateral, interfering with vision, and not the result of ERU or other intraocular inflammation then surgical removal of the lens can be performed. Prior to cataract surgery, evaluation of retinal anatomy and function should be performed using ocular ultrasound and electroretinography. Foals with congenital cataracts should be operated early (<6 months) to avoid possible deprivation amblyopia. Surgery is best performed using the technique of phacoemulsification. As artificial lens implants are now available for horses the postoperative hyperopia can be avoided (1,4,5).
**Retina**

Equine congenital stationary night blindness (CSNB) is a bilateral, congenital, non-progressive retinal disease seen in Appaloosa horses. The degree of visual disturbance varies between horses with mildly affected horses exhibiting signs only in dark conditions, while severely affected horses are totally blind in the dark, exhibit apprehension in daylight, and may have a bilateral dorsomedial strabismus and nystagmus. The diagnosis is suspected on history, breed of horse, clinical signs, and maze testing in an illuminated and darkened environment, but must be confirmed by electroretinogram as fundic examination in affected horses is normal. Electroretinography demonstrates an almost purely negative wave form in the scotopic (dark-adapted) response, characteristic of ENB. The results of the electroretinogram indicate an abnormality in signal transmission from the photoreceptor cells to the inner retina. CSNB in the Appaloosa is associated with the leopard complex gene (1,6,7).

Equine Motor Neuron Disease (EMND) is a neurodegenerative condition resulting form a deficiency of vitamin E. Some affected horses accumulate ceroid-lipofuscin in the retinal pigment epithelium which appears as irregular, often linear pigment changes on fundic examination. The fundic changes are supportive of a diagnosis of EMND, but should be combined with musculoskeletal and other systemic changes to confirm the diagnosis.

**Keywords:** Equine, Intraocular, Equine Recurrent Uveitis, ERU, Cyclosporin, Sustained-Release, Cataract, Glaucoma

**References**

Diagnosis of Early Cranial Cruciate Ligament Disease

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Summary
Rupture of cranial cruciate ligament (CCL) is a common problem in dogs. This condition can cause deterioration in limb function and progression of osteoarthritis (OA). Its significance in veterinary medicine has been well documented in the literature. Despite being the most common cause of lameness in dogs, diagnosis of this condition can be challenging, particularly in its early phase. Manual examinations to elicit cranial translation of tibia relative to femur (“drawer” and “tibial thrust” tests) have been traditionally used to detect CCL rupture. However, only 50 to 70% of dogs with CCL rupture may show positive drawer or tibial thrust signs. Therefore, diagnosis of early CCL rupture should not rely solely on these tests and may require comprehensive approach based on multiple layers of evidence.

Previous studies have uniformly stated that dogs of any breed, size, or age may be affected by a CCL rupture, although this condition is particularly common in large and giant breed dogs. Pathology of CCL disease appears to involve a gradual degeneration of CCL itself, inflammatory disease in the stifle joint, partial rupture, progressive rupturing, complete rupture, and secondary disease such as progressive osteoarthritis and meniscal injury. Partial ruptures can occur at any part of the CCL.

Dogs with early CCL disease (i.e. minor partial CCL rupture) may have little or no palpable instability, but they are often presented with lameness, effusion of the stifle joint, and mild osteoarthritis. Major partial or complete CCL ruptures produce marked instability of the stifle joint, resulting in lameness and progressive degenerative changes within the joint. Clinical observations have demonstrated that these changes consist of periarticular osteophyte formation, capsular thickening, and meniscal degeneration. As these changes progress, the joints become less unstable. Advanced or end-stage CCL disease may have little palpable stifle instability because of the periarticular fibrosis.

Gait Observation
Lameness of dogs with CCL rupture varies widely depending on many factors. In general, dogs with early CCL rupture show mild to moderate weight bearing lameness, which may improve rest or medication. There is no specific sign of gait abnormality with early CCL disease.

Sit Test
Dogs with CCL rupture often dislike full flexion of the affected stifle when they sit, and tend to throw the leg to their side. This finding can be called as “positive sit test”, and indicates stifle pathology and discomfort. The most common cause for this is CCL disease.

Orthopaedic Palpation
Stifle effusion is often present in dogs with early CCL disease, which can be palpated when dogs are
standing and bearing weight on both hind limbs. In normal dogs, patellar tendon should be distinct and easily identifiable with palpation. In dogs with stifle effusion, medial and lateral edges of patellar tendon may become indistinct, and swelling on both sides of patellar tendon may be palpable. These findings are suggestive of stifle effusion from early CCL disease, which should be confirmed with radiographic examination, as discussed later. Mild discomfort may be elicited on palpation of stifle joint space, particularly medial to patellar tendon. Range of motion of the affected stifle can be decreased and pain may be elicited with full extension of the affected stifle.

Orthopaedic Manipulation
As mentioned above, cranial drawer motion and cranial tibial thrust may not be present in dogs with early CCL disease. Sedation may help to facilitate the detection of mild cranial drawer motion and cranial tibial thrust.

Radiographic Examination
Lateral radiography of stifle is sensitive to detect stifle effusion, and very useful to diagnose early CCL disease. However, the presence of stifle effusion is not specific to CCL disease, therefore other joint diseases such as immune-mediated polyarthritis (IMPA), stifle OCD, patellar luxation, neoplastic disease such as synovial cell sarcoma, and septic arthritis need to be ruled out. Early CCL disease may not have radiographic signs of osteoarthrosis.

CT/MRI/Ultrasound
Advanced imaging modalities have been used to detect early CCL disease. Currently, clinical usefulness of these modalities has not been shown.

Joint Fluid Analysis
Synovial fluid analysis in dogs with CCL rupture generally is non-specific and not useful, except to rule-out IMPA, septic arthritis, and neoplasia. A number of studies have investigated osteoarthrosis parameters associated with CCL rupture; however, currently there is no specific marker for CCL rupture.

Arthroscopy
Arthroscopy is an effective procedure to document minor partial CCL rupture and to detect early CCL disease. Advantages include 1) direct and dynamic observation and palpation of partial rupture, 2) magnified and clear view of the lesion, and 3) relatively minimally invasive procedure. Disadvantages include 1) cost of set-up, 2) difficulty to perform high quality arthroscopy, and 3) requirement of general anesthesia and invasive surgical procedure. If arthroscopy is not available, exploratory arthrotomy may be performed; however minor rupture can be difficult to detect.

Management of Early CCL Rupture
Early CCL disease with minor partial rupture usually progresses to complete rupture over time. Early surgical intervention is generally recommended to improve limb function and to slow down progression of arthritic changes. Conservative management with rest and medication may result in temporary improvement of clinical signs; however, it almost always ends up in eventual decline of limb function. Therefore, conservative management of early CCL rupture is recommended only for geriatric or systemically ill patient that cannot undergo general anaesthesia and surgery.

Keywords: Dog, Stifle, Ligament
Patellar Luxation in Young Dogs

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Summary
Medial patellar luxation (MPL) is a common cause of lameness in small and toy breed dogs. A survey performed in Japan, where MPL repair is the most commonly performed canine orthopedic surgery, revealed that approximately 50% of popular small breed dogs are affected by congenital form of MPL. The incidence was reported to be particularly high in Pomeranian, Toy Poodle, Yorkshire Terrier, Maltese, and Chihuahua.

Traditionally, grading schemes (grades 1-4) based on physical findings and clinical signs have been used to classify MPL, and treatment options have been well described for each grade of MPL. However, management of dogs with severe skeletal deformities is often unsuccessful, with very few dogs regaining normal limb function even with aggressive surgical corrections. In addition, MPL is usually treated after growth phase, and little has been discussed on appropriate age of surgical intervention for varying severity of MPL. Therefore, a novel approach is necessary to improve the outcome of treatment of severe MPL. After review of clinical observation in more than 4,000 cases, a new treatment guideline has been proposed. This guideline emphasizes on early identification of severe MPL within 1-4 weeks of birth and early surgical correction of pathogenesis of patellar imbalance and instability in 2-3 months of age. Although this proposal has not been critically examined and no studies have been published in English literature, clinical results have been promising.

Pathogenesis
The etiology and pathogenesis of MPL are not well identified in veterinary literature, although genetic factors appear to be most important in the congenital MPL. Patella is a sesamoid bone embedded in the tendon of the quadriceps muscle, facilitating stifle extension. The extensor mechanism of the stifle is composed of the quadriceps muscle groups, patella, trochlear groove, patellar tendon, and tibial tuberosity. The quadriceps muscle group is formed by rectus femoris, vastus lateralis, vastus intermedius, and vastus medialis. Among them, vastus medialis and vastus lateralis are fixed to patella by the medial and lateral parapatellar fibrocartilages, thus potentially controlling medial and lateral movement and stability of the patella. In severe MPL cases, grossly evident atrophy and fibrosis of vastus medialis (tight band-like appearance) have been observed in fetus and new born puppies. As puppies grow, medial displacement of patella and quadriceps muscle group occurs, and underdevelopment of patella and absence of trochlear groove are seen. Based on these findings, it has been hypothesized that medial luxation of patella occurs because of abnormal tension in quadriceps muscles in the fetal stage, resulting in medial displacement of the quadriceps, and that this muscle imbalance causes deformation of the epiphyseal growth plate in the hind limb skeleton and hypoplasia of the stifle. Patellar displacement and consequent growth deformities may be secondary to muscle imbalance in the quadriceps muscle group, and therefore treatment should be aimed to address the muscular pathogenesis subsequently to prevent skeletal deformities.
**Proposed Classification**

Clinical observation that “mild/occasional MPL” does not generally progress to “severe/permanent MPL” suggests that severity of MPL may be determined in the early phase. Mild/occasional types of MPL mostly become clinical in the late stage of growth, with near normal skeletal formation, with intermittent lameness characterized by carrying affected leg and spontaneous return to the normal gait. In severe/permanent MPL, ectopic patella may be identifiable at the birth, with skeletal deformities that progress dramatically during early stage of growth (4-6 months). This classification may have a clinical importance because each class seems to require different treatment strategy.

Proposed Treatment Guideline for Mild/occasional Luxation (usually Grade 2):

As conventionally described, the major goals of surgical treatment of MPL are to correct alignment of extensor unit (such as tibial tuberosity transposition and medial release) and to increase patellar stability in the trochlear groove (such as groove deepening and lateral tightening). Surgery should be considered when frequency of lameness is increasing. Excellent stifle function can be restored if surgery is performed appropriately, however osteoarthritis may develop due to cartilaginous erosion and defect in grade 2 MPL.

Proposed Treatment Guideline for Severe/permanent Luxation (usually Grade 3-4):

New born puppies should be examined for MPL within 1-4 weeks, particularly in the commonly affected breeds. Puppies predisposed to severe/permanent MPL exhibit bowlegged appearance, with their toes touching each other or legs crossed when held in dorsal recumbency, whereas normal puppies flex and extend hind limb with their plantae pointing upwards. Passive range of motion exercise consisting of flexion and extension of the stifle should be initiated to strengthen quadriceps, to increase range of motion, and to potentially promote normal skeletal growth.

Around 4 weeks when they start walking on four legs, puppies with permanent MPL exhibit wobbly gait, with limited stifle extension and progressed skeletal deformities such as genu varum. In these cases, early surgical intervention is recommended at the age of 2-3 months. Major goal is to restore normal mechanical environment about the patella, before severe deformities occur and when puppies still have growth potential to adjust to the correction. Muscle realignment by correcting imbalance of muscle forces in the quadriceps is particularly important. At this age, deepening groove can be performed; however, tibial tuberosity should not be touched because disturbance of its growth plate can result in severe distal displacement of tibial tuberosity and subsequent multiple problems. In other words, tibial tuberosity transposition is not necessary if the puppy with severe MPL is operated early, at the age of 2-3 months. Placement of a positional screw into the femur just proximal to patella has been used successfully to keep the quadriceps from displacing medially and to maintain the quadriceps mechanism over the femur.

If the puppy with severe MPL is already 4-5 month old, tibial tuberosity transposition can be considered in small breeds, in addition to other procedures including muscle re-alignment and groove deepening, because the puppy may not grow significantly more, particularly in toy breed dogs. If the puppies with severe MPL are left untreated, skeletal deformities continue to progress and become unrepairable by 6 months. These dogs are not likely to restore normal limb function.

**Keywords:** Dog, Stifle, Patella
Developments in Total Hip Replacement

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Summary

Total hip replacement (THR) has been available as a treatment option for severely debilitating hip conditions in dogs. However, THR is not widely performed in veterinary practice, mainly due to its cost and potential risks. Multiple design modifications have been attempted recently to improve surgical outcome and to broaden its application in larger patient population (including cats).

Total hip replacement is a surgical procedure to replace a severely debilitated, painful and dysfunctional hip joint with artificial prostheses, in order to provide a pain-free, fully functional joint for an active lifestyle. This surgery is typically performed on a dog with severe hip dysplasia and osteoarthritis. THR can also be considered for a dog with hip fracture, luxation, and necrosis. Post-operative complications can occur (5-30%) and are most commonly due to luxation, fracture of the bone, infection, or loosening of the implants.

Treatment Options

Genetic control is the best strategy to manage canine hip dysplasia. Dogs with abnormal hips should not be bred. Treatment options can be divided to three major categories:

1. Conservative management
2. Preventive surgery
3. Salvage surgery

Selection of the appropriate treatment option is dependent on many factors such as: age, severity of hip dysplasia, development of osteoarthritis, degree of pain/discomfort, and owner’s expectation and financial constraint.

Many dogs with pain and lameness associated with hip dysplasia can be effectively managed with conservative methods (i.e., without surgical intervention). Conservative methods include weight management (extremely important), moderation of excessive exercise/activity, providing warm comfortable bedding, and the use of non-steroidal anti-inflammatory drugs, physical rehabilitation, and oral supplement/agents as needed.

Preventive surgical options can be considered for puppies with hip dysplasia; however clinical efficacy of these procedures remains controversial.

If the dog has severe hip dysplasia or has developed osteoarthritis, preventive surgery is no longer a surgical option. Salvage surgical options include total hip replacement (THR) and femoral head and neck ostectomy (FHO). Main differences between these procedures are:

1. Function after surgery
2. Cost
3. Post-operative care
4. Potential complication

FHO is the removal of the femoral head and neck. It is also called an excisional arthroplasty. This procedure eliminates the pain caused by the bone on bone contact that occurs in the arthritic hip joint when all the cartilage had been worn away. Scar tissue and muscle help to form a false joint, which minimizes the pain and discomfort felt by the dog when walking. Removing the femoral head and neck does slightly shorten the limb and the range-of-motion is not as good as with a total hip replacement; therefore, some gait abnormalities may persist. Nevertheless, it is a valuable procedure for improving the quality of life for many pets by the elimination of pain. This procedure may be performed bilaterally. Post-operative care involves aggressive physical therapy and activity is not restricted. Complications are rare.

**Total Hip Replacement**

There are several different types of total hip replacement available in veterinary market. They are largely classified as “cemented” and “cementless”. The choice of which technique is used is based on primarily the surgeon’s preference and experience, and the patient’s age and quality of bone. Due to concerns regarding cement-related complications (particularly infection and loosening), there is a recent trend to avoid the use of cement in total hip replacement.

Cementless systems utilize “press fit” or screws for their initial stability. Press fit application is simple but does require technical precision, and can result in fracture of femur and subsidence of the femoral implant (as much as 17%). Multiple modifications of surgical technique and implant design have been attempted to reduce these types of complications.

“Mini”, “Micro” and “Nano” total hip systems have been available for a few years now for small breed dogs and cats. Clinical efficacy remains to be evaluated.

**Keywords:** Dog, Hip, Osteoarthritis
Treatment for Complications of Medial Patellar Luxation

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Summary

Medial patellar luxation (MPL) is one of the most common orthopedic diseases causing hindlimb lameness in small breed dogs. Malalignment of the quadriceps mechanism may be the major cause of the condition. Therefore, appropriate realignment of the quadriceps mechanism is essential to have a successful surgical outcome for MPL.

Surgical correction of medial patellar luxation is based on realignment of the quadriceps mechanism and stabilization of the patella within the trochlear groove of the femur. There are many surgical techniques such as soft tissue and bone reconstructive procedure for stabilizing the femoropatellar joint. The most common surgical procedures, which are performed alone or in combination, are as follows: abrasion arthroplasty, trochleoplasty (block or wedge recession), tibial tuberosity transposition, corrective osteotomy, medial retinacular release, lateral retinacular imbrication and antirotational suture.

Postoperative complication rate have reported 13-48% including reluxation of the patella, infection, implant failure, avulsion of the tibial tuberosity, and degenerative joint disease. Reluxation of the patella is major complication after MPL surgery.

These complications arise when the wrong surgical procedure is applied, or when the appropriate realignment of femoral quadriceps femoris is corrected with soft tissue treatment alone such as antirotational suture, retinacular release and imbrication.

Therefore, it is important to know the advantages and disadvantages of each surgical procedure in order to expect a successful surgical outcome, and to evaluate whether the correct surgical procedure has been performed well during surgery.

In this lecture, author talk about the exact method and advantages and disadvantages of each surgical procedure to reduce complications after MPL surgery. In addition, author will discuss the misconception of the surgical procedure through the case of complications and will introduce the treatment method for that.

Diagnosis of MPL (patellar) can be made easily by simple physical examination, but differential diagnosis is needed for MPL concurrent with polyarthritis and cranial cruciate ligament injury.

Trochleoplasty should be performed considering the depth, width, and length of trochlear grooves so that the patella can be within the trochlear groove while preserving the articular cartilage.

Patellar groove replacement can be performed in severe femoro-patellar osteoarthritis, and recent reports of good surgical prognosis using patellar groove replacement have been reported.

Tibial tuberosity transposition (TTT) is a useful surgical procedure for maintaining the appropriate realignment of the quadriceps mechanism. It is important to reduce the patella to the trochlear groove and move the position of the tibial tuberosity so that the quadriceps muscle, patella, and tibial tuberosity are in the same line. In addition, it is advisable to familiarize yourself with pin insertion and tension band usage to fix displaced tibial tuberosity. Tibial tuberosity fractures after TTT are extremely difficult to treat. In this case, calcaneal tendon allograft with a bone block has been reported to have good prognosis.
Distal femoral osteotomy is performed to correct the femoral varus when the distal femoral varus is greater than 10 degrees above normal to induce proper alignment of the quadriceps mechanism. To measure the varus angle of the femur, accurate radiographs are essential, and CT imaging has the advantage of providing more information. A 3D-printing bone model created using CT data can be used for corrective osteotomy. The 3D-printing bone model aided in evaluating bone deformity accurately, made it possible to make an accurate surgical plan at a 1:1 ratio with the real bone of the patient and allowed the surgeon to confirm the planned surgical procedure through a rehearsal surgery. Furthermore, the rehearsal surgery using the 3D-printing bone model raise familiarity with the surgical procedure and the confidence of the surgeon and make the pre-contouring of bone plates possible.

**Keywords:** Medial Patellar Luxation, Trochleoplasty, Tibial Tuberosity Transposition, Distal Femoral Osteotomy

**References**


AO Principles of Fracture Fixation

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Summary
Goal of fracture repair is a) early ambulation and complete return of function, b) pain free recovery, and c) prevention of fracture disease. “AO principles of fracture treatment” is a useful guideline to achieve this goal: 1) Anatomical reduction, 2) Stable fixation, 3) Preservation of blood supply, 4) Early mobilization. The concept of “biological fracture repair” has been proposed in human and veterinary orthopedics, in order to improve clinical outcome, to reduce patient discomfort, and to simplify surgical procedure. Recently, new techniques, devices, and implants are developed to practice this concept of minimally invasive fracture repair in small animal orthopedics.

“AO principles of fracture treatment” can be summarized: 1) Anatomical reduction, 2) Stable fixation, 3) Preservation of blood supply, 4) Early mobilization.

Fracture Assessment Scoring
The fracture assessment score is an attempt to put together some of the decision-making factors for fracture repair. These include the mechanical factors of the fracture and the biologic factors of the patient.

Mechanical scoring
From our radiographic assessment, we can get an appreciation for the mechanics of this fracture and its intrinsic stability. Simple fracture has a high degree of intrinsic stability whereas comminuted fracture has no intrinsic stability. Use the description of the radiograph to give you an idea of the intrinsic stability of the fracture.

Biological scoring
Based on your knowledge, you can easily think of biological factors that would make an animal more or less likely to heal. Age, general health, and blood supply from soft tissue are major biological factors.

By combining the assessment of the biological and mechanical factors, we can assign the fracture a score on a scale of one to ten. A score of one means that there are many factors working against healing and therefore we need to consider rigid fixation that will maintain stability for an extended period of time. A score of ten means that the fracture will likely heal very quickly and has a high degree of intrinsic stability.

Reconstructable Fracture? Closed or Open Approach?
In planning fracture management, the surgeon must first determine a) whether full reconstruction of the bone column is possible, and b) whether open or closed reduction is preferred. Fracture healing is initially dependent on extraosseous blood supply from surrounding soft tissues. In any fractures, soft tissue attachment should be preserved as much as possible. Fracture hematoma is generally believed to improve fracture
healing. Therefore, closed approach may have some advantages over open approach.

**Completely closed approach** would be ideal for preservation of soft tissue, blood supply and hematoma, and minimization of surgical trauma and risk of infection. This approach can be attempted with external coaptation (full cast) or external skeletal fixators. However, difficulty in gaining adequate reduction and unavailability of advanced imaging techniques such as fluoroscopy may limit its application in veterinary medicine.

**Limited open approach, or Open-But-Do-Not-Touch approach**, is currently recommended for many fractures to practice the concept of biological fracture fixation. In this approach, the major bone segments are manipulated but the fracture fragments are not disturbed, and the bone is distracted to length where the major segments are realigned with an intramedullary pin (in plate rod combination, PRC) or interlocking (ILN), or minimally invasive plate osteosynthesis (MIPO). Autogenous cancellous bone graft can be applied.

**Open approach** is chosen for internal fixation (e.g. bone plate). Most bones can be approached by muscle/fascia separation techniques, with occasional tenotomy or osteotomy (see Surgical Approaches to the Bone and Joints of the Dog and Cat, Piermattei and Johnson). While minimizing surgical trauma, surgical incision should be sufficient to allow adequate exposure for fracture reduction and implant placement.

**Soft Tissue Handling in Fracture Repair**
The surgeon must abide by Halsted’s principles of surgery: preservation of all soft-tissue attachments to bone fragments, sharp and accurate tissue dissection, avoidance of excessive trauma, and careful and gentle handling of soft tissues, nerves, and vessels. Minimization of finger use will decrease contamination, minimize tissue devitalization, and result in improved tissue handling. During dissection, large blood vessels and major nerve trunks must be preserved at all cost. The anatomy of the area should be kept firmly in mind, particularly around radial and sciatic nerves. Whenever possible, the nerve is retracted with an adjoining muscle. If isolation of the nerve is necessary, Penrose drain can be passed around and is then used to maintain traction.

**Fracture Reduction**
The goal of fracture fixation is to restore functional limb alignment (reduction) and to stabilize the fracture (stabilization). Fracture reduction is important for fracture stability during healing and for limb mechanics. Reduction is the process of either reconstructing the fractured bone to its normal anatomical configuration, or restoring the normal alignment of the limb. Normal limb alignment is achieved by

1. restoring normal limb length,
2. maintaining normal spatial orientation of the limb, and
3. restoring the alignment of the joints adjacent to the fractured bone.

**Preoperative Planning for Fracture Reduction**
Type of fracture and choice of fixation method dictate method of fracture reduction. Methods of fracture reduction include closed reduction, open but do not touch approach, limited open reduction, and open reduction.

**Indications for closed reduction** include
- Non-displaced or incomplete fractures
- Comminuted fractures treated with minimally invasive fixation methods

**Indications for open reduction** include
- Articular fracture
- Simple displaced fracture
- Comminuted fractures treated by major segment alignment + cancellous bone graft

**Closed Reduction**
Closed reduction involves reducing fractures or aligning limbs without surgically exposing the fractured bones. This approach has several advantages as it preserves the surrounding soft tissues and blood supply to the bone, and decreases the possibility of iatrogenic contamination associated with surgery. The end result is shorter overall operating time, improved healing potential, and a lower rate of infection. The main disadvantage is that cortical apposition of the fracture fragments can be hindered.

**Open reduction**
Open reduction uses a surgical approach to expose fractured bone segments and fragments, so they can be anatomically reconstructed and held in position with implants. The fracture fragments may be seen and reconstructed and a cancellous bone graft can be used. The major benefit of a fully reconstructed bone column is that it can share the load during fracture healing. Therefore, open fracture fixation is reserved for fractures that can be anatomically reconstructed. The potential disadvantages include iatrogenic contamination, additional soft-tissue damage, and impairment of blood supply.

**Postoperative and Fracture Repair Assessment**
Assessment of postoperative radiographs is important for fracture movement, bone inactivity, bone resorption, loss of alignment or apposition, infection, implant loosening. Assessment of postoperative radiographs may be structured into four steps each beginning with the letter A.

**Alignment**
This is perhaps the most important factor relative to ultimate outcome yet one of the most frequently overlooked. Alignment assesses the linear and torsional realignment of the fracture. Assess spatial relationship of joints above and below fracture. The greatest significance of this is its effect on the joints. Malalignment can lead to: a. osteoarthritis, b. limb malfunction, c. hip luxation, d. ligamentous damage, e. cruciate damage.

**Apposition**
This is evaluation of the degree of reduction at the fracture site. With the exception of articular fractures, this is of less importance than alignment and apparatus.

**Apparatus**
This is an assessment of the apparatus applied and its relationship to the bone. The clinician must assess if the surgically resulting bone and implant composite will be capable of withstanding the forces that will be applied to it and lead to healing.

**Activity**
This assessment is made after time is permitted for biological response to the surgery. We assess the radiographs for appropriate healing: a. callous formation, b. disappearance of the fracture line, c. continued implant stability, d. continued apposition, f. continued alignment.

**Biological Fracture Fixation**
Recently, the concept of “biological fixation” has been proposed. The primary consideration in this concept is the protection of the soft tissue and the blood supply of the fracture fragments. Anatomical reduction in this circumstance means restoring axial alignment in the frontal and the sagittal plane, eliminating torsional deformity (and maintaining bone length to the extent possible). The major goal of this concept is to improve overall healing time, patient comfort level, and difficulty of the fracture repair.
To practice the concept of minimally invasive fracture repair, external skeletal fixators have been used. However, this technique is often associated with practical issues such as soft tissue irritation, pin track infection, pin loosening, and difficulty in long term management. Recently, bone plates have been successfully applied in a minimally invasive fashion in selected fractures (minimally invasive plate osteosynthesis, MIPO), thanks to better understanding of anatomy and development new plates. Interlocking nail (ILN) system is also an excellent choice in practicing minimally invasive fracture repair.

**Keywords:** Dog, Fracture, Fixation
Nonunion: Radius and Ulna Fracture

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Summary
Fractures of the antebrachium account for approximately 17% of all canine fractures (Philips 1979). Motor vehicle trauma is a prevalent cause. However, in toy breeds, fractures of the antebrachium can occur after apparent minimal trauma, such as jumping or falling, and usually affect the distal region of the diaphysis (Waters et al. 1993). The prevalence of delayed union or non-union fractures in dogs is 3.4% (Atilola & Sumner Smith 1984). Atrophic non-union is a rare complication. However, this complication occurs most commonly after fracture of the distal antebrachium, and is particularly prevalent in toy breeds (Vaughn 1964, Sumner-Smith & Cawley 1970, Atilola & Sumner-Smith 1984). Development of delayed or non-union after surgery is a potentially serious complication, because limb amputation may be necessary. Although the prevalence of antebrachial fracture and the prevalence of fracture healing complications after treatment in toy breeds have been recognized for many years, the causative biological mechanism is not understood.

Antebrachial fractures usually develop between 15% and 35% of radial length from the distal end of the bone. A transverse or short oblique fracture is typically seen. Fractures are most common in young adult or adolescent dogs. This pattern of fracture reflects compressive or bending overload during a jump or fall. In this regard, breed-related variation in cross-sectional bone geometry suggests that toy breeds are more vulnerable to fracture after a fall or jump (Brianza et al. 2006). Age-related changes in bone mineral density or bone adaptation may also be a factor. Similar trauma in large-breed dogs usually results in carpal hyperextension injury (Willer et al. 1990).

Various hypotheses regarding the prevalence of delayed bone healing after antebrachial fracture in toy breeds have been proposed, including fracture instability (Hunt et al. 1980), increased and persistent formation of cartilage within the fracture site (Sumner-Smith 1974a), and decreased osteogenesis compared with larger dogs (Sumner-Smith 1974ab). Differences in vascular density at the metaphyseal/diaphyseal junction have also been implicated as a factor (Welch et al. 1997), but fracture site blood supply has not been directly measured.

Surgical treatment
The observation that vascular density in the distal radius is decreased in small breed dogs, when compared with large breed dogs (Welch et al. 1997) and the suggestion that this may be an important factor influencing fracture healing after injury argues that stable internal fixation of antebrachial fractures is particularly important, since restoration of medullary circulation can occur as early as one week after rigid fixation in the dog (Rhinelander 1968).

Overall approach. Historically, external coaptation, intramedullary fixation, or both, have been used to stabilize antebrachial fractures in toy breeds (Lappin et al. 1983, Waters et al. 1993). High complication rates can be expected with these fracture treatments (Lappin et al. 1983, Waters et al. 1993) in both adolescent
and adult dogs. Development of delayed or non-union fractures is often associated with this type of fixation (Muir 1997). External coaptation or intramedullary fixation is not recommended.

**Plate fixation.** Internal fixation with bone plates, typically with 1.5mm, 2.0mm, and 2.4mm implants, is recommended for rigid stabilization of this type of fracture. Dorsal applications of specialized plate for this type of fracture in small and miniature breeds have been highly successful (Hamilton & Langley-Hobbs 2005). Using of locking plates may also help augment construct stability (Voss et al. 2009, Gibert et al. 2015). A high rate of fracture union, can be expected after rigid fixation with bone plates, with a large majority of the dogs experiencing a successful return to function (≥ 90%) (Larsen et al. 1999). Implants are usually only applied to the radius in toy dogs. After the fracture has healed, elective bone plate removal from the antebrachium in toy breed dogs should not be performed, as the risk of refracture is high (Bernard et al. 2008).

**External skeletal fixation** is another treatment option, particularly for dogs with delayed or non-unions where loss of bone has developed (Eger 1990). External fixation is usually performed with small Kirschner wires, with the use of methylmethacrylate as a connecting bar in a freeform frame. Use of a circular external fixator can also be considered. This approach may be particularly useful for malunion fractures (Piras et al. 2011). A disadvantage of external skeletal fixation is the risk of refracture after frame removal through a pin tract, the original fracture site, or a new fracture in the antebrachium (Piras et al. 2011).

**Bone grafting.** In addition to rigid stabilization of the fracture, use of autogenous cancellous bone graft to stimulate fracture healing is recommended in skeletally mature patients. In very small dogs, cortico-cancellous graft material can be collected from the wing of the ilium, if a larger volume of bone graft is needed. Applications of allogenic demineralized bone matrix, recombinant bone morphogenetic protein-2 (rBMP-2), and free autogenous omental graft have also been reported (Bernard et al. 2008, Hoffer et al. 2008, Baltzer et al. 2015).

**Loss of cortical mineralization after plate application**

There is little evidence to suggest that stress protection is an important factor influencing bone adaptation after plate application to the radius in toy breed dogs. Although it has been hypothesized that stress protection may play a role in loss of bone mass from the antebrachium after plate fixation, clinically this is a rare phenomenon and is not confined to toy breeds. The phenomenon of stress protection has been little studied in the dog. After plate repair of long bone fractures, loosening of bone screws is typical, such that the density of bone under the plate is little affected by use of a stiff plate (Glennon et al. 1994, Muir et al. 1995). This concept is further reinforced by recent research showing that normalized stiffness after plate fixation of radii from large- and small-breed dogs is similar (Gauthier et al. 2011).

A neurovascular mechanism is the most likely explanation for the cortical atrophy of bone and regional osteopenia that may be seen after radial plate and external fixator application in dogs, particularly dogs with long limbs and a slender conformation, such as the Italian greyhound and many other toy breeds, as well as larger breeds, such as the Borzoi. This bone loss often affects the ulna as well as the radius (Hamilton & Langley-Hobbs 2005). Such a mechanism would also explain the high incidence of a second antebrachial fracture developing after external skeletal fixator frame removal (Piras et al. 2011). Selecting an appropriately sized bone plate, use of an atraumatic surgical technique, use of bone graft in adult patients, and meticulous rehabilitation can minimize the risk of this phenomenon.

**Aftercare**

With rigid plate fixation and bone grafting, fracture healing is usually rapid. In general, external coaptation should be avoided after surgery. Physical therapy using cold and warm packs with a regimen of passive range-of-motion should be used to encourage early return to weight bearing and to minimize fracture disease and tissue fibrosis as much as possible. Patient activity should be carefully managed until the fracture has healed. Follow-up radiographs should be made to document fracture healing.

Long-term aftercare should include permanent restriction of the dog’s activity to minimize the risk of falls. The conformation of toy breed dogs means that there is a reduced safety margin based on radial
cross sectional geometry. Consequently, dogs of this conformation are vulnerable to fracture from a fall and this vulnerability remains after surgery. If a fall is sustained after plating, the fracture will often develop at the level of the proximal end of the plate because of the change in stiffness of the antebrachium at this level. This phenomenon is not a consequence of stress protection.

Conclusion

Antebrachial fracture is common in small dogs. Prognosis is generally good with appropriate treatment. Plate fixation is the treatment of choice and it is essential to ensure that the appropriately sized bone plate is selected for surgical treatment. Long-term aftercare should minimize the risk of a fall.

Keywords: Dog, Fracture, Nonunion

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Surgical and Nonsurgical Management of Osteoarthritis

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Summary
Osteoarthritis (OA) or degenerative joint disease (DJD) is estimated to be present in one in five adult dogs worldwide. While there are many initiating causes, osteoarthritis is an irreversible process that often results in an end-stage clinical syndrome of the joint (i.e. lameness, pain, and decreased activity) that affects quality of life.

Generally, there is no one etiology and its cause may be multifactorial. Primary OA is often referred to as “wear-and-tear” joint disease. It has an insidious onset, and is thought to be caused by chronic use combined with aging and obesity, and may involve a genetic predisposition to cartilage degradation. Secondary OA, identified most commonly in dogs, results from an initiating cause such as shoulder OCD, elbow dysplasia, hip dysplasia (developmental disorder), cranial cruciate ligament rupture (mechanical/inflammatory disorder), intra-articular fracture, luxation (traumatic injury). Genetics are the major cause while nutrition and environmental factors can worsen the condition, i.e. weight can affect the phenotypic expression of the genotypically predisposed animals to OA.

Definition
“Osteoarthritic diseases are a result of both mechanical and biologic events that destabilize the normal coupling of degradation and synthesis of articular cartilage chondrocytes, extracellular matrix, and subchondral bone. Although they may be initiated by multiple factors, including genetic, developmental, metabolic, and traumatic factors, osteoarthritic diseases involve all of the tissues of the diarthrodial joint. Ultimately, osteoarthritic diseases are manifested through morphologic, biochemical, molecular, and biomechanical changes in both cells and matrix that lead to softening, fibrillation, ulceration, articular cartilage loss, sclerosis and subchondral bone eburation, and osteophyte production. When clinically evident, osteoarthritic diseases are characterized by joint pain, tenderness, movement limitation, crepitus, occasional effusion, and variable degrees of inflammation without systemic effects.”

Pathogenesis
Generally, there is no one etiology and its cause may be multifactorial. Primary osteoarthritis is often referred to as “wear-and-tear” joint disease. It has an insidious onset, and is thought to be caused by chronic use combined with aging and obesity, and may involve a genetic predisposition to cartilage degradation.

Secondary osteoarthritis, identified most commonly in dogs, results from an initiating cause such as shoulder OCD, elbow dysplasia, hip dysplasia (developmental disorder), cranial cruciate ligament rupture (mechanical/inflammatory disorder), intra-articular fracture, luxation (traumatic injury). Genetics are the major cause while nutrition and environmental factors can worsen the condition, i.e. weight can affect the phenotypic
expression of the genotypically predisposed animals to OA.

Pathophysiology
Normal articular cartilage is primarily composed of water, extracellular matrix (a collagen fibril network (types II, IX, XI) and proteoglycan macromolecules-Aggreccan), and chondrocytes. The maintenance of normal cartilage homeostasis requires the coordinated synthesis and degradation of articular cartilage matrix macromolecules (primarily by chondrocytes).

Ongoing, repetitive biomechanical or biochemical insult to the joint can cause a shift in the balance between anabolic and catabolic statuses, initiating a “vicious cycle” of destruction. Cytokines and growth factors appear to play a critical role in the induction and progression of osteoarthritis. Proinflammatory cytokines induce articular cartilage depletion by increasing the synthesis of matrix-degrading enzymes.

Conservative Management
Weight control is essential when dealing with OA in dogs and cats. Owner education and proper dietary management must be considered in every case. Most nutritional supplements are intended to reduce inflammation and pain, although there is no significant evidence that these products help treat OA in dogs and cats. Commonly used nutritional supplements are omega-3 fatty acids (DHA, EPA), glucosamine, and chondroitin sulfate. Animals with OA benefit from regular low-impact exercise. Protecting the osteoarthritic joint from excessive mechanical stress may limit clinical signs. Active and passive forms of physical rehabilitation have been shown to be effective in maintaining range of motion and muscle mass, thus improving limb function. Analgesic and anti-inflammatory agents are the most common final component in the management of OA. However, there are some risks in using these agents, and one must consider all the possible ramifications prior to their usage. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to control acute and chronic pain in veterinary patients. Chondromodulating injectable agents such as polysulfated glycosaminoglycans (PSGAG) and hyaluronic (HA) have been tested in dogs, with conflicting results. Opioids (such as tramadol) can be used to control severe pain in addition to NSAIDs.

Multimodal Therapy
Use of a multimodal therapeutic approach to treat chronic pain has been suggested for the alleviation of osteoarthritic pain in human and veterinary patients.

The rationale for this approach is based on the evidence of functional changes in the central nervous system induced by the constant input of noxious signals from the periphery, i.e. chronic pain. Central acting agents, N-methyl-D-aspartate (NMDA) antagonists such as amantadine, ketamine, and opioids such as tramadol, a synthetic derivative of codeine, have been examined for a potential role in this approach in addition to current therapeutic options.

Surgical Treatment
Identifiable congenital and developmental orthopaedic conditions (e.g. patellar luxation, elbow dysplasia, OCD) should be treated surgically in many cases to minimize the progression of OA. Cruciate ligament disease and intra-articular fractures require surgical stabilization to prevent OA. Established clinical OA in hip can be treated effectively with joint replacement. Elbow replacement and resurfacing procedures are being examined for clinical applications.

Keywords: Dog, Joint, Osteoarthritis
Is Minimally Invasive Surgery Better – What Is the Evidence?

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Summary
This lecture will discuss the evidence in the scientific literature to back-up the use of minimally invasive procedures in companion animal species. The principal literature on the topic will be discussed as well as an introduction to the process of providing high quality studies for the field of laparoscopy and thoracoscopy.

Minimally invasive surgery (MIS) was first described in veterinary patients in the mid-1980's at a similar time to it's initial development in humans. A series of patients undergoing laparoscopic renal biopsies was described in 1983. In 1985 another group of early adopters used laparoscopic uterine horn occlusion on 6 bitches and 3 queens in an attempt to describe an alternative method for mass sterilization of small animal patients.\(^1\)

Over the last 15 years, despite the lack of a broad-based adoption of MIS alternatives to open surgery by veterinary surgeons, a slow but steady evolution and refinement of laparoscopic and thoracoscopic techniques in small and large animal practice has taken place. Rawlings amongst others was an early pioneer of many laparoscopic-assisted and laparoscopic techniques that are user friendly and can often be performed using only basic laparoscopic instrumentation. These techniques include laparoscopic-assisted ovariectomy, cryptorchidectomy, cystotomy, cryptorchidectomy and a variety of laparoscopic organ biopsy techniques.\(^3,6\) They have allowed many veterinarians with limited MIS experience to start performing MIS procedures in referral as well as general practice. A natural progression to more complex techniques has occurred more recently with laparoscopic adrenalectomy, cholecystectomy and thoracoscopic lung lobectomy being performed in some referral centers.\(^7,10\) Other trends in the development of veterinary MIS include the movement towards fully laparoscopic versions of previously described laparoscopic-assisted techniques. These "second generation" techniques are aimed at minimizing incision size and therefore invasiveness even further.\(^11\) Similarly, reducing port size and number, a concept that is gaining great popularity in human medicine, is now well under way in veterinary MIS and is also aimed at reducing the invasiveness of certain procedures.

Despite all of the new procedures and refinements to existing techniques that have been made, the evidence base to suggest that MIS has advantages over traditional surgery remains weak in veterinary medicine. Most authors have suggested that advantages may exist in minimizing pain and improving return to function, decreasing risk of surgical site infection (SSI) and reducing various morbidities when patients undergo a laparoscopic or thoracoscopic approach compared to a celiotomy or thoracotomy. While we would like to think this is true and we are quick to "borrow" evidence from human medicine it is imperative that veterinarians demonstrate these advantages with rigorous scientific evaluation of the techniques.

There is now a steady trickle of evidence to suggest that many of the advantages that humans have enjoyed since the paradigm shift to MIS may also be afforded to our small animal patients. Reliable outcome measures are a challenge in veterinary medicine but most investigations have employed either defineable outcomes
such as surgical time and complication rates or parameters of post-surgical pain or stress such as serum cortisol and glucose measurement as well as a variety of visual analogue scales. In Devitt’s paper, one of the earliest to compare a population of healthy animals undergoing either open or laparoscopic ovariohysterectomy, the MIS group was found to have lower pain scores for 24 hours post-operatively whereas indirect measures of surgical stress, cortisol and glucose, were significantly increased in the open group up to 2 and 6 hours respectively compared to baseline, which was not the case for the MIS group. Surgical time has been compared between open and MIS approaches in a number of studies. Surgical time needs to be interpreted with caution in these studies as many represent the early part of a center’s learning curve in comparison to that of an open procedure that the surgeon is presumably, in most cases, experienced with. However, several studies have documented longer surgical times associated with an MIS procedure compared to the traditional open procedure. As more experience is gained with newer techniques however we may find that there is a time advantage to some procedures that are performed using an MIS technique as has been shown in one study of laparoscopic adrenalectomy. Other studies have evaluated the return to normal function using an objective measure of activity. Accelerometry has been validated for use in both dogs and cats and was used to compare open to laparoscopic OVE in cohorts of small dogs. A detectable difference was found between groups with activity counts in the 48 hours post-operatively being 25% and 62% below baseline activity in the laparoscopic and open groups respectively. In another study using accelerometry devices the authors compared a laparoscopic-assisted gastropexy to a “next generation” technique performed entirely laparoscopically using intracorporeal suturing. Accelerometry revealed activity counts that were 44% and 11-19% decreased compared to pre-operatively in the laparoscopic-assisted and total laparoscopic groups respectively in the 7 days post-operatively a difference that was statistically significant and was attributed to the avoidance of the deep paramedian incision performed for the “assisted” technique. The duration of this effect appeared to be somewhere in the region of 4-5 days post-operatively.

More recently other studies have concentrated not only on improvements in post-operative discomfort but in the effect on morbidity of MIS approaches. In a study comparing surgical site infection (SSI) rates between open and minimally invasive surgery, the authors found that on univariate analysis surgical approach (MIS versus open) had a beneficial effect on SSI rate post-operatively. The SSI rate for the open group was 5.5% compared to an SSI rate of 1.7% in the MIS group. However, it should be noted that on multivariate analysis, this difference was at least in part driven by other potential confounders. Further studies will be required to confirm this hypothesis.

In thoracic surgery few studies have been pursued evaluating the difference between open and MIS approaches. One study evaluated the difference between an open and a VATS approach in a canine pericardectomy model. In this report the VATS approach was associated with lower post-operative pain scores in the post-operative period as well as higher blood glucose and cortisol concentrations in the thoracotomy group. Rescue analgesics were used with greater frequency in the thoracotomy group and fewer complications were observed in the VATS group. A recent clinical study has documented the results of VATS lung lobectomy in 22 canine patients with primary lung lobe tumors compared to a population that underwent thoracotomy for the same reason. The authors were able to demonstrate that the VATS approach was feasible and not associated with significantly greater morbidity but were not able to demonstrate apparent advantages in the length of hospital stay, ICU time or indwelling thoracic drain time advantages which have all been documented in humans after VATS lobectomy. In this study no attempt was made to evaluate post-operative pain, discomfort or activity between groups. Additionally, outcome measures were not indexed to any clinical benchmarks and significant confounders caused by the decisions of different managing clinicians may have influenced outcomes. Future studies are required in this area to investigate in a prospective fashion the effects of these interventions versus their traditional open counterparts.

In conclusion, much research remains to be done in establishing which procedures will lend themselves well to an MIS approach and in which cases a traditional open approach might remain the wiser choice. We must also continue to strive to critically evaluate the procedures we perform to ensure that our MIS procedures are really delivering the advantages that we hope they will.
Keywords: Laparoscopy, Thoracoscopy, Minimally Invasive, Evidence-Based

References
Laparoscopy in Small Animals
- Instrumentation, Anesthesia and Access

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Summary
In this lecture we will discuss the basic principles of laparoscopy, the instrumentation that is required to start a program in laparoscopy and the principles of safe and efficient abdominal access. We will also discuss the principles of hemostasis and energy devices commonly employed by minimally invasive surgeons to achieve efficient and complete hemostasis in their patients.

Instrumentation:

Imaging - Rigid Telescopes - The telescope allows transmission of the visual image from the surgical site to the camera. Quality of telescopes has risen rapidly in recent years especially with the introduction of the rod lens system. These telescopes employ a series of glass lenses arranged in a narrow tube to transmit light. The important variables to consider when choosing a telescope are the diameter, the length and the angulation at the tip. Probably the most versatile telescope for laparoscopy and thoracoscopy in small animals is the 5 mm diameter telescope. This is adequate for most dogs and cats. A smaller 3 mm diameter 14 cm long telescope (Karl Storz Veterinary Endoscopy) or a traditional 2.7 mm arthroscope can be used for smaller dogs and cats. Angulation of the tip of the telescope dictates the direction of the field of view. The 0° telescope will provide an image of what lies directly in front of the tip of the scope which is adequate for most abdominal procedures. The 30° telescope provides a view that is offset by 30°. Although somewhat more difficult to manipulate initially, they are helpful as by rotation of the light post (and therefore the telescope), a greater field of view is obtained.

Imaging – Cameras - The image that is transmitted through the lenses of the telescope is captured by the camera and turned into a video image. The camera attaches to the head of the telescope and has a cord that feeds into the camera control box housed on the tower. The quality of the endoscopic video camera depends on the camera control unit or “chip”. One-chip cameras use a single computer chip to process the colors the camera sees whereas in a 3-chip camera each chip processes a separate primary color, namely red, green or blue. One-chip cameras are satisfactory for most applications but three-chip cameras have superior optical clarity and color reproduction and provide images that are of photographic or broadcast quality. Alterations of the focus and field of vision size are usually controlled by rings located on the camera head with more modern cameras allowing the operator control of multiple functions such as white balance, other menu alterations, and image and video recording via buttons also located on the camera head.

Tower components:

The Light Source - Modern light sources are usually powered by either halogen or xenon. Xenon is preferable as it emits a high intensity light that reproduces the color of natural light closely. Light sources between
150-300 watts are recommended to ensure good picture quality. Care of the fiberoptic light cable is also essential for optimal performance as if the fibers break or the tips are not regularly cleaned image quality will suffer. Care should always be taken to know where the tip of the telescope or light cable (when not attached to the telescope) is while the light source is fully powered. When resting on the table adjacent to drapes or in body cavities, or when resting against tissues, thermal burns can occur rapidly. When not directly in use, the power to the light source should always be turned down.

The Insufflator - During laparoscopy a mechanical insufflator regulates the flow of gas (usually CO₂) into the abdomen during creation of a pneumoperitoneum. Pneumoperitoneum allows the working space necessary to manipulate instruments and organs during laparoscopic procedures. Modern insufflators allow monitoring of the pressure within the abdominal cavity and prevent pressure rises above preset levels. In dogs, intra-abdominal pressure of 15 mm Hg is considered to result in physiologically acceptable levels of cardiorespiratory depression while providing more than adequate working space in most patients.¹ Intra-abdominal pressures of 8-10 mm Hg generally provide adequate working space in most dogs and cats.

The Monitor - Usually located at the top of the “tower”, a high quality medical grade cathode ray tube or flat panel monitor is essential to be able to view structures clearly and should be large enough so that the surgeon can see images from across the surgical table. It is very important to consider optimal tower location for any given procedure during preoperative planning. This will minimize the need for cumbersome intra-operative relocation of the tower while maximizing the ability to maintain the straight line viewing axis from the surgeon to the lesion being operated and on to the monitor.

Data Recording Devices - Providing a record of images or video of the procedure is very helpful for patient medical records as well as client and veterinarian education and publication purposes. The simplest device for image capture is the video printer that will print single images in surgery. Video recorders can be used to record whole procedures that can then later be edited. Digital capture devices are now commonplace which provide perhaps the most versatile storage and distribution medium. Still images and videos can be captured, stored on the units’ hard drive or be easily downloaded to CD, DVD or connected via USB ports to external storage devices such as flash drives or portable hard drives (e.g. AIDA-vet device, Karl Storz Veterinary Endoscopy).

Trocars and Cannulas - Establishing access ports using cannulas is essential for laparoscopic procedures to allow atraumatic repeated instrument exchanges. For laparoscopy where insufflation is used, maintenance of an airtight seal to prevent leakage of insufflation gas during laparoscopy is also accomplished using cannulae.

A large variety of sizes and designs of trocars and cannulae are available. Choices to be considered include the use of single-use disposable versus resterilized non-disposable cannulae, blunt versus sharp trocars and trocar-cannula assemblies versus trocar-less cannulae. Single-use disposable cannulae are generally made of lightweight plastics and are less likely to slide out of port incisions; a common occurrence in small dogs and cats. Blunt trocars should always be used during establishment of the first (camera) port to avoid iatrogenic damage to underlying organs prior to the establishment of a pneumoperitoneum or pneumothorax. Instrument ports can then safely be established using sharp-tipped trocars that can be placed under direct visualization from within the body cavity.

Surgical Instruments - Almost all of the instruments used for open surgical procedures are available in laparoscopic versions including scissors, various grasping forceps and cup biopsy forceps. The 5 mm sized instruments are usually used for small animal procedures although 2, 3 and 10 mm are also available. A simple blunt probe is one of the most useful instruments for carefully moving organs in a non-traumatic fashion. Other components of a basic instrument set required for most laparoscopic and thoracoscopic interventions include a Metzenbaum and hook (suture-cutting) scissors, Kelley hemostats, Babcock forceps (5 and 10 mm versions), and cup biopsy forceps. For procedures requiring more intricate dissection various sizes of right-angle forceps (5 and 10 mm) are useful. A knot-pusher is an instrument required when extra-corporeal knot tying is used. Knots tied outside the body cavity are pushed into position through the
cannula and around the vessel or other structure being ligated. Various forms of laparoscopic retractors are also available including fan retractors, and various forms of inflatable retractors that are necessary when performing certain procedures where visualization is obscured by certain other organs or by the movement of the organ being operated. Laparoscopic needle holders are necessary for intracorporeal suturing. These are generally used in pairs and are available with various styles of tip, one of the most popular of which is the parrot-jaw.

Principles of abdominal access:
Veress Needle Technique - A Veress needle is a specialized instrument used for creation of pneumoperitoneum or pneumothorax. It consists of a sharp-tipped needle component that houses within it a blunt-tipped obturator loaded on a spring-like mechanism. The sharp component of the needle will penetrate the body wall whose resistance will force the blunt obturator to retract into the shaft. Upon penetration of the body wall, however, resistance is lost, allowing the blunt-tipped obturator to spring forward thereby shielding the abdominal viscera from injury.

Modified Hasson Technique - The modified Hasson technique avoids the blind introduction of a sharp needle and cannula into the peritoneal cavity. It is performed by making a 1 cm skin incision just caudal to the umbilicus which is followed by blunt dissection to the linea alba. A 3-4 mm incision is made through the linea alba and a trocar-cannula assembly is introduced into the peritoneal cavity. A blunt-tipped trocar should be used to prevent injury to the abdominal organs. Penetration into the peritoneal cavity can be confirmed by observation of falciform fat through the incision prior to inserting the trocar. The insufflator line is attached to the cannula and the abdomen is insufflated. Upon establishing a pneumoperitoneum, the abdominal cavity should become tympanic.

Placement of instrument portals - Once the camera portal has been established as many instrument portals as are necessary for a given procedure can be placed. Instrument portals are usually placed using direct visualization from inside the body cavity now that access for the telescope has been achieved. A stab incision with a scalpel blade is made over the proposed site of the portal and a trocar-cannula assembly or trocarless cannula is inserted through the deeper layers of the body wall. The surgeon can then observe from within the body cavity as the cannula enters thereby avoiding any iatrogenic damage to organs.

Achieving hemostasis:
A variety of methods for achieving hemostasis within body cavities during laparoscopic and thoracoscopic procedures are available.
Laparoscopic hemostatic clips - A variety of disposable or non-disposable laparoscopic hemoclip applicators are available. Reusable sterilized clip applicators (M/L-10 reusable multi-fire hemoclip applier, Microline Pentax) are loaded with clip cartridges and are more cost effective than disposable devices. Multi-fire clip applicators that allow several clips to be applied without withdrawal of the device minimize instrument exchanges and surgical time. The use of laparoscopic hemoclips in veterinary patients has been evaluated in a laparoscopic ovariohysterectomy model and was shown to be safe and effective for ovarian pedicle ligation; however, hemoclip application was shown to be more time-consuming than the use of a vessel-sealing device for this application.

Monopolar and Bipolar Electrosurgery - Both monopolar and bipolar electrosurgery can be used in minimally invasive surgery. Monopolar electrosurgery should however be used with great caution in MIS as several potentially hazardous problems can occur. A defect in the insulation of the instrument shaft can result in the passage of current to tissues that are not in the visual field resulting in iatrogenic injury. Direct coupling injuries can occur when the instrument through which the electric current is passed comes into contact with the telescope, or other instrument, resulting in iatrogenic damage to tissues that may lie outside the visual field. Bipolar electrosurgery is safer as it is usually of lower voltage current and the electrical current
is only passed between the tips of the bipolar instrument used.

**Vessel-Sealing Devices** - A more recent development in MIS is the increasing use of vessel-sealing devices. Vessel-sealing devices are very helpful for hemostasis and simultaneously sealing and cutting a variety of tissues. They work by a combination of pressure exerted on tissue when the tissue is crushed in the tips of the device, followed by the application of bipolar or ultrasonic energy applied to the tissue. This process allows the elastin and collagen in the vessel wall to be sealed together permanently. A variety of units are currently available. Two bipolar electrocautery devices are the Ligasure™ (Valleylab, Tyco Healthcare Group) and the Enseal™ (SurgRX Inc.). Both devices have tips that are indicated to seal arteries and veins up to 7 mm in diameter. The Ligasure has the advantage of sensing the tissue impedance within the jaws of the tip that then adjusts the energy output from the generator accordingly to ensure a safe and effective seal. A second-generation Ligasure device known as the Force Triad™ is now available that provides a much more rapid seal cycle. The Harmonic Scalpel® (Ethicon Endo-surgery) is a device that uses ultrasonic energy to cut and coagulate tissue. The Harmonic Ace® tip is indicated to seal vessels up to 5 mm in diameter.

Several reports have compared these devices with respect to the degree of lateral thermal spread, bursting pressures and sealing time although the results of these studies are often conflicting. Overall, however, all produce supra-physiological bursting pressures of at least three times systolic blood pressure. For the Ligasure lateral thermal spread ranged from 1.5-3.2 mm in one study with a greater degree of thermal spread seen as vessel size increased.

**Keywords:** Laparoscopy, Telescope, Minimally Invasive, Vessel-Sealing, Veress

**References**

Basic Laparoscopic Techniques That Any Veterinarian Can Do

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Summary

During this session we will discuss some of the basics of laparoscopic surgery and go through procedures that general practitioners can perform in everyday practice. These will include ovariectomy / ovariohysterectomy techniques, cryptorchidectomy, gastropexy and abdominal organ biopsy.

As the popularity of laparoscopic surgery in veterinary medicine grows so does the armamentarium of procedures that are deemed “possible” with this approach. In human medicine a much more advanced evaluation of different minimally invasive surgical (MIS) techniques has taken place and is still evolving to try and establish the relative advantages of MIS approaches. In many cases clear superiority is reported in well-designed studies with large case numbers but in others significant controversy still exists.

Laparoscopic surgery in small animals is going through a sustained evolution that began with the description of some simple laparoscopic and laparoscopic-assisted procedures that are excellent starter procedures for veterinarians wanting to introduce MIS in their practice. More recently next-generation, less invasive versions of laparoscopic-assisted procedures as well as more advanced procedures performed totally laparoscopically have been developed. Currently the following laparoscopic procedures are carried out on a very regular basis by large numbers of practitioners in the USA: OVE/OVH, cryptorchidectomy, gastropexy, abdominal organ biopsy, feeding tube placement and cystotomy. Although the results of large numbers of these types of cases have not been reported they are generally very successful techniques and associated with low complication and conversion rates in the hands of most experienced surgeons. One of the key advantages of these techniques is that they replace open procedures for which anatomy is known and the steps involved in completing the procedure are familiar to most practitioners. The learning curve involved is therefore generally less arduous than for example, arthroscopic surgery.

Laparoscopic ovariectomy (OVE) and ovariohysterectomy (OVH) is a commonly performed procedure that has undergone an evolution in technique over the last few years. Several papers have described both one, two and three port techniques and the author generally favors a single port or two port technique using transabdominal suspension sutures to enable the ovaries to be elevated in order to be sectioned. A vessel-sealing device greatly facilitates efficient completion of OVE and OVH procedures although the procedure can also be completed with hemostatic clips, intra or extracorporeal suturing techniques or non-vessel-sealing bipolar electrosurgical devices.

Laparoscopic cryptorchidectomy has been described using a laparoscopic-assisted as well as a totally laparoscopic approach. The author prefers a totally laparoscopic approach and in this procedure the vascular supply and spermatic cord are ligated within the peritoneal cavity followed by removal of the testicle(s) from the abdomen. If the testicle is directly visible it can be grasped with laparoscopic Kelly or babcock forceps and elevated. In this way the vascular pedicle and spermatic cord can be moved away from surrounding structures in readiness for ligation. A vessel-sealing device can be placed into another instrument port and the gubernaculum, spermatic cord and vascular pedicle can be sealed and subsequently...
sectioned. The vascular pedicle can be very substantial in large dogs and care should be taken to ensure adequate hemostasis. Vessel-sealing devices have been used by the author to seal the pampiniform plexus effectively even in large dogs. If a vessel-sealing device is not available hemostasis can be achieved using hemostatic clips delivered using a laparoscopic clip applier. Although 5mm laparoscopic clip applicers are available medium/large clips are generally delivered in a 10mm clip applier. To reduce costs associated with the use of expensive single use disposable clip applicers, multifire resterilizable clip applicers which can be loaded with cartridges of clips are available (ML-10, Microline Pentax, Beverly, MA). When laparoscopic cryptorchidectomy has been performed the testicle(s) must be withdrawn through one of the ports. The author prefers to remove the testes through the subumbilical port as enlargement of the port on the linea alba is usually technically easier and less painful than when a paramedian port needs to be substantially enlarged.

Laparoscopic gastropexy is generally recommended in high-risk breeds and has been shown to be highly effective in prevention of gastric-dilation volvulus syndrome in these dogs. A classical laparoscopic-assisted gastropexy technique was described by Rawlings and is still used commonly today. It is very simple and efficient and generally has low morbidity although seroma formation and abscessation are rare complications. The authors group described intracorporeally-sutured gastropexy several years ago and showed that it was associated with improved post-operative activity in dogs compared to the laparoscopic-assisted technique that involves a full-thickness incision through the body wall in a paramedian location. Seroma formation is highly unlikely with this technique but a significant disadvantage is the technical challenges inherent in intracorporeal suturing and the longer surgical time associated with the technique. Newer barbed sutures have recently greatly facilitated intracorporeal suturing and made this procedure easier and faster.

Biopsies of the small intestine are usually performed using a laparoscopic-assisted technique. Exteriorization of bowel segments through a small "assist" incision followed by standard small intestinal biopsy collection from the antimesenteric border is usually the best technique in dogs and cats. Once any totally laparoscopic procedures (such as liver biopsy) are completed the telescope is removed from its subumbilical location and the port incision is enlarged to 2-3cm to allow placement of a 2-4cm laparoscopic wound retractor (Alexis®, Applied Medical Inc.). Once in place the circumferential force exercised at the wound margin holds open a small circular orifice into the peritoneal cavity. This relatively inexpensive device has several advantages. It prevents compression of the mesenteric root and subsequent vascular compromise thereby allowing large sections of intestine to be exteriorized for examination at any one time. It allows other structures such as the pancreas and mesenteric lymph nodes to be elevated enough to be easily biopsied. There are limitations to the use of small "assist" incisions for abdominal organ biopsy. It is difficult to exteriorize the proximal descending duodenum and the ileo-ceco-colic junction. The colon can be exteriorized although full-thickness colonic biopsy is strongly discouraged due to the high morbidity associated with dehiscence in this area and the excellent diagnostic quality that can be obtained from colonoscopic biopsies. Using either technique once the intestine is exteriorized, biopsies can be taken using a similar technique as would be used if an "open" celiotomy had been performed.

Constant re-evaluation and refinement of the procedures that have been described is an absolutely necessary part of the evaluation of new procedures in both human and veterinary MIS. Currently there is significant interest in human laparoscopy in single port surgery. The idea is particularly attractive in people as it allows the prospect of a single umbilical surgical site that is hidden from view and is thus to some extent "scarless". A number of device companies have developed single access devices to facilitate these procedures and some of them (e.g. SILS, Covidien Inc.) have been used in small animal patients. The author currently uses the SILS port for all gastropexy/OVE/OVH combinations, cryptorchidectomies and has used them for splenectomy in small dogs. Originally designed to be used with specialized articulating instruments they can also be used with regular laparoscopic instruments and allow for the placement of three portals (two 5mm and one 5-12mm portal). Using the SILS device despite the close positioning of the three portals significant triangulation is still possible due to the malleable nature of the device.
Keywords: Laparoscopy, Minimally Invasive, Ovariectomy, Gastropexy, Cryptorchidectomy

References
Advanced Laparoscopic Procedures – What Is the Limit?

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**Summary**
This lecture will discuss the more advanced procedures that can become realistic for surgeons in private practice to perform once they have grasped basic principles and practice with more straightforward procedures. Procedures to be discussed include laparoscopic adrenalectomy, cholecystectomy, splenectomy and ureteronephrectomy.

Once basic knowledge of laparoscopic instrumentation, technique and safe access has been mastered surgeons will be able to swiftly move on to more complex techniques if they are familiar with performing these techniques using open surgical approaches. One critical component of performing more advanced techniques is the recognition that appropriate case section becomes more and more important. As an example adrenalectomy is a complex intervention but is one that lends itself very well to a laparoscopic technique as long as vascular invasion has been ruled out. Small non-invasive lesions are very good candidates for a laparoscopic approach but larger more invasive lesions are poor candidates. If these more challenging cases are selected in the early phase of a surgeons learning curve it is likely that a much greater conversion rate will occur and the possibility that complications will occur is greater. The following procedures are more advanced interventions that the authors group perform regularly but only after careful consideration of whether or not there is a likelihood that the patients can be treated as optimally with an MIS approach as with an open surgical approach.

Laparoscopic adrenalectomy is performed regularly in humans for resection of both adrenocortical tumors as well as pheochromocytomas and has been reported in small cohorts of veterinary patients.\(^1\)\(^,\)\(^5\) We have performed this procedure now in >50 dogs and around 10 cats and feel that it can be performed efficiently, safely and with a very low complication and conversion rate. Case selection is absolutely paramount in these cases with careful exclusion of cases that have vascular invasion into the caudal vena cava being critical. For this reason we always perform a CT angiogram study pre-operatively to rule out vascular invasion. Tumors up to 5cm can readily be excised with experience although early in the learning curve it is better to select smaller masses that are technically less challenging to resect. We believe that LA is one procedure that lends itself so well to a laparoscopic approach that in the future, adrenal gland tumors might be routinely approached in this fashion, by those with the appropriate equipment and experience.

Laparoscopic cholecystectomy (LC) is the most frequently performed procedure in human MIS. In dogs and cats a very different spectrum of disease of the extra-hepatic biliary tract is encountered compared to humans. Currently, LC has been described principally for treatment of uncomplicated gall bladder mucoceles in two small cohorts of veterinary patients.\(^6\)\(^,\)\(^7\) The development of laparoscopic biliary tract procedures is hampered by the relatively low numbers of cases affected. In the future, if therapies such as endoscopic or laparoscopic bile duct exploration can be performed reliably and efficiently, a greater subsection of cases may become available for a laparoscopic approach. Currently, this author recommends that only uncomplicated gall bladder mucoceles (those without any evidence of obstruction or rupture) or symptomatic cholelithiasis
restricted to the gall bladder, be considered good cases for an LC approach. This procedure is technically demanding especially in the smaller dogs that are most commonly affected by mucoceles. Although first performed using extracorporeal ligation for common bile duct closure the use of stapling devices or self-locking clips may significantly shorten the surgical times reported for this procedure.

Laparoscopic splenectomy (LS) is also commonly performed in human patients and open splenectomy is one of the most commonly performed procedures in small animal surgery. Small numbers of LS have been described in experimental and clinical canine patients in the literature.5-10 The principal indication for splenectomy in cats and dog is for resection of potentially neoplastic lesions, which in many cases can be very large. If splenic size and/or lesion size is substantial a careful assessment needs to be made as to whether a laparoscopic approach is recommended. One difficulty with this procedure is that physical manipulation of heavy organs can be challenging laparoscopically and care has to be taken not to cause iatrogenic rupture of splenic lesions, which could lead to seeding of neoplastic cells within the abdominal cavity. However, there is a significant subset of dogs and cats that present for splenectomy that have smaller lesions or diffuse splenic disease that may be good candidates for this procedure.

Laparoscopic ureteronephrectomy is performed in human surgery as a treatment for a variety of conditions. Simple nephrectomy is used in the management of chronic pyelonephritis, obstructive calculus disease, traumatic injury, renovascular hypertension and congenital dysplasia. Radical nephrectomy which includes removal of the kidney as well as the associated adrenal gland, lymph nodes and surrounding tissues is the treatment of choice for most renal cell carcinoma cases. Prior to ureteronephrectomy being performed kidney function in the contralateral kidney should ideally be documented by measurement of glomerular filtration rate. Only one small cohort of cases of laparoscopic ureteronephrectomy exists in the veterinary literature.11 Appropriate cases include modestly sized primary renal neoplasms, chronic renal failure with infection, renal dysplasia and idiopathic renal hematuria. Contraindications should initially include large renal masses including neoplasia, hydronephrosis and pyelonephritis with abscessation, and if there is any infection that extends beyond the renal capsule. In clinical cases pre-operative imaging with ultrasonography and preferably computed tomography (or MRI) would be very helpful in ruling out conditions that might make a laparoscopic approach undesirable.

**Keywords:** Laparoscopy, Adrenalectomy, Cholecystectomy, Splenectomy, Ureteronephrectomy

**References**


Thoracoscopy – How to Achieve Safe Access and Great Working Space

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Summary
Basic principles of thoracoscopy including anesthetic management, the equipment required and principles of access will be discussed in this lecture. The creation of working space is especially important in the thorax and therefore discussions will occur on the principles of one-lung ventilation and carbon dioxide insufflation.

The major difference between minimally invasive procedures in the thoracic and peritoneal cavities is that thoracoscopy does not usually require insufflation because the ribs form a rigid frame that maintains a working space in which the surgeon can manipulate organs and operate instruments. For the same reason, there is no need to maintain a tight seal around cannulae that are placed into the thoracic cavity. To allow pneumothorax to develop, access to the thoracic cavity can be initiated either using an open technique or an optical entry technique which is the technique favored by this author. These techniques can be used for initial thoracic access using a subxiphoid location or an intercostal location depending on which procedure is being performed. In general techniques that would be performed in dorsal recumbency using a median sternotomy approach will be performed using an initial subxiphoid telescope portal (e.g. pericardectomy). Techniques that would normally be performed using an intercostal thoracotomy will be performed in lateral recumbency using an initial intercostal telescope portal placement (e.g. lung lobectomy, thoracic duct ligation). While a pneumothorax will allow some surgical procedures to be performed adequately there are interventions in which greater working space will be required to complete procedures safely. In this lecture we will also discuss anesthetic techniques that aid in creating greater working space for those procedures that require it.

Establishment of initial telescope portal
Open technique – The traditional open technique is similar to the Hasson technique used for laparoscopy in that a small skin incision is made after which dissection down through the deeper tissue layers is continued until the parietal pleura is penetrated. Dissection can be pursued either using a combination of blunt and sharp dissection or with the aid of monopolar electrosurgery. Many surgeons like to perform this deeper dissection using blunt dissection with a mosquito hemostat. Penetration of the actual pleura itself with electrosurgery is avoided to prevent iatrogenic injury to the lung tissue beneath. Once penetration of the pleura is complete the incision is widened to allow passage of the cannula. Correct cannula placement can be confirmed by passing the telescope down the cannula to allow visualization of intrathoracic structures. If a subxiphoid telescope portal is being placed, final cannula penetration into the pleural cavity is usually performed at least partially with the trocar-cannula assembly as penetration through the most ventral part of the diaphragm is challenging with surgical instruments due to it’s deep location.

Optical entry – Optical entry involves the process of visualizing penetration of the thoracic wall tissues with the telescope as cannula placement is being performed. This necessitates the use of either a trocarless
cannula (e.g. Ternamian EndoTip cannula, Karl Storz Veterinary Endoscopy) or a specialized disposable optical entry cannula (e.g. Versaport Bladeless Optical trocar, Covidien Inc, Mansfield, MA or Kii Fios First Entry, Applied Medical, Rancho Santa Margarita, CA). Optical entry is gaining widespread use in the human field and is usually performed using specialized disposable cannulae that incorporate a translucent trocar with a lumen that can accommodate the telescope. An initial 1-1.5cm incision is made in the desired location of the portal. The subcutaneous tissues can be incised although penetration into the deeper layers is achieved by advancement of the cannula-trocar assembly during placement. Placement of the cannula is initiated just until the cannula starts to become “seated” in the deeper tissues. Once this has occurred the telescope is placed into the cannula (or trocar-cannula assembly in the case of disposable versions) and the cannula is advanced into the thorax by firm pressure combined with a twisting motion around the telescope. This can be done both for subxiphoid cannula placement or intercostal placement. During this procedure the telescope is able to visualize penetration of the deeper muscle layers as it occurs. Additionally penetration of the visceral pleura is seen as soon as it happens thus avoiding placement of the cannula into an excessively deep position. This technique is currently the preferred technique of the authors and is performed very effectively using the trocarless EndoTip cannula in veterinary species. Use of disposable cannulae for this technique is also very successful although the cost of these specialized cannulae can be high.

Establishment of instrument portals
Once access to the thoracic cavity is obtained instrument ports are generally placed in a triangulating pattern around the area of interest under direct visualization. Port positions for different techniques are dictated by the surgical technique being performed and to some extent by surgeon preference. When establishing instrument ports for thoracoscopy, it can be helpful to make a small skin incision with a scalpel blade and then use mosquito or Kelly forceps to bluntly dissect a small hole that penetrates the pleural cavity before passing the instrument cannula under direct visualization. Many options exist for instrument portal cannulae and cannula size needs to be chosen based on the anticipated size of the instruments that will be passed through each cannula (e.g. if surgical staplers are to be used most are 12mm diameter devices and so cannulae capable of accommodating 12mm instrumentation need to be placed). In procedures that are performed with the patient in dorsal recumbency, the first step after access to the thoracic cavity has been established is to section the ventral mediastinal attachments. These are usually thin “curtains” of tissue that hang down from the sternum and are generally poorly vascular. However, because any hemorrhage can cause significant loss of visualization, these ventral mediastinal attachments are best sectioned using a vessel-sealing device or electrosurgical unit. In some dogs with long-standing pleural effusion or pleuritis more substantial neovascularization can be encountered within the mediastinal tissues. Once this tissue has been sectioned, access to both hemithoraces is established and the surgical procedure can proceed.

Anesthetic techniques for thoracoscopy
For certain thoracoscopic interventions such as pericardial window, thoracic duct ligation and lung biopsy, a pneumothorax that forms within the chest when the first cannula is placed and air is allowed to enter the pleural cavity, will provide adequate working space for the procedure to be completed safely. For these procedures anesthesia concerns are similar to those for any “open” thoracotomy.

To increase the working space in the thoracic cavity during more advanced thoracoscopic procedures there are several techniques that can be used. Intermittent ventilation can be used for shorter procedures and is variably tolerated by animals under anesthesia. Animals with normal pulmonary parenchyma may tolerate long breaks between ventilation, however those with cardiorespiratory disease may be less tolerant. Intermittent ventilation is generally frustrating for more complex procedures where intermittent inflation of lung fields obscures visualization making iatrogenic trauma to pulmonary parenchyma during instrument exchanges more likely and generally prolongs the procedure.

Thoracic insufflation with a closed chest can be performed if thoracic cannulae with one-way valves are used, as is done for laparoscopic surgery. Carbon dioxide is used for thoracic insufflation just as it
is for creation of a pneumoperitoneum. The big advantage of thoracic insufflation is that it is very easy to institute and can significantly increase the volume of working space. The big disadvantage is that the thoracic cavity is poorly tolerant of positive pressure insufflation due principally, to the many thin-walled low pressure vascular compartments within it which include the right side of the heart, cranial and caudal vena cava and pulmonary veins. Even at low insufflation pressures (3mmHg), significant cardiopulmonary depression has been shown to occur in dogs\(^2\) although recent work by the authors group suggests that it is better tolerated in cats.

One-lung ventilation (OLV) is generally the preferred method for increasing the working space during more advanced thoracoscopic interventions. Improved visualization can help avoid iatrogenic trauma to tissues that can occur when visibility is impaired by repeated lung inflation. Whenever OLV is used, significant physiological changes must be anticipated as a significant ventilation-to-perfusion mismatch occurs as a result of non-ventilated lung remaining perfused. However, studies have shown that no large effect on oxygen delivery in healthy dogs occurs during one-lung ventilation.\(^3\) It should be remembered that tidal volume must be reduced usually by a factor of 30-50% to avoid barotrauma and to compensate, the respiratory rate is usually increased by approximately 20%. Positive end-expiratory pressure (PEEP) of 5cm H\(_2\)O can be helpful during OLV and has been shown to increase PaO\(_2\) and decrease shunt fraction without having a detrimental effect on cardiac output.\(^4\) Subjectively, the author has noted that PEEP can, however, significantly impair visualization in the chest of smaller or flat-chested dogs where even small amounts of residual inflation during ventilatory cycles can decrease visualization of organs.

Various techniques can be used to create OLV including use of endobronchial blockers (EBB), selective intubation, or double lumen endobronchial intubation (DLT).\(^5\) All usually require bronchoscopic-assisted placement although blind thoracoscopic-assisted placement of DLTs has been described and is feasible in certain breeds of dog.\(^6\)

Selective intubation involves the placement of a smaller diameter long endotracheal tube into one mainstem bronchus. A bronchoscope is placed down the lumen of the tube and guided into either the left or right mainstem bronchus depending on which side requires ventilating (contralateral lungfield to the side of the lesion). Selective intubation is used principally in the author’s institution for very large dogs in which DLT’s are usually too short and in which the balloon on the tip of the EBB is sometimes not large enough to completely occlude the lumen of the mainstem bronchus.

EBBs are relatively easy to place and consist of an endotracheal tube with either a small diameter balloon-tipped catheter attached to the end of the tube or running within the lumen of the tube. In the most common model used (Amid Endobronchial Blockerä, Cook Medical Inc.) the bronchoscope is passed through a suture loop on the tip of the EBB as it is passed down the lumen of the endotracheal tube. This allows the balloon-tipped catheter to be guided by the bronchoscope into one or other mainstem bronchus so that when the balloon is inflated, fresh gas inflow is prevented from entering the now obstructed right or left mainstem bronchus. They are available in 5,7 and 9 Fr sizes with either spherical or round balloons. These work well although in very large dogs the balloons on even the largest blocker sizes may not inflate to a large enough diameter to completely occlude a mainstem bronchial lumen. The 5Fr EBB is probably the best option for induction of OLV in the smaller patients as DLT cannot be used in dogs smaller than about 10kg.

Double-lumen endobronchial tubes (DLT) are slightly more challenging to place but have the advantage of allowing alternating one-lung ventilation that can be useful in situations in which both sides of the thoracic cavity need to be examined or in which the precise location of the lesion is unknown, such as occurs sometimes in cases of spontaneous pneumothorax caused by pulmonary bullae or blebs. It is also the only method, in theory, for which intra-operative bronchoscopic-assisted manipulation of the tube is not necessary if alternating left and right ventilation is required. Ventilation can be alternated between the right and left lungfields by swapping the fresh gas inflow from the tracheal to the bronchial side using DLT’s.

In all cases, whether selective intubation, endobronchial blockade or DLT’s are used, great care needs to be taken monitoring anesthesia. The most significant clinical problem is tube displacement
intra-operatively. Most commonly this results in loss of OLV intraoperatively, impairing the surgeon’s ability to proceed with the procedure. Less commonly if a bronchial blocker or DLT slips cranially out of the mainstem bronchus into the trachea it is possible for an acute airway obstruction to occur resulting in total cessation of ventilation. This must be noticed immediately and will necessitate tube repositioning. Placing the OLV tube in the operating room rather than in the anesthesia preparation area will minimize movement of the patient and minimize risk of initial displacement.

Keywords: Thoracoscopy, Optical Entry, One-Lung Ventilation, Thoracic Insufflation

References
Advanced Thoracoscopic Procedures in Dogs and Cats

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Summary

This lecture will present tips and video presentations to illustrate thoracoscopic biopsy techniques, pericardectomy, lung lobectomy and cranial mediastinal mass resections amongst others. The technical challenges of these techniques will be discussed as well as anesthetic techniques required to complete them successfully.

Thoracoscopic surgery offers an exciting new modality for treatment of a variety of thoracic disease processes. In humans suggested advantages of VATS include a reduced volume of thoracic drainage, less post-operative pain, shorter hospital stay and a more rapid return to normal function.\textsuperscript{1,2} Limited objective comparisons of “open” versus VATS procedures have been reported in the veterinary literature but similar advantages are likely to be present in small animal patients.\textsuperscript{3} One study using a pericardectomy model showed that dogs undergoing thoracoscopic pericardectomy had lower pain scores and decreased requirements for analgesic medications compared to dogs undergoing open thoracotomy.\textsuperscript{3}

Thoracoscopic Pericardectomy

Pericardectomy is indicated for treatment of various cardiac and non-cardiac diseases. Creation of a pericardial window (PW) can relieve clinical signs associated with idiopathic or neoplasia-associated pericardial effusion.\textsuperscript{3,4} Subphrenic pericardectomy (SPP) is the treatment of choice to relieve the symptoms associated with constrictive pericarditis and has been recommended as an adjunctive therapy in patients with idiopathic chylothorax.\textsuperscript{5,6}

For creation of a thoracoscopic PW generally no increase in working space is required during the procedure and so CO\textsubscript{2} insufflation or one-lung ventilation (OLV) are generally not required. The need for OLV in thoracoscopic SPP is debatable but in this author’s opinion OLV can be very helpful in allowing a maximal amount of pericardium ventral to the phrenic nerve to be removed whilst minimizing the possibility of iatrogenic damage to surrounding organs especially in flat-chested dog breeds.

For both PW and SPP dogs are generally positioned in dorsal recumbency. Thoracoscopic access is achieved through placement of a subxiphoid camera port. Once access to the chest is obtained, a pneumothorax is allowed to form and an instrument port is established at the 4-6\textsuperscript{th} intercostal space on the right or left sides. The ventral mediastinal attachments to the sternum are removed with a scissor or vessel-sealing device. Once visualization of both hemithoraces is obtained a second instrument port is established at the 4-6\textsuperscript{th} intercostal space on the contralateral side in identical fashion to the first.

Once unobstructed visualization of the cardiac apex is achieved the pericardium is incised over the apex. This can be the most challenging part of the procedure as care needs to be taken not to damage the underlying epicardium or coronary vessels. If a significant pericardial effusion is present incision into the pericardium is usually easier and safer as the fluid will act as a protective barrier to avoid iatrogenic damage to the underlying epicardium. Generally, the pericardium is either elevated using a Kelly or babcock forcep and a laparoscopic...
scissor is used to incise into the pericardial sac. A scissor or preferably a vessel-sealing device is then used to excise an approximately 4x4cm window of pericardium over the apex of the heart. Whichever device is used to create the window care should always be taken to avoid damage to the underlying coronary vessels and epicardium. Resected pericardial tissue should be removed from the thoracic cavity either through a large cannulae (10-12mm) or in a specimen retrieval bag.

For SPP initial penetration of the pericardium occurs in the same way as described above for PW. Through the established pericardial incision the vessel-sealing device is used to section the pericardium ventral to the phrenic nerve on both sides so that almost all the pericardial sac is removed. Techniques for SPP have been described for SPP with or without the use of OLV. Principal complications of pericardectomy include iatrogenic damage to underlying epicardium or coronary vessels, or damage to the phrenic nerve if a SPP is performed.

**Thoracoscopic lung biopsy and lobectomy**

Many primary lung tumors may be diagnosed on plain radiography. However, advanced imaging may be beneficial to aid in surgical planning and to rule out metastatic disease. Computed tomography is generally considered in human medicine to be the diagnostic imaging modality of choice for pulmonary imaging of small masses and metastatic disease and is usually performed by the author in any case where thoracoscopic lung lobectomy (TLL) is being considered.

The use of OLV is mandatory for a thoracoscopic partial or complete lobectomy in order to provide adequate visualization. A thoracoscopic lung biopsy can be harvested in a number of ways. One of the easiest and cheapest ways is to use a loop ligature that is placed around the periphery of one of the lung lobes to be biopsied. Alternatively an endoscopic stapler (EndoGIA) can be used to harvest a lung biopsy. Again with the help of babcock forceps to stabilize the periphery of a lung lobe the stapler is placed across the lung tissue to be biopsied and the staples are deployed. The stapler will place three lines of staples on either side of the cut line thus providing very secure closure of all airways and blood vessels within the lung tissue. For TLL the mass in the lung can usually be visualized after OLV has been established. If the mass cannot be obviously visualized it may be safer to attempt a full lung lobectomy to ensure that the mass is not missed. In the case of the caudal lung lobes the pulmonary ligament which attaches the caudal lung lobes to the mediastinum must be sectioned from caudal to cranial up to a point close to the pulmonary vessels. The EndoGIA is used for sectioning the pulmonary artery vein and bronchus. Several cartridge lengths (30,45 and 60mm) and staple sizes (2.0, 2.5, 3.5, 4.8mm) are available. For lung lobectomy in dogs it is generally recommended that 60mm long cartridges are used with 3.5mm staples. The EndoGIA stapler comes in a straight linear form or in a “roticulating” form where the tip of the cartridge can be moved from side to side. This is a very useful feature when attempting to maneuver the stapler into position around the base of the pulmonary hilus.

Once the lung lobectomy is completed the cut surface of pulmonary hilum is closely visualized for hemorrhage or air leakage. The lung lobe should then be placed in a specimen retrieval bag and the bag removed through one of the portals.

Several important intra- and post-operative complications can be associated with thoracoscopic lung lobectomy. Hemorrhage from intercostal vessels has been reported causing significant post-operative hemorrhage. Hemorrhage or air leakage from the pulmonary hilus can also occur if the EndoGIA stapler does not function correctly or is incorrectly placed.

**Thoracoscopic thoracic duct ligation (TDL)**

Chylothorax is a complex disorder that results in accumulation of chylous fluid within the thoracic cavity. In some cases it is associated with an underlying disease process but in many cases it is idiopathic in nature. Medical management of chylothorax is rarely successful and it is considered a surgical disease by most clinicians. Although many different surgical procedures have been used for management of chylothorax
the combination of thoracic duct ligation and subphrenic pericardectomy (SPP) has been associated with some of the highest success rates albeit in small populations of dogs. The outcome of a minimally invasive approach for TDL and SPP has now been described in small cohorts of dogs and it appears to be associated with similar success rates as when an open approach is used. The MIS approach can obviate the need for single or double intercostal thoracotomy which has often been used for management of this condition. The SPP technique is the same as used in the section listed above and uses different portals from those used for the TDL. Therefore the two techniques are largely performed as two individual procedures performed in sequence during the same anesthetic episode.

For TDL dogs are placed in left lateral recumbency. The first thoracic portal for the placement of the telescope is placed at the mid- to dorsal third thoracic level in the 8th or 9th intercostal space allowing visualization of the caudal mediastinal area. Two further instrument portals are placed at the 7th or 8th and 9th or 10th intercostal spaces in a slightly more dorsal location and in a triangulating pattern around the caudal mediastinum.

Once port placement is complete, a 3-4cm laparotomy incision is made in the patients’ right cranial abdominal quadrant. Mesenteric lymph nodes in the area of the ileo-ceco-colic junction are exteriorized through this incision and methylene blue dye diluted 1:1 with sterile saline is slowly infused into a lymph node to a dose not exceeding 0.5mg/kg. As soon as the methylene blue is visualized in the thoracic duct branches, blunt dissection around the duct branches in the mediastinal root dorsal to the aorta is initiated. A space between vertebral arterial branches is selected as far caudal within the thoracic cavity as possible while still allowing dissection and clip placement. As much dissection across the mediastinal base as possible is performed and all visualized thoracic duct branches are clipped using a laparoscopic clip applier.

**Thoracoscopic cranial mediastinal mass resection**

Cranial mediastinal masses in dogs are most frequently diagnosed as either thymoma or lymphoma with ectopic thyroid carcinoma, branchial cysts and chemodectomas being much less common. If a cytological or histopathological confirmation of a thymoma is made surgical resection is usually recommended. Many thymomas will be too large to be amenable to an MIS approach although those that are up to 5-6cm in diameter in middle to large breed dogs and are not invading surrounding organs may be amenable to a thoracoscopic resection.

Surgical anatomy of these masses is quite variable and so preoperatively a CT scan can be very helpful in clinical decision making. One-lung ventilation may also be very helpful during the procedure to maximize visualization and potentially reduce iatrogenic damage to pulmonary parenchyma during dissection.

The dog is placed in dorsal recumbency and a telescope portal is established in a subxiphoid location. A 3cm incision is then made at the left 4th intercostal space at the level of the dorsal third of the thorax in an area adjacent to the mass (if the mass is in more right sided which is less common this incision can be made on the right side). This incision can be used to place a finger into the thoracic cavity to aid in manipulation of the mass during dissection and also to remove the mass at the termination of the surgery. A second port will usually be placed on the right side at the 6-9th IC costal in the ventral third of the thoracic cavity. Once all ports are in place, a blunt grasping instrument, blunt probe or gloved finger are used for manipulation of the mass and a vessel-sealing device is used to initiate the dissection of the mass from the surrounding tissue planes. In some cases the masses are attached to the internal thoracic artery and careful dissection is required to dissect these two structures from one another. Every effort should be made to resect the mass without penetrating the tumor capsule. Once completely dissected the mass is placed into a specimen retrieval bag and removed through the cranial left-sided portal.

**Keywords:** Thoracoscopy, Pericardectomy, Thoracic Duct, Thymoma
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Is There a Role for Minimally Invasive Surgery in Surgical Oncology?

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Summary
This lecture will give attendees an introduction to the principles of oncologic surgery and the discuss whether or not minimally invasive approaches can meet the standards that we expect from in surgical oncology. Using current examples of procedures performed in minimally invasive surgery we will discuss whether we can meet these standards for procedures such as adrenalectomy and lung cancer.

The role of minimally invasive surgery (MIS) in cancer management remains a topic of great debate in human medicine where long-term studies of certain procedures that have only been performed for 15-20 years in some cases is still not available. As an example surgical management of adrenocortical carcinoma (ACC) remains highly controversial in people. Although laparoscopic adrenalectomy (LA) for benign disease is considered standard of care, LA for resection of ACC has been seriously questioned. Some studies suggest equal results between LA and open adrenalectomy (OA) after resection of ACC whereas others have found that recurrence and disease free interval are inferior to results obtained with traditional OA. ACC is, however, a highly malignant neoplasm in humans with nodal and distant metastases present in 26.5% and 21.6% respectively at the time of diagnosis. By comparison, in dogs, ACC is a less malignant tumor with distant metastasis reported to occur in only 5-14% of cases and reports of local recurrence after adrenalectomy being extremely rare. This example highlights the need for good evidence based studies to evaluate the oncological outcomes of different surgical approaches as it may be that an MIS approach to certain malignancies may be inferior to more traditional open approaches. Furthermore this example highlights the danger in extrapolating human data to our veterinary species.

A variety of studies do now exist to support the hypothesis that an MIS approach may aid in minimizing pain, improving return to function and decreasing the risk of surgical site infection (SSI) after surgery compared to open approaches. In humans there is gathering evidence that the biological response to minimally invasive surgery may be superior to that seen after open surgery. These studies mainly concentrate on acute phase reactants and cellular immune responses and have shown that MIS approaches cause less interference with these normal post-operative immune responses which may account for some of the advantages in short term perioperative morbidity seen in patients undergoing MIS techniques compared to open surgery. To date the data that documents oncological outcomes after MIS in humans is variable but remains weak in veterinary medicine. While we strive to reduce perioperative pain and minimize morbidity in our patients by employing these less invasive techniques we must remember that any approach that compromises oncological surgical principles may be detrimental to long-term oncological outcomes.

Some evidence is starting to emerge in veterinary patients although little long-term data on recurrence or disease-free interval/overall survival is available. We have used laparoscopy successfully for resections of renal, splenic, urogenital and gastrointestinal malignancies although at this point in time inadequate numbers of these cases have been performed to critically analyze the use of a minimally invasive technique compared
to traditional approaches. An example of an oncologic laparoscopic approach that we have built up more substantial experience with is adrenalectomy. In recent years our group at UCD has performed LA for resection of benign or malignant adrenal masses in 46 dogs and 9 cats. We recently published data on the cohort of canine patients with adrenocortical masses that underwent LA and compared it to a population that had similar tumors resected in an open fashion in the years that predated the introduction of the LA technique. In this study we were able to show that intraoperative complication rates were no greater with the LA technique than with OA. Surgical time was decreased in the LA group compared to the OA group and hospitalization time was decreased for the LA group compared to the OA group. Although the incidence of capsular rupture was higher in the LA group, this intraoperative problem may not have been accurately documented in every open case, data on which was collected retrospectively from old medical records. Capsular rupture occurred with some frequency (7/22) in our cohort of LA cases, however, a finding which has been similarly described by other groups. The significance of impingement on the tumor capsule is unknown although no group has ever described a tumor recurrence after LA. In two dogs that were worked up at UCD for recurrent signs of Cushings disease after unilateral LA both had contralateral adrenal tumors only with no evidence of tumor recurrence on the original operative side. We suspect that capsular rupture occurs with similar frequency between OA and LA techniques and that its significance is limited. However it is these kinds of questions that require critical evaluation going forward. We also recently reported outcomes of a cohort of 10 dogs that underwent LA for resection of non-invasive pheochromocytomas. These dogs all recovered well from surgery with no perioperative deaths and no known recurrences. Interestingly in none of these cases was gross capsular rupture seen intra-operatively.

In the thorax the use of minimally invasive approaches might be particularly beneficial given the potential for avoiding a thoracotomy or sternotomy approach. One study has evaluated the difference between an open and a video-assisted thoracoscopic (VATS) approach in a canine pericardectomy model. In this report the VATS approach was associated with significantly lower pain scores in the post-operative period as well as higher blood glucose and cortisol concentrations in the thoracotomy group. Our group started using VATS approaches for certain types of intra-thoracic neoplasia some 10 years ago and have performed significant numbers of lung lobectomies for removal of primary or metastatic lung neoplasia, resection of thymomas, palliation of cardiac or pericardial neoplasia by pericardectomy as well as staging of intrathoracic malignancy by lymph node biopsy or collection or pleural or mediastinal biopsies. As in the examples used for laparoscopic management of abdominal neoplasms it is imperative that minimally invasive management of intrathoracic neoplasms be as complete and as effective as when an open thoracotomy or sternotomy is employed. In a recent study our group evaluated results from a cohort of 22 dogs that underwent VATS lobectomy for resection of primary lung tumors and compared them to a group of 24 dogs that underwent the procedure using an open approach. We were able to show that the morbidity between groups was no different although the surgical time for the VATS approach in this study was significantly longer. We evaluated the surgical margin of resection where possible and found that incomplete margins of resection were found in only 1/13 dogs in the VATS group and 1/14 in the open thoracotomy group suggesting that the VATS approach does not in any way compromise the ability to take a wide margin of normal tissue in these cases. A surgical technique for VATS resection of tracheobronchial lymph nodes for cancer staging has been reported in healthy dogs as well as a small number of clinical cases with primary lung tumors. However, it remains to be seen whether a VATS technique can be as effective for tracheobronchial lymph node sampling as an open approach. Long-term oncological outcomes of VATS lobectomy for management of primary lung tumors in dogs has not been evaluated in a large cohort of dogs but a recent case-matched study compared 9 dogs undergoing VATS lobectomy to 9 dogs undergoing thoracotomy with no differences in long-term survival reported. Further larger scale studies are required to confirm these findings.

It should be remembered, however, that challenges exist with many minimally invasive surgeries. Many are technically challenging and will require the acquisition of special equipment and additional training. For example lung lobectomy requires familiarity with one-lung ventilation techniques and is greatly facilitated by the availability of suitably trained anesthesia staff. If these resources are available, however, and appropriate
case selection is exercised we firmly believe that many abdominal and thoracic oncological challenges can be approached using less invasive approaches that may greatly benefit the patient. In conclusion much research remains to be done in establishing which procedures will lend themselves well to an MIS approach and in which cases a traditional open approach might remain the wiser choice. We must continue to strive to critically evaluate the procedures we perform to ensure that our MIS procedures are really delivering the advantages that we believe they have the potential to.

**Keywords:** Oncology, Minimally Invasive, Laparoscopy, Thoracoscopy

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Principles of Surgical Oncology

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Summary

Radiology is a cornerstone of veterinary diagnostics and therapeutics, and other specialties routinely rely on this discipline to optimize care. Many advances have been made in veterinary radiology over the last 20 years, and we are currently experiencing an abundance of new and exciting opportunities particularly in our ability to provide image-guided interventions.

Interventional Radiology (IR) is a specialty that utilizes different imaging modalities to perform minimally invasive diagnostics and therapeutics. Interventional Oncology (IO) is sub-specialty of IR in which these minimally invasive options are utilized in the treatment of neoplastic disease. Many of the treatments that are performed with IO techniques are palliative, and the goal is to improve the patient's quality of life. However, IO techniques are quickly expanding into potential options as a primary treatment or in the neoadjuvant/adjuvant setting. The two major treatment categories currently being pursued in veterinary IO are the administration of locoregional therapies such as intra-arterial chemotherapy and embolic agents and the stenting of malignant obstructions.

Locoregional Therapies: Intra-arterial chemotherapy

Conventional chemotherapy involves administration of a drug into a peripheral vein resulting in systemic dosing of the drug. When given intravenously, the drug also undergoes dilution prior to reaching the tumor.(1) The administration of intra-arterial chemotherapy is common practice in human medicine. The major reason for administration of chemotherapy directly into the arterial supply of a tumor is that a higher concentration of drug can be accumulated locally (at the tumor site), with the potential for less systemic side effects.(2)

In humans, intra-arterial chemotherapy is often administered in conjunction with radiotherapy in an attempt to achieve better outcomes.(3,4) This concept has also been exploited in clinical veterinary cases of bladder carcinoma and osteosarcoma.(5-8) In a study evaluating the combination of intra-arterial chemotherapy (cisplatin) with radiation therapy for treatment of bladder cancer, two dogs demonstrated an objective reduction in tumor size.(5) Side effects and toxicity were minimal in these two dogs.(5)

Locoregional Therapies: Embolization/Chemoembolization

Transarterial embolization (TAE) and transarterial chemoembolization (TACE) are well-established treatment modalities in human medicine. TAE and TACE are generally not considered first-line therapies when other standard treatments such as surgery remain as a viable option.(9)

TACE has been promoted for several reasons. Eliminating the blood flow to an area that has received
chemotherapy will reduce the wash-out of that drug.(10) Additionally, vessels that are exposed to chemotherapy will also become ischemic (secondary to the embolization), making them more susceptible to the toxic effects of the drug. Blood stasis secondary to embolization may also cause the drug to be retained in the tumor for an extended period of time.(10) As the chemotherapy is given directly into an artery that is feeding the tumor, the systemic side effects may be less.(10)

The effects of TACE and TAE have been most studied in the liver. The liver has a unique dual blood supply that allows for embolization, while still maintaining an adequate blood supply to healthy hepatic tissue. The portal vein supplies the majority of the liver’s blood supply (75-85%) with the hepatic artery supplying the rest.(11) Additionally, the hepatic artery is the major blood supply to most primary hepatic tumors (85-100%) and tumors that metastasize to the liver (80-100%).(12) This unique arterial blood supply allows for occlusion of the blood supply to the tumor without causing ischemia to normal liver tissue.(9)

Clinical veterinary literature documenting both TAE and TACE therapies is limited, and those available have demonstrated mixed results. The embolization of hepatocellular carcinoma, hepatocellular adenoma, fibrosarcoma, nasal adenocarcinoma and metastatic osteosarcoma has been attempted.(13-16) In the report evaluating two cases of hepatocellular carcinoma, one dog received TAE and one dog received TACE.(14) In those cases, the survival times post embolization were approximately four months and 28 days, respectively; however, subjective decrease in the size of the tumor in the dog undergoing TAE was noted and blood flow to the embolized region was decreased.(14)

Investigation into the use of TAE and TACE in the treatment of other tumor types has also been performed in humans, and embolization of neoplasia of the nasal cavity, thyroid and prostate is being investigated in clinical veterinary cases. Further research into this arena may reveal other tumor types that might respond to these treatments, potentially improving outcomes for companion animals with neoplasia.

Malignant Obstructions: Urethral/Bladder/Prostate Neoplasia

Tumors resulting in malignant obstruction of the urethra can originate from the bladder, urethra or prostate. Traditional treatment options for tumors causing obstruction of the urethra generally fall into three general categories: surgery, chemotherapy and radiation therapy. While these treatments may be good options for the initial treatment of non-obstructive urethral neoplasia, these are not good options for immediate relief of complete urethral obstruction secondary to neoplasia. Palliation of urethral obstruction by urethral stent placement has been extensively described in human clinical medicine(17) and experience is increasing in veterinary clinical medicine.(18)

Malignant Obstructions: Ureteral Obstruction

Most ureteral obstructions occur secondary to neoplasia that originates in the bladder or urethra. A technique for percutaneous placement of ureteral stents in dogs with malignant ureteral obstruction was recently reported.(19) In this series of 12 dogs, the obstructed ureters were accessed in antegrade fashion through the placement of a needle within the renal pelvis (successful in 11/12 dogs). In all cases, a double-pigtail stent was placed, and all patients with azotemia demonstrated improvement in BUN and creatinine concentrations post-stent placement. Overall, ureteral stent placement was determined to be safe and well-tolerated in this cohort of dogs.(19)

Malignant Obstructions: Tracheal Neoplasia

Several treatment options exist for tracheal masses. Surgical resection and anastomosis can be performed in select cases where it is deemed to be possible and appropriate. Additionally, endoscopic debulking has also been recently employed to decrease tumor burden and palliate clinical signs.(20) Chemotherapy and radiation therapy can be considered in cases that are sensitive to these treatments. In those cases
where surgery, bronchoscopic reduction, chemotherapy and radiation therapy are not considered good treatment options or not elected by the owner, palliative stenting of the trachea to relieve clinical signs may be warranted. A single report of malignant tracheal stenting exists in the veterinary literature. In that study, a cat with a tracheal adenocarcinoma, experienced alleviation of clinical signs and maintained a good quality of life until metastatic disease was noted.\(^{(21)}\)

**Malignant Obstructions: Colorectal Neoplasia**

Surgery is considered the treatment of choice for most non-lymphomatous colorectal tumors and several surgical treatments have been developed to resect both small and large tumors. In certain cases, humans with colorectal masses may undergo stenting in the treatment both benign and malignant tumors. Colorectal stents in humans have two primary indications: as a “bridge to surgery” which allows for patient stabilization prior to undergoing an elective procedure or to palliate clinical signs.\(^{(22,23)}\) In the “bridge to surgery” group, stent placement prior to undergoing an elective procedure has been shown to improve outcome (less complications, less unnecessary operations).\(^{(23)}\) As opposed to major surgical resection or colostomy stoma formation, some patients elect to have a stent placed to palliate clinical signs.\(^{(24)}\)

A report of two cats and a report of one dog undergoing colonic stent placement can be found in the veterinary literature.\(^{(25,26)}\) In the feline cases, colonic obstruction was relieved after stent placement and both cats maintained fecal continence. The authors reported that colonic stenting provided an effective palliative option for those two cases.\(^{(25)}\)

**Keywords:** Interventional Radiology, Interventional Oncology, Stent, Embolization, Chemoembolization

**References**


Abdominal Neoplasia

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Summary
Abdominal neoplasia is common in veterinary patients. Abdominal masses are often diagnosed on physical examination, and useful diagnostics may include bloodwork, radiographs and ultrasound or more advanced imaging techniques such as computed tomography and magnetic resonance imaging. Surgery is often the treatment of choice for abdominal neoplasia cases and outcomes is associated with diagnosis. In this lecture, we will discuss the most commonly diagnosed abdominal neoplasms focusing on tumors of the spleen, liver and urogenital tract.

Splenic Tumors

Depending on the evaluated study, 45-92% of splenic tumors have been shown to be hemangiosarcoma.(1) Other tumor types that have been identified include histiocytic sarcoma, lymphoma, mast cell tumor, and leiomyosarcoma; metastatic disease to the spleen has also been diagnosed. German shepherd dogs, Labrador retrievers and golden retrievers are overrepresented for the development of splenic hemangiosarcoma.

When considering the diagnosis of a splenic tumor, many clinicians rely on the so-called “two-thirds” rule. This rule refers to the rough estimate that two-thirds of splenic masses are malignant and approximately two-thirds of those cases are hemangiosarcoma. Several canine studies have evaluated factors that may assist clinicians in determining a splenic diagnosis prior to obtaining a sample for histopathology. In one study, dogs with hemangiosarcoma had significantly lower total solids concentrations and platelet counts.(1) Additionally, dogs with hemoperitoneum are more commonly diagnosed with hemangiosarcoma as compared to hematoma.(2)

In cats with hemoperitoneum, approximately 60% of malignant neoplasia cases are splenic in origin.(3) As opposed to dogs, the liver is the most common location for hemangiosarcoma. The spleen is the second most common location accounting for 23% of cases.(4)

As many splenic tumor cases present after rupture, treatment prior to surgery includes stabilization. Blood products may be needed to add both red blood cells and clotting factors to the patient’s circulation. After stabilization, or if stabilization is unable to be achieved with aggressive treatment, surgery should be pursued. The main surgical techniques for splenectomy include a hilar splenectomy or a modified procedure where only the short gastric arteries, left gastroepiploic artery, and splenic artery are ligated.

The prognosis with splenic hemangiosarcoma is considered poor. An early study found a median survival time of 86 days for stage 1 and 2 splenic hemangiosarcoma.(5) Chemotherapy significantly improves prognosis;
in one study, a median survival time of 172 days was achieved in dogs receiving doxorubicin.(6) The survival time with other splenic tumors is also poor with lymphoma median survival times of 5-14 months being reported, and a 55% survival at 12 months after splenectomy for histiocytic sarcoma.

Hepatic Tumors

There are four major types of liver tumors including hepatocellular carcinoma, bile duct carcinoma, neuroendocrine tumors and mesenchymal tumors.(7) Metastasis to the liver is much more common than primary liver neoplasia. In dogs, malignant tumors are diagnosed more readily whereas benign tumors are more common in cats.

The treatment of choice for liver tumors is surgery whenever possible. In the largest study evaluating massive hepatocellular carcinomas (the most common type), the recurrence rate after resection was 0% and the metastatic rate was low at 4.8%.(8) Increased concentrations of ALT and AST were associated with a poor prognosis. In cases undergoing surgery, the median survival time was not reached at >1,460 days.(8) For bile duct tumors, the prognosis for carcinoma is considered poor with the majority of cases dying within 6 months due to recurrence and metastatic disease. Bile duct adenoma cases have an excellent prognosis with liver lobectomy.

There is virtually no data on the use of chemotherapy for liver tumors. For nonresectable liver neoplasia, the emerging treatments of embolization and chemoembolization can be considered. During this treatment, the arterial blood supply to the tumor is targeted and an embolic agent is delivered. The delivery of the embolic agent can also be combined with chemotherapy to allow for the administration of a dose of intra-arterial chemotherapy.

Urogenital Tumors

Malignant obstruction of the urethra can originate from the bladder, urethra or prostate. Transitional cell carcinoma is the most common tumor affecting the urethra and bladder of dogs.(9) The most commonly diagnosed neoplasms affecting the prostate include adenocarcinoma, undifferentiated carcinoma and transitional cell carcinoma.(10) Traditional treatment options for tumors causing obstruction of the urethra, generally fall into three general categories: surgery, chemotherapy and radiation therapy. Median survival times for surgical resection alone have been reported as 86-125 days.(11-13) Electrosurgical, laser, and vaporization techniques have also been described and carry a concern for urethral rupture.(14) Several surgical options have been proposed for the treatment of prostatic neoplasia and results have shown mixed success.(15-19) Both subtotal and total prostatectomies have the potential to result in significant morbidity and surgical resection is often not recommended or indicated.(15-19)

Chemotherapy may be included in the treatment of tumors causing malignant urethral obstruction.(9,16) Additionally, radiation therapy is often utilized to treat prostatic neoplasia in dogs and may be considered for urethral and bladder tumors as well. While surgical debulking/resection, chemotherapy and radiotherapy are good options for initial treatment of non-obstructive urethral neoplasia, these are not good options for immediate relief of complete urethral obstruction secondary to neoplasia. Palliation of urethral obstruction by the placement of a urethral stent has been extensively described in human clinical medicine(20,21) and experience is increasing in veterinary clinical medicine.(22)

Keywords: Abdomen, Neoplasia, Spleen, Liver, Urogenital
References


Thoracic Neoplasia

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Summary
Thoracic neoplasia, while uncommon, presents unique challenges for clinicians when considering diagnostics and treatments. Most evaluations of suspected thoracic neoplasia start with thoracic radiographs and bloodwork, but in many cases, advanced imaging with computed tomography is necessary. Treatments focus on surgical removal whenever possible, although thoracic surgery can be challenging and present anesthetic difficulties. Surgery often improves survival when pursued in the treatment of thoracic neoplasia, although, metastatic disease with certain tumors is common.

Esophageal Neoplasia

Esophageal neoplasia generally occurs in three scenarios: primary neoplasia of the esophagus, neoplasia metastatic to or invading the esophagus from another organ or neoplasia that occurs in conjunction with *Spirocerca lupi* infection. Primary tumors that have been reported in the esophagus of companion animals are varied. Thyroid tumors are the most likely tumors to secondarily invade the esophagus, however, respiratory tract tumors and stomach tumors have also been reported.(1)

Dogs are the definitive hosts of *Spirocerca lupi* which is a nematode capable of being released into the stomach and migrating to the thoracic aorta after ingestion.(2) Once located within the thoracic aorta, these organisms can penetrate into the esophageal wall.(2) *Spirocerca lupi* infestation causes nodules to form within the esophagus, and these nodules can undergo malignant transformation to form a sarcoma. The earliest report of this disease described five osteosarcomas and two fibrosarcomas; however, undifferentiated sarcomas have also been identified.(3,4)

Pulmonary Neoplasia

Pulmonary tumors can arise from any tissue in the lung but most commonly originate from epithelium of the airways or alveolar parenchyma. Tumors derived from epithelium of large airways are typically located near the hilus whereas parenchymally derived tumors tend to be peripherally located. However, the most recent World Health Organization (WHO) guidelines on classification of pulmonary neoplasms largely classifies lung tumors of domestic animals by histologic pattern and not by site of origin.(5)

Approximately 85% of canine lung tumors are bronchioalveolar in origin, whereas adenocarcinoma, adenosquamous, and squamous cell carcinoma collectively comprise the remaining 13-15% of primary lung tumors.(6,7) Adenocarcinoma represents 60-70% of feline lung tumors, whereas bronchioalveolar, squamous cell carcinoma, and adenosquamous carcinoma are less common.(5,8) Small cell carcinoma represents
approximately 25% of human pulmonary neoplasms but rarely occurs in the dog or cat.

Lung tumors can spread by local invasion, or hematogenous and lymphatic routes resulting in loco-regional spread to other areas of the lung or lymph nodes or distant metastasis. Intrapulmonary metastases are believed to occur through vascular and lymphatic invasion or intra-airway seeding. Seventy-one percent of canine pulmonary malignant tumors had evidence of local vascular or lymphatic invasion, and 23% had distant metastasis beyond hilar lymph nodes.(7) Squamous cell carcinoma and anaplastic carcinomas have metastatic rates that exceed 50% and 90% of cases respectively, and thus are believed to be more likely to metastasize than adenocarcinoma or bronchioalveolar carcinoma.(9)

Metastasis is common in the cat, with a metastatic rate of 76% in feline patients with pulmonary tumors.(8) The incidence of lymph node metastasis and intrathoracic metastasis are equivalent at 30% of patients, whereas extrathoracic metastases occurred in only 16% of cats. Metastasis to bone or the nervous system is not uncommon in dogs or cats. Metastasis to the digits is a common and well-described clinical phenomenon in cats.(10)

**Rib Neoplasia**

Neoplasia of the rib, while uncommon, requires a combination of thorough preoperative diagnostics and surgical planning to ensure a positive surgical outcome. Preoperative imaging should include radiographs to assess the number of ribs that are affected by the tumor, and a CT scan is essential to assess the invasiveness of the tumor into surrounding structures. The CT scan images should also be utilized to determine if a normal rib in front of and behind the rib affected by tumor can be removed in order to ensure a substantial margin of normal tissue. It has been stated that up to 6 ribs can be removed without an increased risk of complications associated with ventilation.(11)

The most common tumors affecting the ribs are osteosarcoma and chondrosarcoma.(12) The prognosis after surgical resection of these two types is significantly different; in one study(12), dogs with primary rib osteosarcoma had a median survival time of 290 days whereas median survival time was not reached for dogs with rib chondrosarcomas. For all rib tumors, the treatment of choice is rib resection. When performing rib resections, the potential to develop postoperative complications increases in dogs that undergo synthetic material reconstruction.(11) Additionally, when sternal reconstruction is included in the treatment of rib tumors, the risk of complications increases significantly.(11)

**Thymic Neoplasia**

A thymoma is a tumor of the thymic epithelial cells, however, variable amounts of lymphocytes are generally found in a thymoma and may predominate.(13,14) Thymomas are uncommon tumors, but when a mass is noted in the cranial mediastinum, the level of suspicion is heightened. Other tumors that are commonly noted in the cranial mediastinum include lymphoma, chemodectoma, and ectopic thyroid/parathyroid tumors.(14) As mentioned previously, diagnosis of a thymoma can be achieved in some cases by cytology; however, the addition of flow cytometry increases the chance of obtaining a definitive diagnosis.(15)

Surgical resection is the most well-documented treatment modality for thymomas.(14,16,17) In two retrospective canine case series, outcomes post-thymectomy were variable.(14,16) The presence of megaesophagus significantly decreased survival time in one of the studies, likely resulting in the poorer outcomes in that group of dogs.(16) In dogs without megaesophagus, 1-year survival rates range from 64-83%.(14,16) Another study reported overall median survival time in dogs post-thymectomy as 790 days.(14) In two dogs treated with thoracoscopic resection of thymomas, one died five days postoperatively from aspiration pneumonia, and one was alive 18 months postoperatively.(18)
Two of twelve cats did not survive the immediate postoperative period in one study; in the non-surviving cats, one cat died secondary to postoperative hemorrhage and the other cat developed a pleural fungal infection. However, none of the other cats in that study developed local tumor recurrence or metastasis for a median follow-up time period of 21 months. The median survival time post-thymectomy for 9 cats with thymoma in a separate study was 1,825 days, and the 1-year survival rate was 89%. In both dogs and cats, thymomas with a higher percentage of lymphocytes live significantly longer.

Keywords: Thoracic, Neoplasia, Esophagus, Pulmonary, Lung, Rib, Thymus

References
Mast Cell Tumors and Soft Tissue Sarcomas

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Summary
Canine patients are commonly presented to veterinary clinics for evaluation of cutaneous and subcutaneous masses. When evaluating these cases, clinicians need to have an established protocol for diagnosis and treatment as the biologic behavior of these masses can range from benign to malignant, and the approach can vary widely. The two neoplasms with malignant potential that are most regularly diagnosed are mast cell tumors and soft tissue sarcomas. The approach to the management of these cases is similar, and the importance of choosing an appropriate treatment strategy is paramount.

Mast Cell Tumors

Mast cells play several important roles in dogs and are integral to host defense mechanisms. Mast cell tumors (MCTs) are the most common canine cutaneous neoplasm, and several breeds including Boxers, bull terriers, and Labrador retrievers are noted to regularly develop MCTs. When a MCT is suspected, the initial step in the diagnostic process is generally fine-needle aspiration. As mast cells exfoliate readily, diagnosis via cytology tends to be a relatively straightforward. After a MCT has been confirmed, clinicians must decide what further diagnostics should be considered prior to primary tumor treatment. For MCTs on the lower limb, fine-needle aspiration of the draining lymph node is highly recommended as this can affect surgical dose and post-operative adjuvant therapy recommendations. If a MCT is located on the pelvic limb proximal to the popliteal lymph node, the treating clinician should consider an abdominal ultrasound to evaluate the intra-abdominal lymph nodes. An abdominal ultrasound can also be recommended to assess other locations where MCTs can be found most commonly the liver and spleen. Chest radiographs are often not recommended specifically for the staging of MCTs; however, radiographs could be extremely useful in older dogs or dogs undergoing a surgical procedure to improve anesthetic preparation.

The mainstay of treatment for MCTs is surgical removal. With MCTs, the palpable mass often does not provide a complete picture for the location of the MCT as these tumors tend to have “tentacles” of mast cells extending from the bulky tumor itself. These areas of mast cells should also be removed during resection of the bulky tumor as incomplete resection may increase the risk of recurrence at a future date. Several studies have evaluated outcomes with varying levels of margins. Additionally, there are now several studies about the outcome in cases with incomplete resections. There are many prognostic factors that have been evaluated in patients with MCTs. Histologic grade and certain cellular characteristics (eg. mitotic index) are likely the most consistently evaluated factors, and these factors may impact recommendations. Chemotherapy is generally recommended for higher grade MCTs (grade 3 or grade 2 with high mitotic index) or situations where metastatic disease has been diagnosed. In cases with an incomplete resection, a second surgery or radiation therapy can be pursued.
Soft Tissue Sarcomas

Soft tissue sarcomas (STS) account for approximately 15% of all skin and subcutaneous tumors in dogs. (1) This group of tumors, which is comprised of 11 different histological variants, shares a similar biological behavior. The tumors included under the title of STS do not include those with higher metastatic rates such as hemangiosarcoma and osteosarcoma. Instead, canine STS share the characteristics of being locally invasive with a less aggressive metastatic potential. (1,2)

Surgery is the mainstay of treatment for STS, although surgery alone is not always sufficient to completely treat STS. Special consideration as to the surgical dose necessary to remove a STS is mandatory, as these tumors tend to have poorly defined histological margins and invade through fascial planes. (3) STS often appear as isolated, well-encapsulated masses that can be found anywhere on the body; however, these tumors are often surrounded by a pseudocapsule which is constructed of a compressed layer of tumor cells. 1 Due to the above features, wide surgical excision is recommended and scar excision is advocated in cases with incomplete resections. (4)

Radiation therapy is more often utilized in the adjuvant setting rather than as a primary treatment for STS in dogs. (1) Gross disease is considered to have less response to radiation therapy, however, the use of radiation therapy for treatment of a surgical wound after cytoreductive surgery is well documented in dogs. (1,5) In dogs with surgical excision of gross disease and subsequent delivery of radiation therapy to the surgical wound, outcomes are excellent and long-term survival is high. (5) However, metastasis and recurrence after radiation therapy significantly affect survival times. (5)

Chemotherapy is generally reserved as a treatment for patients with STS that are considered to be high-grade or grade 3. (2) Grade 3 STS have been shown to have a higher metastatic rate and the mitotic index is the variable most likely to influence the development of metastasis. (6) Chemotherapy as a sole treatment for gross disease is not recommended.

In general, the prognosis for STS is considered good to excellent. (6) The metastatic rate for low grade (grades 1 and 2) STS is less than 20%, and surgical excision with or without radiation therapy to treat local disease is often curative. (1,4,6) Tumors greater than 5 cm in size, tumors with a high mitotic index or tumors that are grade 3 have a less favorable prognosis. Metastatic rate associated with grade 3 tumors is approximately 50%. (1)

Keywords: Mast Cell Tumor/s, Soft Tissue Sarcoma/s, Margin/s

References
Oral Tumors

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Summary
The use of maxillectomy and mandibulectomy techniques are well-described in veterinary medicine. Dogs tolerate these procedures extremely well, and are often able to account for changes in jaw position, change in tongue position and loss of bone with little adjustment on their part. Cats, likely due to the smaller size of the maxilla and mandibles, are less tolerant of major maxillary and mandibular surgeries, however, some options still exist.

Oral Tumors

Surgical oncologists are often presented with surgical challenges. Surgical challenges may include those patients that have excessively large tumors or tumors found in difficult locations. With the constant improvement in veterinary medicine and the overall increases in companion animal survival times, it is likely that these challenges will continue to be encountered. Surgical oncologists should have a thorough understanding of advanced preoperative diagnostics including ultrasound, computed tomography and magnetic resonance imaging as well as the adjuvant therapiies and surgical techniques that will allow for a successful outcome in these difficult cases.

Diagnostics such as blood work, chest radiographs and abdominal ultrasound are often employed as staging tools for companion animals with neoplasia. Abdominal ultrasound also has the advantage of providing the opportunity for guiding fine-needle aspiration and pretreatment biopsies. Additionally, ultrasonographers can often determine the integrity of tissue planes for soft tissue tumors and assess the extent of penetration of a tumor. Further, ultrasound of tumors that are adjacent to bone can be performed to evaluate for bone penetration.

Surgical oncologists should become comfortable evaluating computed tomography (CT) images and magnetic resonance (MR) images. Often these diagnostics are crucial for pre-operative planning, and fully understanding these imaging modalities allows for a careful assessment of the anatomical structures of importance that may be near the tumor. CT is an excellent means of assessing bone structures while MR is considered superior in the evaluation of soft tissue abnormalities.

The major categories of maxillectomy include unilateral rostral maxillectomy, bilateral rostral maxillectomy, total unilateral maxillectomy and caudal maxillectomy. The major categories of mandibulectomy include unilateral rostral mandibulectomy, bilateral rostral mandibulectomy, total unilateral mandibulectomy, caudal mandibulectomy, segmental horizontal body mandibulectomy and mandibular rim excision. Preoperative planning for tumors located on the maxilla generally includes CT evaluation to determine the extent of the tumor. The CT can be utilized to assess the surgery that will be necessary to remove the tumor.

When performing maxillectomies and mandibulectomies, local reconstructive options should be considered. Understanding these reconstructions is important to facilitate the apprehension of food, to improve
tongue position and to improve cosmesis. Several techniques for reconstructing oral mucosa and the skin of the face have been described.

**Keywords:** Oral, Tumor/s, Maxillectomy, Mandibulectomy
Interventional Oncology

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Summary
Surgical oncology is an ever-changing discipline. The fact that stands the test of time, however, is that surgery provides the best option for providing a cure for our oncology patients. The role of the surgical oncologist is to approach each case with a multidisciplinary outlook, considering adjuvant treatments such as chemotherapy and radiotherapy. With the development of newer treatments such as antiangiogenic agents, immunotherapy and gene therapy, the role of the surgical oncologist is likely to expand as we move into the future.

Goals for each case should be established before a surgical approach is even made. The effectiveness of the surgical oncologist relies on his or her ability to consider all aspects of the patient as well as strict adherence to certain mainstays of treatment such as staging, tumor diagnosis and surgical planning. To maximize effectiveness, each case should be strategically planned from the point of preoperative assessment to the surgical options to the postoperative care. This careful initial preparation will allow for the most successful outcome for the patient.

Preoperative Planning: Signalment

The patient's age, gender, breed and weight are important factors in the determination of proper recommendations. As the majority of our oncologic patients are considered geriatric, clients may feel that putting their pet through staging diagnostics and surgery is overly traumatizing for the patient. As stated elsewhere, there is "no cancer in which the older age of the patient has any direct bearing on the tumor-related prognosis."(1) Other comorbidities that we see in older dogs such as renal insufficiency, hepatic disease or osteoarthritis may limit our surgical or anesthetic options; however, the age of the patient alone should not.

Preoperative Planning: Staging/Concomitant disease

Staging diagnostics such as a complete blood count, chemistry profile, chest radiographs and abdominal ultrasound are essential components for the preoperative assessment of our veterinary oncologic patients. While there is debate about the timing of some of these diagnostics (ie. before or after biopsy), for many patients proper preoperative staging diagnostics can unmask an underlying condition that may alter the plan for an individual case or better assist the surgeon in providing a proper prognosis. A different surgical approach or dose may also be recommended based on the results of staging diagnostics.
Preoperative Planning: Neoadjuvant Therapy

The surgical oncologist is often presented with extremely large tumors or tumors located in difficult anatomic locations. It is important to consider neoadjuvant treatments such as chemotherapy and radiotherapy before proceeding with surgery. Most commonly, recommendations about chemotherapy are made after the grade of the tumor and the surgical margins have been determined. In tumors that are suspected to be sensitive to chemotherapy based on published literature or previous experience, a postoperative protocol can be discussed prior to surgery.

Surgical Planning: Biopsies

Biopsies should be well-planned with several factors in mind: location of the mass, differential diagnoses of the mass, biopsy technique, eventual definitive treatment and neoadjuvant/adjuvant therapies that may need to be incorporated. A major component of this planning is deciding whether to perform a pretreatment biopsy (before treatment initiation) or a posttreatment biopsy (at the time of definitive tumor resection).

Surgical Planning: Surgical technique

Certain surgical technical principles will improve the chance of success. Tension should be avoided whenever possible, especially in cases that have undergone radiotherapy. Proper knowledge of tension-relieving techniques such as tension-relieving sutures and flaps can assist in closure. Additionally, when establishing a margin during surgical dissection, this margin must be maintained around the periphery of the tumor down to the deep margin. Straying from this may result in an incomplete resection. Similarly, the pseudocapsule present around a tumor should not be penetrated, as this pseudocapsule is constructed of a compressed layer of neoplastic cells. Seeding of these cells will likely result in recurrence, and healing may be inhibited. Lastly, control of hemostasis and prevention of seroma or abscess development due to dead space is encouraged.

Surgical Planning: Margins

The evaluation of margins of an excised mass is an essential component to offering appropriate care to our veterinary cases. Excised masses should be submitted in their entirety for evaluation of the completeness of excision. The surgeon should mark the margins so that the margin can be assessed accurately by the histopathologist.

The assessment of margins has some general guidelines. An intracapsular technique of biopsy leaves residual neoplastic disease. Similarly, biopsies with less than 1 mm of normal tissue on the margins are considered incompletely resected. Margins within 1 cm of the mass are considered clean but close or marginal. Included in this group are those tumors that are easily “shelled out” of their environment (i.e., lipomas). Tumors are considered to have been removed with a wide excision if 1-3 cm of normal tissue margin is present. For a wide excision, the mass needs to be removed en bloc and the excision should include the pseudocapsule and reactive zone. The dissection for a wide excision is intracompartmental, and this dissection plane is what separates a wide excision from a radical excision. A radical excision is considered an excision of normal tissue surrounding the mass of greater than 3 cm. This is an extracompartmental (beyond an anatomic compartment that is considered to have a cancer-resistant tissue barrier) excision and examples include digit or limb amputations.

Surgical Planning: Palliative and Debulking Surgery

Often a clear surgical option is present when a surgeon is evaluating an oncologic case. The surgeon
needs to be cognizant of those cases, however, when pursuing surgery may actually result in more morbidity than not pursuing surgery. The decision to perform a palliative or debulking surgery is often a difficult one, and the surgeon needs to be the patient advocate when cases such as this arise. Palliative and debulking surgeries are performed regularly in human oncology(6-9), but there are likely less indications in veterinary medicine.

**Postoperative considerations: Tumor Fixation**

Small biopsy samples should be placed in fixative immediately to prevent drying of the sample. Early fixation will initiate changes in the sample that will prevent autolysis and bacterial alteration of the sample.(10) In large biopsy submissions, the sample should be sliced evenly to allow for more complete fixation.(11) Many fixatives including formalin, Bouin’s fluid, chilled isopentane, Zenker’s fluid and glutaraldehyde have been described in veterinary medicine(10,12), but in general, 10% buffered formalin is sufficient for almost all biopsies.(13) A biopsy sample should be fixed in formalin in a 1:10 solution of tissue to formalin.(13)

**Postoperative considerations: Wound healing**

The veterinary oncologic patient has several reasons for complications associated with wound healing.(14) Certain factors such as nutritional state and concomitant disease can be treated to improve the outcome of wound healing, but other factors like tumor type and completeness of surgical excision have to be addressed as well. Neoadjuvant/adjuvant therapies such as chemotherapy, radiotherapy, and antiangiogenic medications have been well documented to impair wound healing.(14-16)

Proper surgical technique as described above can be employed to decrease the chance of complications associated with the wound. Regular communication with clients both before and after surgery will help to preemptively prepare for complications or address complications quickly when they occur. Preventing self-trauma by the patient can also be incorporated by bandaging the wound or having the patient wear an Elizabethan collar.

**Keywords:** Preoperative, Neoadjuvant, Biopsy, Margin, Palliative, Debulking, Fixation, Wound Healing

**References**


Applications and the Use of Molecular Diagnostics in Aquaculture and Aquatic Animal Medicine

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Summary
Molecular diagnostics and their application in aquaculture can assist with the rapid, early detection of pathogens before the development of signs of infectious disease. Methods such as PCR, qPCR, RT-PCR, cPCR and iPCR are increasingly being used by diagnostic laboratories, veterinarians, fish health biologists and farmers to make informed decisions about fish and shrimp health management. Point-of-care tests (POCT) are also being utilised in aquaculture with molecular based and antibody based tools proving the most commonly used and robust tools for practitioners operating in remote locations with at times limited infrastructure.

Fish health in aquatic animal medicine is a balance between the pathogen (in the case of infectious disease), the host (fish or shellfish) and the environment, plus in the case of aquaculture the influence of humans through management and husbandry decisions. For disease diagnosis in aquaculture we can apply the three pillars of clinical veterinary medicine namely 1) clinical history, 2) clinical examination (and post mortem findings) and 3) results of laboratory tests (including point-of-care tests (POCT)). Our aims with disease diagnosis and surveillance in aquaculture are to:

a) establish whether or not there is infection presence
b) establish infection levels
c) develop plan/s to reduce or control disease
and at farm level these can focus on diagnostic tests, biosecurity and practices such as vaccination, treatment, and/or change of husbandry. These may be instituted all at the same time to enable effective control and value for money.

Within aquaculture the laboratory diagnostic tests utilised include bacteriology (culture, biochemistry, agglutination, etc.), virology (cell culture, electron microscopy, etc.), parasitology (microscopy, histology, etc.), mycology, histology (including immunohistochemistry), haematology, blood biochemistry (enzymes, electrolytes, lipids, etc.), serology and antibody tests (ELISA, IFAT, DFAT, lateral flow), radiography, cytology, nutritional analysis, toxicology, harmful algae and zooplankton monitoring, water quality analyses and molecular diagnostics.

Molecular diagnostics includes the polymerase chain reaction (PCR) tests which work to amplify a single or few copies of a segment of DNA through several orders of magnitude. In addition to the standard PCR test which is specific and sensitive for a pathogen, variations that are used in aquaculture diagnostics include:

a) convective PCR (cPCR or iPCR) which involves a thermal gradient in the test, rather than repeated heating and cooling, and hence reduces power requirements and run time leading to a more rapid result and portable unit
b) multiplex-PCR which involves multiple primer sets and hence can screen for more than one pathogen in the same test sample
c) quantitative PCR (qPCR) which measures the amount of the target sequence (commonly in real-time) and usually use fluorescent dyes, such as Sybr Green or TaqMan
d) reverse transcription PCR (RT-PCR) which amplifies DNA from RNA and is widely used for expression profiling, to determine the expression of a gene, etc..

In addition, in aquaculture molecular diagnostics we utilise, or various groups are developing, next generation sequencing (NGS), multiplex analysis (bead array technology), nanotechnology (magnetic beads, etc.), matrix assisted laser desorption/ionisation (MALDI-TOF), pyrosequencing (for viruses, bacteria and parasites), nanopore technologies and proteomics.

An example of where PCR surveillance can be used as an early warning for presence of an infectious agent has been demonstrated with pathogens such as with Neoparamoeba perurans, the causal agent of amoebic gill disease (AGD), where a positive signal of infection can be shown at least two weeks prior to any other signs of AGD (1). This is used by many farming companies now in Northern Europe to give early warning and preparation time for treatment. The sensitivity of the PCR can also be improved with different PCR techniques and the use of non-lethal gill swabbing rather than tissue samples. PCR testing for Renibacterium salmoninarum, the causal agent of bacterial kidney disease (BKD), has also been shown to be the fittest of tests when compared to culture, IFAT and ELISA for screening subclinically-infected adult Atlantic salmon (Salmo salar) (2).

Subtyping of disease pathogens is increasingly utilising molecular diagnostics, in particular PCR, such as with screening for the subtypes of salmonid alphavirus (SAV), where there is evidence for differing clinical impact and prognosis (3) and regulatory implications as with infectious salmon anaemia virus (ISA) and non-pathogenic genotypes (4). It is also being used increasingly for aquaculture epidemiology and for the tracking and identification of point source of disease outbreaks. Molecular diagnostic techniques such as fluorescence in situ hybridisation (FISH) can be valuable in confirming the association of newly identified pathogens with previously described pathology, as in the case of Candidatus Branchiomonas cisticola (5) with salmon gill pathology. Other applications include the identification of pathogen species or strains and the detection in the environment and influence on the microbiome of fish thereby adding to the knowledge of reservoirs, epidemiology, risk factors and improvements in prevention and control (6).

Point-of-care tests (POCT) currently developed or in use in aquaculture include antibody based lateral flow cassettes for pathogens such as betanodavirus, white spot syndrome virus (WSSV) in shrimp (7) and cyprinid herpesvirus-3 (also known as koi herpes virus or KHV) (8). Loop-mediated isothermal amplification (LAMP) assays have advantages for working in field or farm situations as they do not require an expensive thermal cycler, but rather can operate under isothermal conditions and have been successfully developed for several fish and shellfish pathogens (9). cPCR or iPCR based tests are also in use for some aquaculture pathogens such as WSSV in shrimp, SAV and N. perurans in salmon and betanodavirus in sea bass (D. labrax) with the advantages of being inexpensive to operate, delivering results within 60 to 90 minutes plus machines are portable.

Keywords: Molecular, Diagnostics, Aquaculture

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Bath Treatments in Aquaculture: 10 Key Aspects for Successful Health Management

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Summary
Disease treatment in aquaculture via immersion (bath, flush, dip or flowing) allows for the control of a variety of parasites (usually ectoparasites), external fungus, some bacteria and can also be utilised to reduce challenge with viruses. However, immersion or bath treatments in aquaculture have several risks for the fish, the operator and the environment, when compared to alternatives such as oral or injectable therapy. To minimise the risks of adverse reactions or impacts in the patients there are a number of precautions and practices that can be instituted.

Disease treatment in aquaculture via immersion (bath, flush, dip or flowing) allows for the control of a variety of parasites (usually ectoparasites), external fungus, some bacteria and can also be utilised to reduce challenge with viruses. However, immersion or bath treatments in aquaculture have several risks for the fish, the operator and the environment, when compared to alternatives such as oral or injectable therapy. To minimise the risks of adverse reactions or impacts in the patients there are a number of precautions and practices that can be instituted. These can be outlined as follows:

1) Know the condition and health status of the fish before treatment. Existing gills conditions or pathology such as proliferative gill disease, amoebic gill disease, necrosis, etc., myopathies (such as those induced via viruses or nutritional imbalances) or deformities (such as opercular shortening, mandibular deformity) will all make the fish less able to cope with the rigours of treatment and hence any treatment time should be shortened, dosage decreased or background level of oxygen increased to assist the fish with the treatment. In some cases treatment may have to be postponed until a specific background condition improves, such as with allowing fish to pass through the viraemic phase of salmonid alphavirus (SAV) infection. To assess the fish condition and health status of the fish pre-treatment diagnostics and clinical examination should be undertaken will in advance as well as close to treatment time.

2) Water quality parameters should be monitored and established pretreatment and these should include temperature, oxygen, pH, salinity, hardness (in the case of freshwater) as well as phyto- and harmful zooplankton assessments. Some bath treatment dosages are more hazardous at higher temperatures i.e. hydrogen peroxide, others at lower pH and hardness. If a harmful plankton bloom is underway gill condition may be compromised and in addition concentrating the fish in the surface layers of such a bloom could prove very counterproductive. Dosage and treatment time for some medicines should be adjusted to take into account water quality parameters. These parameters, and in particular oxygen (and carbon dioxide in the case of lengthy treatments), should also be monitored throughout, and after, the treatments. Supportive care for the fish during treatment such as elevation of dissolved oxygen during and/or pretreatment and also sedation (with isoeugenol where permitted) can also
be employed.
3) Preparation of equipment and staff pretreatment, with contingency plans in place for when things do wrong i.e. how to flush and exchange water rapidly, if fish show signs of distress, will be vital to minimise any adverse impacts on the livestock. All methods and protocols should be written down and available for all to view and understand. Training of staff in techniques, fish welfare and handling and operator safety will be important as one of the largest losses of fish biomass during bath treatments is as a result of poor technique/s and misunderstandings over fish stress and welfare.

4) Check dosage calculations and have the arithmetic double checked. Measure the volume of the water in the pond, tank, well-boat or pen and take into account pipework, tarpaulin stretch, etc..

5) Decide when to treat via trigger levels of parasite burdens or monitoring of pathogen level in the fish or water. It will be better to treat before clinical disease manifests and fish become immunocompromised. Have established treatment trigger levels on a holding unit basis and treat on this basis. This requires regular monitoring of each unit for infectious agent burden or level and as an example salmon farms will monitor lice burden on a weekly basis (1).

6) Pharmaceutical and chemical background questions that should be answered pretreatment include: What medicines are available in the region? Which are licensed and what are the environmental discharge and impact considerations? What are the tissue residue withdrawal times, especially if used off label? How long will efficacy be and can the treatment be cost effective?

7) Undertake bath treatments at a water body level to synchronise treatments and maximise the efficacy (area based management) in a region especially in the case of treatments that do not kill the parasite but only dislodge or temporarily immobilise.

8) Ensure accurate disease diagnosis to avoid unnecessary treatment if not warranted and to use the correct medicine. Increasingly some crustacean parasites are exhibiting resistance to medicines that have been used repeatedly so bioassays or molecular diagnostics of lice are undertaken to assess likelihood of efficacy. Measures during treatment to reduce the risks of resistance development should also be adhered to i.e. avoid using skirt bath treatments in pens (2).

9) Consider the conditions of the holding units for the fish to ensure effective treatment and such factors include biofouling on nets and on tank walls which may reduce efficacy of treatment and further dislodgement of such during crowding may lead to gill and skin irritation. Roughened surfaces on tank floors, pipe junctions, nets, etc. may all lead to physical abrasions to skin or fins which will then be at risk of opportunistic infection post treatment. In recirculating aquaculture systems (RAS) the biofilter/s may have to be bypassed during treatment or there may be a dramatic reduction in percentage activity.

10) Re-examine the fish after treatment to assess the treatment efficacy and if needed then organise to start again.

Keywords: Bath, Treatment, Aquaculture

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Applications and Use of Digital Histopathology in Aquaculture and Aquatic Animal Medicine

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Summary
The use of digital histopathology or whole slide imaging (WSI) in pathology has developed in human and veterinary medicine to be a valuable tool for clinical pathology, research and training. Its use is now being applied to aquaculture and aquatic animal medicine, research as well as training and specific examples are considered in this paper.

The use of digital histopathology or whole slide imaging (WSI) in pathology has developed in human and veterinary medicine to be a valuable tool for clinical pathology, research and training. Its use is now being applied to aquaculture and aquatic animal medicine, research as well as training and specific examples are considered in this paper.

WSI, which is otherwise termed virtual microscopy or digital microscopy, provides scanned slides, produced by conventional histology processes, which are available to view through the internet using specialised software. Current WSI scanners (which are basically microscopes under robotic and computer control) can produce high-resolution digital slides within minutes. A scanner is attached to a highly specialised camera containing advanced optical sensors for image capture. WSI has transformed many aspects of human pathology and is likely to catalyse further novel applications to the benefit of veterinary pathology, veterinarians and their clients (1). Within aquaculture and aquatic animal medicine the following are examples of current use:

1) Virtual pathology, second opinions and consultancy in remote locations i.e. pathologists can screen the slides scanned in any part of the world in real time. This allows for sharing of slides between pathologists or a group of pathologists meaning that the expertise and specialist knowledge of one or many can be shared and consensus and validation established for each specific case. Many disease diagnoses are based on a panel of histopathological criteria being present e.g. amoebic gill disease (AGD) is diagnosed when gill histopathology with all the four criteria of hyperplasia, lamellar fusion, gill vesicles and presence of amoeba (with parasomes) are present (2) and these can be confirmed through WSI from Tasmania to Trondheim within minutes (Fig. 1).
Figure 1 Amoebic gill disease histopathology (hyperplasia, lamellar fusion, vesicle formation and amoebae (with parasomes) in gills of ballan wrasse (Labrus bergylta) (H & E).

2) Training of students, pathologists and technologists can be undertaken through the platform to a far wider audience and with reduced costs. On-line courses can be established for species, body systems and disease conditions and slides stored in teaching sections for easy access, reference. Within Fish Vet Group (www.fishvetgroup.com) the pathologists meet virtually every few weeks to present and discuss cases of emerging diseases or conditions of note.

3) Quantification of items or areas of interest can be undertaken on each slide with relative ease due to software tools for calculating areas, size, etc. and by marking each on a slide these can then be shared with other pathologists. In addition, automated image analysis can also be incorporated and developed for rapid assessment of response to different treatments, diets, experimental regimes, etc. (Fig. 2).

Figure 2 Measurement of diameter of chlamydial cysts (epitheliocystis) in gills of sea bream (Sparus aurata) (Giemsa).
4) The results, reports and findings can have a direct interface with laboratory information management systems (LIMS) thereby allowing tracking, reporting and analysis. Further ease of data mining of pathology, specific diseases and diagnostic tags will all assist in improving the pathology service and outcomes for epidemiological investigations, research and knowledge for the pathologists, clinicians, clients and further by providing business intelligence will be a valuable resource for decision making for the future.

5) The storage of WSI means that there is no degradation of samples due to time, light or environmental conditions and they can be made available to anyone at any time, through the internet.

6) Specific slides or conditions can be displayed and discussed (and made available) to clients hence increasing the communications and interactions between pathologists and clients.

**Keywords:** Whole Slide Imaging (WSI), Digital, Histopathology

**References**


Emerging Gill Health Challenges in Finfish Aquaculture: Causes, Effects and Control

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Summary
Gill health and associated disease challenges have emerged to become one of the major health issues affecting the global salmonid industry in the past five years. Infectious diseases such as amoebic gill disease (AGD), proliferative gill disease, complex gill disease and salmon gill poxvirus (SGPV) as well as non-infectious conditions such as harmful algal blooms (HABs) and harmful zooplankton have emerged to cause direct mortality, loss of growth, increased susceptibility to other diseases and economic impact on markets and investment. The causes, effects and methods to control these conditions are considered and presented in this paper.

Gill health and associated disease challenges have emerged to become one of the major health issues affecting the global salmonid industry in the past five years. Infectious diseases such as amoebic gill disease (AGD), proliferative gill disease, complex gill disease and salmon gill poxvirus (SGPV) as well as non-infectious conditions such as harmful algal blooms (HABs) and harmful zooplankton have emerged to cause direct mortality, loss of growth, increased susceptibility to other diseases and economic impact on markets and investment. The causes, effects and methods to control these conditions are considered and presented in this paper.

Infectious gill disease can be induced through viruses, bacteria, parasites and fungi and in salmonids there are a long list of pathogens implicated and those recently reported to have emerged include salmon gill pox virus (SGPV) (Fig. 1), Neoparamoeba perurans, Candidatus Branchiomonas cysticola, Desmozoön lepeophtherii (syn. Paranucleospora theridion), plus the more well-known challenges with Trichodina spp., Ichthyobodo spp., Loma sp., Flavobacterium spp. and Saprolegnia spp. In many cases there may be multiple agents involved or one leads or facilitates another to infect or infest (1). In the Mediterranean, the sea bream (Sparus aurata) are increasingly challenged with the gill monogenean Sparicotyle sp. and Atlantic cleaner fish (used for sea lice control) by N. perurans and epitheliocystis. Non-infectious gill disease can be induced through harmful algae (over 200 harmful species) which have their effects via either toxins, physical damage or deoxygenation, through nematocysts from harmful zooplankton including some biofouling organisms (such as Ectopleura larynx), through water quality issues (such as rapid changes in pH, heavy metals, high suspended solids, elevated carbon dioxide, trauma, veligers and deformities) (2).
Gill health can be monitored through a combination of water quality parameters (oxygen, carbon dioxide, plankton, pH), physical examinations (deformities and gross pathology), routine fresh microscopy on gill scrapes, PCR screening of pathogens of concern and histology. Gills should be thoroughly examined and screened before any bath treatment, livestock movement or major husbandry event (such as vaccination). Treatments for gill disease depends on the aetiology but with infectious causes options include freshwater, salt, formalin, chloramine T or hydrogen peroxide baths for ectoparasites, bacteria and fungus depending on pathogen, species, water quality, regulations, fish health and local environmental conditions. Oral broad spectrum antibiotic for bacterial infections have been attempted and if fish are feeding well these can assist in some cases of chronic Flavobacteria spp. infections. Cessation of feeding during gill disease for several days has also shown to be of benefit in SGPV viraemias and harmful plankton periods. In freshwater hatcheries affected by Branchiomonas sterilisation of the inflow of water through ozone and UV have proven effective. Improving the rearing environment through improved water exchange, higher levels of cleaning and disinfection and reduced stocking densities will all be of benefit in both infectious and non-infectious challenges. In addition, supportive therapy such as increased oxygenation or aeration as well as dietary modifications have been shown to assist in recovery. Genetic selection for tolerance or resistance to specific gill pathogens has started with some salmonid broodstock programs and early indications are that there is heritability for some pathogens (3).

Keywords: Gills, Disease, Pathology, AGD

References
Global Spread and Control of Avian Influenza

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Summary
H5 and H7 high pathogenicity avian influenza (HPAI) viruses emerge from the mutation of H5 and H7 low pathogenicity avian influenza viruses (LPAI) after circulation in terrestrial poultry for a few weeks to years. There have been 42 distinct HPAI epizootics since 1959. The largest being the H5N1 A/Goose/Guangdong/1/1996 (Gs/GD lineage) HPAI which emerged in China during 1996 and has spread to infect poultry and/or wild birds in 76 countries during the past 21 years. Since 2014, large outbreaks of Gs/GD lineage HPAI have occurred in poultry in USA, Taiwan, South Korea and African, Middle Eastern and European Union countries. Historically, the majority of the Gs/GD HPAI cases have been H5N1 but assortment of the virus has produced H5N2, H5N3, H5N5, H5N6 and H5N8 HPAI viruses of the 2.3.4.4 clade. Other sporadic outbreaks of H5 and H7 HPAI have been reported around the world, most recently in France, United Kingdom, Germany, Italy, and USA. In 2016, the H7N9 LPAI virus mutated to HPAI after circulating in poultry for 4 years. Control leading to eradication has been achieved by stamping-out programs and the most frequent strategy for dealing with HPAI, but some countries have utilized vaccination as an adjunct management tool. However, vaccination alone will not lead to eradication.

Introduction

Avian influenza (AI) viruses are Orthomyxoviruses in the genus Influenzavirus A. They are a diverse group divided into 144 different subtypes based on different combinations of the 16 hemagglutinin (H1-16) and 9 neuraminidase (N1-9) subtypes, and two different pathotypes (low [LP] and high pathogenicity [HP]). The HP pathotype has only been identified with the H5 and H7 hemagglutinin subtypes. Forty-two epidemics of high pathogenicity avian influenza (HPAI) have occurred in the world since 1959 (Figure 1). The largest of these outbreaks has been the H5N1 A/Goose/Guangdong/1/1996 (Gs/GD lineage) HPAI which has caused problems in poultry and wild birds in 76 countries in Asia, Europe, North America and Africa since 1996. This lineage of viruses has crossed multiple species barriers to infect captive and wild birds, carnivorous mammals and humans. Human infections have been associated with direct or indirect contact with live or dead poultry while in carnivores, consumption of infected birds or their products have been associated with infections.
Figure 1 The 42 high pathogenicity avian influenza epizootics from 1959-2017.

Update on High Pathogenicity Avian Influenza, 2014-2017

For 3/2014 to 2/2017, H5N1 HPAI has been reported in 64 countries. Sixty-three countries experienced outbreaks in poultry and/or wild birds with the Gs/GD lineage of H5Nx. This virus group is enzootic in six countries: 1) self-declared enzootic (Egypt and Indonesia), 2) continue to report occurrences of outbreaks over multiple years (Vietnam and Bangladesh), or 3) have published data in the literature of continuous reports of infection and molecular evidence of virus continual presence in country (China and east India). There were six epicenters of H5N1 HPAI: 1) Egypt; 2) Ganges Delta (India, Bhutan, Nepal and Bangladesh), 3) Mekong Delta (south Vietnam and Cambodia), 4) Indonesia, and 5) east to southeast Asia (China, Hong Kong, North Korea, northern to central Vietnam and Myanmar). For 2014-2017, six genetic subclades of H5N1 HPAI virus have been reported in poultry and wild birds: 1) subclade 2.3.2.1, most frequently reported with wide geographic dispersion including northern and central Vietnam, India, Bangladesh, China, Hong Kong, India, Nepal, and Bhutan; 2) subclade 2.2.1 viruses in Egypt; 3) subclade 7.2 in northern China and Vietnam; 4) subclade 2.3.2.1 in Indonesia; 5) subclade 1.1 in southern Vietnam and Cambodia; and 6) subclade 2.3.4.4 which has spread intercontinentally to North America, Europe, Middle East and Africa causing current outbreaks.
The H5N8 Gs/GD “pure-Eurasian” lineage and reassortant H5N2 Gs/GD lineage HPAI viruses were identified in USA in late 2014. These latter H5 HPAI viruses are of the genetic subclade 2.3.4.4A and spread through the Pacific Flyway and to the Midwest USA. The infected premises in Western and initial premises in Midwestern USA were point source introductions from wild birds, while most cases in the Midwest had secondary spread from common sources because the initial viruses were waterfowl adapted but later were adapted to gallinaceous poultry. This clade was eradicated from USA poultry in June 2015 and was rarely been detected in wild birds in 2016. H5Nx Clade 2.3.4.4A HPAI viruses are still causing outbreaks in Chinese Taipei, but are rare in South Korea. In June 2016, H5N8 subclade 2.3.4.4B re-emerged in Russia after the last reports of the virus in S. Korea during 2014, and has spread forward by wild birds to Europe, Middle East and north and central Africa. H5N6 subclade 2.3.4.4c has emerged causing outbreaks in South Korea, Japan and Vietnam. In 2016, a North American H7N8 low pathogenic avian influenza (LPAI) virus caused a limited outbreak in turkeys in Southern Indiana.

Outbreaks of other HPAI viruses have been reported in France (Eurasia H5Nx, different from Gs/GD lineage); USA (H7N8, single turkey farm in southern Indiana, 2016; and H7N9, two farms, 2017); United Kingdom, Germany, and Italy (H7N7, separate LPAI introductions and mutation to HPAI); and mutation of H7N9 LPAI virus to HPAI in China during 2016.

Avian Influenza Control Methods

Stamping-out: The preferred method for dealing with HPAI is eradication of the virus in infected poultry flocks by culling and safe disposal of infected carcasses. This involves several contributing process including rapid diagnostic testing to identify infected poultry early in the disease process; surveillance to learn the extent of infection and freedom from infection within a defined geographic zone; movement controls on poultry, their products and potential fomites to prevent inter-farm spread of the virus; and education of all personnel in their responsibility and actions in the eradication effort. Of the 42 HPAI disease events (Figure 1), 36 have been eradicated through stamping-out, most in less than 6 months and in a single restricted area of a country,
but a few did take a few years and involved multiple countries. Five of the epizootics have used vaccine along with stamping out, and the H7N9 HPAI in China is ongoing.

**Vaccines and Vaccination:** In 1995, vaccination was implemented as a large scale control measure in poultry during the Mexican H5N2 high pathogenicity avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) outbreaks. Vaccination against H5N1 Gs/GD lineage of HPAI was initiated in Hong Kong in 2002, followed by implementation in 2004 in China and Indonesia, 2005 in Vietnam, 2006 in Egypt and 2011 in Bangladesh. From 2002-2010, >113 billion doses of AI vaccine were used in poultry in 15 countries. The majority of vaccine (99%) has been used in the four H5N1 HPAI enzootic countries (China [91%], Egypt [4.7%], Indonesia [2.3%], and Vietnam [1.4%]) where vaccination programs are directed to all poultry. Implementation of vaccination in these four countries occurred after H5N1 HPAI became endemic in domestic poultry and vaccination did not result in the endemic infections. The other 11 countries used less than 1% of the vaccine, administered in a focused, risk-based approach. Inactivated AI vaccines accounted for 95.5% and live recombinant virus vaccines for 4.5% of vaccine used. Clinical disease and mortality were prevented in chickens, and rural livelihoods and food security were maintained by using vaccines during HPAI outbreaks. Fewer outbreaks of H5/H7 low pathogenicity avian influenza (LPNAI) have been reported than HPAI and only six countries used vaccine in control programs which accounted for 8.1% of the total H5/H7 AI vaccine usage.

Since the late 1990’s, field outbreaks of H5N1 HPAI, and H5N2 and H9N2 LPAI have occurred in some vaccinated flocks from both failure of the vaccines (i.e. vaccine efficacy) and failure in proper administration in the target species (i.e. vaccination effectiveness), resulting in an inadequate protective immune response. The former challenge has been met by developing new vaccine strains, by use of reverse genetic technologies to produce LPAI virus seed strains and recombinant technologies to produce live virus vectors with avian influenza hemagglutinin gene inserts, to provide closer antigenic match and *in vivo* protection against ever changing field avian influenza viruses. Multi-vaccinations with monovalent, antigenically diverse seeds can compensate for some antigenic diversity of field viruses, but will not provide predictable protection after a single vaccination. Increased use of bivalent vaccines (with antigenically diverse seeds) can provide protection against diverse field viruses and increasing antigen content of each seed strain can partially compensate for some antigenic diversity of field viruses. However, neither of these strategies will replace the periodic need to update seed strains. Emerging technologies such as computer optimized antigens show promise in generating more broadly protective vaccines within a single hemagglutinin subtype. There is a need for more rapid means of licensing updates in vaccine seed strains through national regulatory processes.

**Keywords:** Avian Influenza, Control, Global Spread

**References**

1. Biosecurity is the most important tool to prevent the introduction of wild bird avian influenza virus or poultry influenza virus onto a naive farm,
2. Highly pathogenic and H5/N7 low pathogenicity avian influenza viruses are nationally and internationally reported and controlled diseases,
3. Stamping-out of highly pathogenic avian influenza virus is the most direct and efficient form of eradication, and
4. Avian influenza vaccines can reduce disease but cannot absolutely prevent infection or result in eradication, and may complicate disease surveillance. Vaccination can reduced poultry illness and death and reduce spread of the HPAIV.
Infectivity, Transmission and Pathogenicity of Avian Influenza Viruses for Domestic and Wild Birds

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Summary
Individual avian influenza (AI) virus strains vary in their ability to infect, transmit and cause disease and death in different bird species. Low pathogenicity AI (LPAI) viruses are maintained in wild birds, and must be adapted to pass to domestic poultry, where they replicate in respiratory and intestinal tracts causing low mortality rates. High pathogenicity AI (HPAI) viruses arise from mutation in the hemagglutinin of H5 or H7 LPAI viruses while circulating in terrestrial poultry. HPAI viruses typically produce a severe, systemic disease with high mortality in chickens and other galliforme birds. However, these same HPAI viruses usually produce no infection or only mild disease in domestic ducks and other wild aquatic birds. Some HPAI viruses have increased in virulence for chickens as evident by shorter mean death times (MDT) and a greater propensity for massive replicate in vascular endothelial cells. Recently, the H5N1 Goose/Guangdong-lineage HPAI viruses have changed from producing inconsistent respiratory infections in 2 week-old domestic ducks to some strains being highly lethal with virus in internal organs and brain.

Introduction
Several criteria are important in understanding the complex disease caused by AI viruses including host adaptation, infectivity, transmissibility, tissue tropism, and lesion and disease production. Overall, such pathobiological features vary with host species and virus strain. Low pathogenicity avian influenza (LPAI) viruses produce a localized infection in epithelial cells of the digestive and respiratory tracts, with no illness or deaths in the native hosts, i.e. wild aquatic birds (ducks, geese and shorebirds), and in terrestrial poultry, respiratory disease and egg production drops accompanied by low mortality rates. Wild aquatic birds are the genetic reservoir for LPAI viruses and to be transferred to, infect and spread among poultry, such LPAI viruses must go through an adaptation process. In the laboratory, we assess this adaptation by intranasal infectious dose and ease of contact transmission (1).

The HPAI viruses, which have all been H5 and H7 hemagglutinin subtypes, arise from mutation of LPAI viruses during unchecked circulation in terrestrial poultry. Such HPAI viruses typically produced a severe, systemic disease with high mortality in chickens and other galliforme birds. However, these same viruses usually produce no infection or only mild disease in domestic ducks and wild birds. Over the past decade, the emergent H5Nx Goose/Guangdong (Gs/GD) HPAI viruses have shifted to increased virulence for chickens as evident by shorter mean death times (MDT) and a greater propensity for massive replicate in vascular endothelial cells. Especially important, the H5N1 Gs/GD-lineage HPAI viruses have changed from producing inconsistent respiratory infections in 2 week-old domestic ducks to some strains being highly lethal with
virus in internal organs and brain. However, the high lethality for ducks is inversely related to age. The most recent H5N1 Gs/GD lineage HPAI viruses have infected some wild birds producing systemic infections and death. Across all bird species, the ability to produce severe disease and death is associated with high virus replication titers in the host, especially in specific tissues such as brain and heart. Lethality in domestic ducks has increased but is not equivalent to HPAI in chickens.

**Changing infectivity, transmission and pathogenicity H5Nx Gs/GD HPAI NORTH AMERICA, 2014-2015**

Between December 2014 and June 2015, USA experienced an unprecedented outbreak of H5 Gs/GD-lineage 2.3.4.4. clade HPAI as the first intercontinental spread of a Eurasian HPAI virus to North America. Initially, a reassortant H5N2 virus was identified in British Columbia, Canada, and within a few days the original Eurasian H5N8 and a Eurasian-North American reassortant and variant H5N2 HPAI viruses were isolated from a gyrfalcon and a wild duck in Washington State, respectively. This H5N8 HPAI virus was spread by migratory waterfowl from Eastern Asia to Russia, Europe and North America (2,3). In North America, the Asian H5N8 HPAI virus reassortated with North America LPAI virus genes to produce H5N2 and H5N1 HPAI viruses (4,5). Subsequently, the H5 HPAI viruses spread through the Pacific Flyway and to the Midwest USA. In total, the H5 HPAI outbreaks has affected 21 states, with detections in 4 captive wild birds, 75 wild birds, 21 backyard flocks and 211 commercial flocks, totaling over 48 million birds. The majority of the affected poultry were Leghorn chickens and turkeys in commercial farms, and various minor gallinaceous species and domestic ducks in backyard flocks, but no broiler farms were affected. In the USA, the eradication effort cost more than $1 billion and the negative economic impact was over $3.2 billion.

Infectivity, transmissibility and pathogenesis studies with an H5N8 and H5N2 clade 2.3.4.4 HPAIVs isolated in December 2014 were undertaken using chickens, turkeys, Japanese quail and mallards (5-8). Chickens and Japanese quail were frequently listless and had ruffled feathers before death, but a few had neurological signs. Pancreatic necrosis, splenomegaly, renomegaly and petechial hemorrhages on myocardium were the most common lesions in birds that died. Some chickens also had cyanosis of combs and wattles and peri orbital edema. Neurological signs were the primary presentation of disease in turkeys. The mean death time (MDT) in all species was late (3-9 days post exposure) when compared to traditional H5N1 HPAIV (2-3 days) with Japanese quail having the shortest MDT, chickens as intermediate and turkeys the longest, while the mallards had no deaths. Infection by these HPAI viruses lead to death as survivors lacked H5 hemagglutination inhibiting antibodies. However, neither virus appeared to be well adapted to chickens or turkeys since 6 log10 50% egg infectious doses (EID50) per bird were required to achieve 50% infection and the virus did not transmit to contact exposed chickens, but did to turkeys. Quail were slightly more susceptible with a 50% infectious dose of 4 log10 EID50 per bird for each isolate. Both viruses caused asymptomatic infection in 100% of mallards at 2 log10 EID50 dose, and consistent contact transmission between mallards. These results suggest the intercontinental H5N8 and H5N2 HPAIVs have reduced virulence and transmissibility for gallinaceous host compared to historical H5N1 HPAIV, but they are highly infectious and transmissible to mallards.

In broad-breasted white turkeys the mean bird infectious dose of A/northern pintail/WA/40964/2014 and A/turkey/MN/12528/2015 was 5log10 EID50 per bird, but was 3log10 EID50 per bird for A/chicken/IA/13388/2015, suggesting the latter virus had some greater adaptation to gallinaceous birds (6). All three isolates presented with a similar pathogenesis: unusually long mean death time of 5.3-5.9 days post challenge, and the primary clinical signs were neurological (e.g. torticollis, ataxia, tremors), and severe lethargy which started no more than 24 hours before death (the average pre-clinical period was 4 days) and neurological signs (e.g. torticollis, ataxia, tremors). Infected turkeys also shed high levels of virus by both the oropharyngeal and cloacal routes. The unusually long mean death times, and high levels of shed virus in manure, and the increased adaptation of the later viruses may have contributed to the rapid spread...
of the virus during the April to June 2015 period of the outbreak.

In commercial 5-week-old broilers, 8-week-old broilers, and >30-week-old broiler breeders the mean bird lethal dose (BLD50) for A/turkey/Minnesota/12582/2015 (H5N2) virus (TK/MN/15) was 5.0 log10 mean egg infectious dose (EID50) for all age groups (9). The mean death time (MDT) was statistically not different among the three age groups, ranging between 3.2 and 4.8 days. All broilers that became infected shed high levels of virus with transmission to contacts and demonstrated severe pathology. Mortality and virus shedding results indicated that age is not a determinant factor in susceptibility of broilers to H5N2 clade 2.3.4.4 HPAI virus. Previously, the Tk/MN/15 virus had a BLD50 of 3.6log10 EID50 and MDT of 2 days in White Leghorn chickens and a BLD50 of 5.0 log10 EID50 and MDT of 5.9 days in turkeys, suggesting that the broiler breed was less susceptible to Midwestern H5N2 virus than the layer breed but similarly susceptible to turkeys. Therefore, genetic resistance of broilers to infection may have accounted only partially for the lack of affected broiler farms in the Midwestern outbreaks, with other contributing factors such as fewer outside to "on farm" exposure to contacts, type of production management system or enhancements to biosecurity.

A limited outbreaks of H7N8 LPAI (n=9) and HPAI (n=1) occurred in Indiana turkeys in 2016 (10). The H7N8 LPAI virus was highly adapted to mallards and turkeys, required low challenge dose to produce infections and was readily spread by contact, but was less well adapted to chickens. The H7N8 HPAI virus required less virus to infect poultry than the LPAI virus.

**Keywords:** Avian Influenza, Infectivity, Pathogenicity, Poultry, Transmission, Wild Birds

**References**


Review on the Evolution of Zoonotic Influenza A Virus in Asia; Implication on the Future Surveillance

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Summary
The highly pathogenic avian influenza (HPAI) H5N1 virus has been circulating in Asia since 2003 and diversified into several genetic lineages, or clades. Until 2014, only HPAI H5N1 viruses circulated. Since 2014, apart from H5N1, outbreaks have been attributed to infections with H5N6, H5N8 and H5N2 and H5N3 subtype viruses. These new virus subtypes appear to have first emerged in China through multiple genetic reassortments of clade 2.3.4.4 influenza A(H5N1) and other subtype viruses within resident domestic and wild bird populations and continued to circulate in domestic poultry populations, leading to wider geographical spread through poultry trade. An overview of evolution of H5N1 clades in Asia will be given, followed by the emergence and spread of other H5Nx viruses since 2014. Key drivers and implications for national, regional and global surveillance will be discussed.

Introduction
Of the transboundary animal diseases, HPAI caused by H5N1 virus is considered one of the priority diseases in Asia because it results in high mortality both in poultry and in humans. H5N1 HPAI is entrenched in the region and is repeatedly reported or detected in a number of countries in Asia including Bangladesh, Bhutan, Cambodia, China, Indonesia, India, Lao PDR, Myanmar, Nepal and Viet Nam. Another concern is the emergence and evolution of a wider range of influenza A viruses that have zoonotic potential. This includes the continued evolution of clades of H5N1 and the emergence of new H5 family (H5Nx) viruses that have spread widely and caused infection in wild waterfowl, poultry and in some cases humans, in China, East Asia, South Asia, Central Asia, Middle East, Africa, Europe and North America. Wild water fowl are known reservoirs for influenza viruses and viruses could be introduced into poultry populations through their migratory patterns between Northern and Central Asia areas to South-Eastern or African regions. Human activity, poultry production practices and marketing systems are most often responsible for dissemination of the diseases. A diversity of non-H5N1 influenza virus subtypes associated with disease outbreaks in poultry and wild birds were reported in Asia, Europe and the Americas. In Asia, H5N3, H5N2, H5N6 and H5N8 HPAI in poultry were reported in China (mainland and in Taiwan, Province of China), while Japan and the Republic of Korea reported H5N8 HPAI, and Viet Nam reported H5N6 HPAI. An overview of the latest spread of H5Nx subtypes is shown in Figure 1.

The evolution of H5N1 in Asia from 2003 to 2017
Since its first occurrence in 1996, the A/goose/Guangdong/96-like viruses have continued to evolve. This molecular evolution is captured in the identification of new H5Nx clades. This clades are regularly updated by the WHO/OIE/FAO H5N1 Evolution Working Group. Currently the main circulating clades are 2.3.4.4 (worldwide), 2.3.2.1A (central Asia), 2.3.2.1.C (Africa, East Asia and Southeast Asia), 2.2.1. (Egypt), and 2.1.3.2 (Indonesia). Over time, certain genetic clades occurred and persisted, while other appeared and went extinct. The evolution of genetic clades over time in Asia is shown in Table 1.
The occurrence and spread of H5Nx clade 2.3.4.4
These new virus subtypes appear to have first emerged in China through multiple genetic reassortments of clade 2.3.4.4 influenza A(H5N1) and other subtype viruses. Clade 2.3.4.4 H5N6 emerged in China in 2013, spread to Lao PDR and Viet Nam in 2014/2015, with evidence of sustained transmission and further geographical spread in both countries. Eastward spread of H5N6 to Japan occurred in short order in 2014, and a first long-range transmission pathway resulted in spread of H5N8 viruses into Germany, The Netherlands and the United Kingdom in late 2014. Another intercontinental wave of distinct H5N8 viruses occurred in late 2016, spreading H5N8 to Europe and Africa. The incursion of H5N8 viruses in Canada and the USA in late December 2014 with rapid reassortment to H5N2 and spread across several Western and North-Central States devastated the regional poultry industry and overwhelmed Federal veterinary services. Control of the epizootic was only achieved by culling over 48 million birds.

Drivers of emergence and spread
Several drivers have been identified that can play a role in the emergence, local and inter-continental spread, and persistence of influenza viruses. To control future novel occurrences and spread of influenza viruses, a better understanding of these drivers for disease will help controlling outbreaks and prevent further viral spread. Main drivers identified are:
- The poultry value chain (production and transport)
- Wild bird movement
- The presence of a ‘gene pool’ in low pathogenic viruses with potential to reassert
- Agricultural and geographic factors (rice fields, water bodies)

Implications for surveillance
Avian influenza control and prevention is being managed in most of the endemic countries through programs that include active and passive surveillance, early reporting and response, culling of poultry in infected farms and in some cases with the use of mass or targeted vaccination. However, factors such as inadequate veterinary services, lack of adequately qualified program staffs, poor biosecurity at all levels of poultry production systems and long complex value chains may hamper surveillance and control efforts. The knowledge acquired over the last ten years regarding drivers of influenza A virus diversity in changing environments, will enable the design of a more integrated risk-based surveillance approaches. These strategies can be implemented by integrating and analyzing data from wild bird migratory patterns, understanding of the complex and dynamic nature of evolving animal value chains, and understanding socio-economic factors and human behavior.
In view of the potential threat of influenza viruses, it is necessary to maintain a strong risk-based influenza A surveillance programme in the regions that will enable an improved risk analysis and will support early preparedness and response plans.
Figure 1. Spread of H5Nx and H7Nx viruses in from January 2016 to April 2017 (FAO, EMPRESi)

Table 1. Transition of H5N1 HA clade during 1996-2014 in Asia

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Source of sequence data: Influenza Research Database (n=2944)
Japanese Experience of Highly Pathogenic Avian Influenza

Takehiko SAITO

Division of Transboundary Animal Disease, National Institute of Animal Health, National Agriculture and Food Research Organization, Japan

Summary

Japan has experienced five seasons of the Asian H5 HPAI outbreaks and one isolated case since 2004. First HPAI outbreak in 70-some years occurred in 2004, following in 2007, 2010-2011, 2014-2015 and 2016-2017 and an isolated case occurred in April 2014. Until 2011, causative agents were the H5N1 subtype and the cases in 2014-2015 were by the H5N8. Outbreaks caused in 2016-2017 were caused by the H5N6 subtype. All 48 but 2 cases were occurred in chicken farms and those 2 were in Muscovy duck farms in 2016. There were sporadic cases of whooper swans in 2008 and in that year, we did not have poultry cases. Dozens of dead wild bird incidents occurred accompanied with poultry outbreaks during both 2010-2011 and 2016-2017 seasons. Particularly in 2016-2017 seasons, number of wild bird incidents were unprecedented, counting 214 cases that included captivity animals as of 28th February 2017.

In this lecture, I will outline the HPAI outbreaks in Japan since 2004 and describe virological properties of the HPAIV isolated from poultry outbreaks and some of those from wild birds. Also, I will show the Spatial Phylogenetic Reconstruction of Evolutionary Dynamics (SPREAD) analysis applied to the H5HA genes of the isolates involved in wild bird incidents occurred in Ibaraki prefecture where our institute located.

In Japan, outbreak of the H5N1 subtype highly pathogenic avian influenza viruses (HPAIVs) occurred for the first time in 2004. Number of outbreaks was four and they were caused by the H5N1 strains belonging to clade 2.5 (1). It was followed by the outbreaks of low pathogenicity avian influenza (LPAI) by H5N2 strains in 2005-2006 (2). The causative strain was genetically related to the H5 viruses circulating in Central America. In 2007, H5N1 HPAIVs belonging to clade 2.2 again hit Japan in two prefectures. From April to May 2008, several whooper swans found dead in northern part of Japan were revealed to be infected with H5N1 HPAIV of clade 2.3.2.1 (3). It was fortunate that there were no poultry cases in this year, although South Korea reported several outbreaks caused by related strains during the same period. Unprecedented series of HPAI outbreaks hit Japan during November 2010 to March 2011 (4). Number of poultry outbreaks recorded 24 and dead wild birds diagnosed to be infected with H5N1 HPAIVs were 61 during the same period. HA gene of those isolates belonged to clade 2.3.2.1. Infectivity and transmissibility of poultry isolates and a wild bird isolate were compared in chickens to show that 50% chicken lethal dose (CLD\textsubscript{50}) of the wild bird isolates was equal to or lower than chicken isolates, while transmissibility of the wild bird isolates was lower than chicken isolates. In April 2014, 3 months after first case of H5N8 HPAIV outbreak in South Korea occurred, one isolated case of the related strain, belonging to clade 2.3.4.4, occurred in Japan (5). HPAIV of clade 2.3.4.4 came back to Japan during December 2014 to January 2015, when the massive outbreaks with related viruses hit North America as well as Europe (6.7). CLD\textsubscript{50} of a chicken isolates and an isolate from fecal sample of wild bird was determined. It was again shown that CLD\textsubscript{50} of the wild bird origin was lower than the chicken isolate.

In November of 2016, the H5N6 HPAIVs, belonging to clade 2.3.4.4, have detected from carcasses and feces from wild bird and environment in several places in Japan. Detection of the viruses from wild birds

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have followed all over Japan, and total number of the cases were 214 as of 28th February 2017. Outbreaks of poultries caused by the H5N6 HPAIV have started since end of November, thereafter, ten outbreaks in total have been reported all over Japan as well. Among 10 outbreaks, 2 outbreaks were occurred in Muscovy duck farms and the others were in chicken farms. Their HA genes that caused those outbreaks belonged to clade 2.3.4.4 and were genetically related to the H5N6 subtype HPAIVs detected in South Korea. Sixty-three cases in wild bird have successively occurred in Ibaraki prefecture where our institute locates from 29th November 2016 to 24th January 2017. Forty-four cases were concentrated in an area within a radius of 5 km at central part of Ibaraki prefecture. We analyzed hemagglutinin genes from wild bird cases in Ibaraki prefecture by the method of Spatial Phylogenetic Reconstruction of Evolutionary Dynamics (SPREAD) to see genetic correlation of HPAIVs detected from wild birds in Ibaraki prefecture.

It was found that there were two clusters of incidents at central and southern part of the prefecture within approximately 10km radius respectively. Two clusters were almost 50km apart from each other. Although two clusters were apparent, each consisted of several incursions of the viruses. The cluster at the central part consisted of three genetically distinct viruses, while that at the southern part consisted of four. One in the southern cluster was spill over from the most frequently observed virus in the central cluster. The same virus spread within a 6km radius in the central clusters. Thus, although the viruses causing wild bird incidents in Ibaraki prefecture were genetically similar, SPREAD analysis clearly demonstrated the spatial and temporal relationship of those viruses.

[Acknowledgement] I sincerely appreciate the effort of North Ibaraki Animal Health Center for their dedication to the diagnosis of wild bird cases and Drs. Yuko Uchida, Ryota Tsunekuni, Nobuhiro Takemae, Taichiro Tanikawa, Junki Mine for their contribution to this paper as well as to the confirmatory diagnosis of poultry incidents in Japan.

Table 1 Poultry outbreaks caused by the Asian H5 HPAIVs in Japan

<table>
<thead>
<tr>
<th>Day of first occurrence</th>
<th>Day of final occurrence</th>
<th>Subtype</th>
<th>HA clade</th>
<th>Number of outbreaks</th>
</tr>
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<tbody>
<tr>
<td>2004/1/12</td>
<td>2004/3/1</td>
<td>H5N1</td>
<td>2.5</td>
<td>4</td>
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<tr>
<td>2007/1/13</td>
<td>2007/2/1</td>
<td>H5N1</td>
<td>2.2</td>
<td>4</td>
</tr>
<tr>
<td>2010/11/29</td>
<td>2011/3/16</td>
<td>H5N1</td>
<td>2.3.2.1</td>
<td>24</td>
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<tr>
<td>2014/4/13</td>
<td></td>
<td>H5N8</td>
<td>2.3.4.4</td>
<td>1</td>
</tr>
<tr>
<td>2014/12/16</td>
<td>2015/1/18</td>
<td>H5N8</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>2016/11/31</td>
<td>Not dissolved yet*</td>
<td>N5N6</td>
<td></td>
<td>10*</td>
</tr>
</tbody>
</table>

*: As of the end of 28th February 2017

Keywords: Influenza, Phylogeography, Japan

References

Case Report of Fowl Adenovirus Outbreak in South Asia

Keat FU

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Summary
Fowl Adenovirus (FADV) outbreaks were reported in South Asia 2015-2016 suspected for serotype 4, 8 and 11 in broiler breeders and broilers. However, qPCR revealed only FADV serotype 8 was positive from the sample collected. Whereas in 2015, serotype 4 (2%) and 11 (87%) were also positive reported in the same area by Rehman et al [4]. High mortalities in the progenies 0.5-1% per day with inclusion body hepatitis (IBH) were reported persisted for 1 month period from the same breeder flock. Hydro-pericardium was rare in this episode of outbreak. The farm management decided to induce molting in affected parent stocks and double vaccinations (FADV serotype 4 and 8) by local autogenous aqueous base vaccine and Boehringer Ingelheim (FADV serotype 4) vaccines were done during the molting period. This strategy stimulates maternal immunity, egg production ceased and the virus shedding gradually diminished. qPCR tested for viral shedding from breeder cloaca swabs and hatching eggs after 10 weeks post vaccination were negative. Progenies hatched from this post vaccinated and molted flock did not exhibit clinical signs of FADV infections or viral shedding indicates a success of this disease management. We recommend vaccinate both FADV serotypes 4 and 8 as a routine program twice during pullet period for future prevention. Monitoring emerging or reemerging FADV serotype 11 and ready to vaccinate are also beneficial to prevent production loss.

Clinical signs and Post Mortem
Progenies affected start showing high mortality 0.5-1% per day from 18 days to 35 days of age. Post mortem were found consistent pale, light yellow, discolored, friable, enlarged and petechial hemorrhages liver lesions (Figure 1 and 2).

Figure 1 pale, friable, enlarged petechial hemorrhage liver
Results
qPCR results were positive for FADV at 1219bp and nucleotide sequence was FADV species E, serotype 8b.

Histopathology
2 sections of liver were presented on a prepared histologic slide for histologic examination. Processing artifacts on the tissue were moderate, with morphologic detail being indistinct on nuclei and cellular borders. Both livers had diffuse coarse vacuolar change in hepatocytes cytoplasm, accompanied by multifocal coagulation necrosis of hepatocytes. The periphery of foci of necrosis were degenerating hepatocytes with basophilic, homogeneous to coarsely granular intranuclear inclusion bodies [5]. These histologic findings are consistent with inclusion body hepatitis (IBH) reported by pathologist Frederic J. Hoerr.

![Figure 2](image1) very light yellow, discolored liver in some birds

![Figure 3](image2) Sample 23887, liver. The liver has diffuse coarse vacuolar change in hepatocytes, with multifocal necrosis (orange arrow) and basophilic intranuclear inclusion bodies (blue arrows)
Figure 4 Sample 23887, liver. Two inclusion bodies from figure 1 are digitally magnified to show cellular detail. Processing artifacts have caused indistinct nuclear detail; however, the size and nuclear location of these inclusion bodies are consistent with adenoviral hepatitis. H&E 1000X

Results and discussion
FADV is ubiquitous and resistant to disinfection and cleaning [1],[2],[3]. In an average biosecurity level farm, breeders maybe acquire FADV antibodies prior to laying period by natural exposure to farm existing virus. Therefore in ideal situation, the exposed hens are immunized, produces antibodies, at least not shedding virus during whole production period. However, in this case of a naïve GP flock entering the production period and get infected will shed virus to the progeny and causing IBH with mortality for 3-6 weeks. Breeder hens in contrast show non-defined clinical signs during exposure due to age resistance but some degree of egg production drop was also reported. This is common scenario in non-vaccinated parents in newly build farm or a farm (new/clean house syndrome) that was rested long period and disinfected well enough to break the virus circulation therefore FADV infections reemerged. The best strategy for FADV prevention is vaccination and monitoring sero-conversion by ELISA and ready to identified other serotypes by molecular methods.

Keywords: Adenovirus Species E, Inclusion Body, Serotype 8b.

References
Case Report of Recent Infectious Bronchitis in a Broiler Breeder Farm from Northern Asia

Keat FU

Global Veterinary Team, Aviagen Inc., Malaysia

Summary

This is a report on a flock of broiler breeder experienced severe egg drop (40% below standard) and poor hatchability (approximate 10% below standard) due to Infectious Bronchitis Virus (IBV) in Northern Asia (2016). 793B variant strains and conventional Massachussets strains vaccines were used in this flock during pullet stage at 1 - 3 weeks of age initially may not be protective from early infections as first week of age or possible vaccination failure due to poor applications. Post mortem revealed severe cystic oviduct complicated by bacterial as secondary infections were seen in most infected hens. White pale, misshapen hatching eggs were observed. Hens infected with IBV found in “penguin posture” and eventually died of bacterial complications. IBV was positive in qPCR test from cecal tonsil samples. Sequencing and phylogenetic analysis reveal closed related to a China isolate SC021202. However, this flock was depopulated early due to poor performance. Since this outbreak, the farm management decided to use 793B variant live vaccine at day old in combination with Newcastle disease live vaccine followed by Massachussets strains live vaccines at day 7 to provide early protection in the next intake flock. There is no reoccurrence of this IBV infection until present.

Introduction

IBV is an enveloped, single stranded RNA virus, which have four structural protein –Spike (S1, S2); Matrix (M), envelope protein (E) and nucleocapsid (N). These spikes glycoprotein comprises two most important glycoproteins (S1 and S2) which appear under electron microscope as a crown like structure [1][2]. S1 glycoprotein is recognized for host immune response for producing neutralizing antibody against IBV infections and serotype hence can be determined [4]. For the virus to be survived from the host immune system, IBV known for its genetic drift and shift to diversify the S1 glycoprotein identity and thus multiple serotypes with distinguish pathogenicity were observed in the last few decades. The most common serotype is Massachussets (Mass) strains being used as an IBV live or killed vaccines. Despite many newly isolated variants strains such as variant 2, QX, 793B, Arkansas, Connecticut etc [5],[6] were adapted as live or killed vaccines, cross protections are reported ranged from 10%- over 90% depending on the prototypetype or homogeneity when facing field challenge [7]. Infertility in mature cockerels caused by IBV in USA and Brazil were reported. [3], [9]. In recent 5 years, IBV infections have shown some reducing trends in number of case report but remain top 5 most reported in breeder, broiler and considered as emerging diseases when surveyed in Asia [8].

Post mortem and sample submission

Post mortem revealed cystic oviduct and swollen kidney. Infectious bronchitis variant strain, Newcastle genotype VII, Avian Influenza (H9) and (H5) were suspected. Tissue samples such as brain, cecal tonsil, liver and kidney were sent for qPCR test. Standardized viral isolation procedures were performed. RNA was extracted by Viral Gene-spin™. Samples were sent to Macrogen Inc. for sequencing by using Sanger’s method.
Results

IBV was positive detected by qPCR. However, NDV and AIV were negative. The IBV strain sequence results revealed closer to a China strain SC021202.

Figure 2 Phlogenetic relationship of the highlighted green “FU” (test sample) and SC021202 IBV strain based on S1 nucleotide sequence.
Discussion
This is an IBV strain closest with a strain called SC021202 found in China [10]. Cross protection studies between Mass, SC021202, 793B, QX and other local China strains are necessary. A novel IBV, CK/CH/2010/JT-1 was isolated believed to be as important, occurred in similar type of vaccination program flock (793B and H120) [11]. Cross reaction studies could be conducted between these two strains will be beneficial.

Keywords: IBV, SC021202, Cystic Oviduct

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(9) Villareal (2007): Orchitis in Roosters with Reduced Fertility Associated with Avian Infectious Bronchitis Virus and Avian Metapneumovirus Infections
Chicken Astrovirus Infection for RSS, Visceral Gout and White Chick Syndrome

Youngho HONG

Global Veterinary Team, Aviagen Inc, AL, USA

Summary
Chicken Astrovirus (CAstV) had been described as an enterovirus-like virus before it is identified in 2004. CAstV is relatively recently identified even though Runting and Stunting Syndrome (RSS) which exhibit poor pigmentation of the shanks, decreased weight gains, elevated feed conversion, poor feathering, diarrhea, etc. has been an issue in broiler industry for decades. After first identification of CAstV from RSS case, there were a couple of more issues of CAstV in chicken such as Visceral Gout (VG) and White Chick Syndrome (WCS). WCS has been observed in several countries in Europe, South and North America since first outbreak in 2006. As well as white chicks syndrome shares characteristics of symptoms and lesions in RSS such as kidney and liver lesion, runting, poor development, weakness and abnormal feathering, it has been described transient egg production drop in breeder and reduction of hatchability with dead in shell embryos together with white plumage in day old chick. VG is officially reported in 2013 from cases of 2011 and 2012 from India. However, similar clinical sign and lesions have been observed in India more than 10 years. There are also several outbreak of VG in Middle East countries. Severe visceral and articular gout were found in affected chicks from 1st week to 6weeks old broiler by field observation. CAstV spread both vertically and horizontally.

Runting and Stunting Syndrome
In 2004, CAstV was termed after genetic sequencing found it is different from astrovirus in turkey and avian nephritis virus. An investigation into CAstV strain diversity from historical and circulating field strains was reported by comparing ORF2 sequence. This allows phylogenetic tress to elucidate the association of specific strains of CAstV causing RSS, VG and WCS (3).
Figure 1 Phylogenetic tree of CAstV based on complete ORF 2 amino acid sequence (3).

A disease syndrome of broiler chicken characterized by stunted growth, poor feathering and an increase in lameness has been described from 1970’s (1). RSS usually starts at 4-8 days old with clinical sign of reluctance to move, ruffled feathers, feces and litter eating, pale shanks with possible secondary signs and lesions of Rickets, atrophied bursa or thymus, gizzard erosion, etc. Chick may huddle as they feel cold. Uneven flock performance occurs when the variance in weights at slaughter is larger than expected. It is most likely that a virus usually always involved in this syndrome such as Reovirus, Rotavirus, Enterovirus as well as CAstV. Most of these viruses are commonly found in many existing chicken houses with/without RSS issues. In most cases of RSS on histopathology, it can be found evidence of intestinal damage often attributed to enteric virus. However, when viruses are identified, isolated then challenge the viruses into normal birds, it is often difficult to re-produce the total RSS diseases. CAstV is one of viruses identified from RSS but as yet a single etiological agent has not been identified. Other management factors such as small hatching egg, high incubation temperature and improper brooding condition in first week can also affect incidence of RSS. It is also possible that early CAstV and other enteric viral infections may create an abnormal intestinal environment that facilitates later dysbacteriosis, and imbalance of naturally colonizing bacteria, usually occurring between days 20 and 30 post hatch and which could further impair performance due to diminished nutrient digestibility and weakened intestinal barrier protection (2). Coinfections of CAstV with other enteric viruses have been observed such as rotavirus, avian nephritis virus, etc.

**Visceral Gout**

Severe kidney disease of young broiler chicks with outbreaks of visceral gout and up to 40% mortality was reported in India in 2012 with CAstV (4). CAstV isolated from VG-affected kidney by inoculating embryonated specific pathogen free eggs showed dwarfing in embryos and a cytopathic effect in chicken embryo kidney cells (4). Inoculation of 1-day-old SPF and broiler chicks with CAstV caused gout and mortality between 4 and 10 days post inoculation (4). Ongoing diagnostic surveillance in 2016 indicates highly similar strains are still circulating in broiler flocks in the Middle East (3). However, the VG has been reported in India industry for more than 10 years at least from personal communication with people in broiler industry in India. They also found same vertical transmission case of VG in broilers from same breeder source both in India and a Middle East country where they exported hatching eggs at the same time. CAstV is causing VG has been
reported only in India and Middle East area. VG is widely spread in all over India. Analysis from 77 samples taken from Indian customers of Aviagen found CAstV isolated in VG broilers was 2nd most frequent findings followed by Fowl Adenovirus group I in between Sep 2011 and Feb 2015 (5). VG found from 3days old to 6weeks old broilers in India. Most of VG cases in India were found in customers moved from other breed in the beginning and then there were only few cases reported in 2016 after they settle down. Tests from vaccines from several vaccine manufacturers supplying CastV vaccine off-label and it turned out a killed vaccine was not inactivated with kidney debris and the other one didn't have any CAstV antigen.

White Chick Syndrome
Recently CAstV has become associated with hatchery with hatchery diseases, most noticeably “White Chicks” (3). WCS has been observed in several countries in Europe, South and North America since first outbreak in 2006. As well as white chicks syndrome shares characteristics of symptoms and lesions in RSS such as kidney and liver lesion, running, poor development, weakness and abnormal feathering, it has been described transient egg production drop in breeder and reduction of hatchability with dead in shell embryos together with white plumage in DOC. Even though it is not officially reported, similar clinical sign and gross lesion were found in China from a vertical transmission suspected case.

Keywords: Chicken Astrovirus, Runting and Stunting Syndrome, Visceral Gout, White Chick Syndrome

References
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Mortality Pattern of Non-Infectious Diseases in Broiler Breeder

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Summary
As biosecurity has been improved in modern broiler breeder industry, less infectious diseases are found in many breeder farms. Nevertheless, broiler breeder farms with higher biosecurity level are still experiencing higher weekly mortality than primary breeder company’s performance objective. US industry reported 13.92% of broiler breeder hen mortality in 2014, which is about 6% higher than primary breeder company’s guideline, otherwise Japanese broiler breeder industry reported <7% hen mortality until 64weeks old. Broiler breeder mortality surveys from 4 companies said that not only company with good hen mortality but also company with much higher mortality than performance objective showed similar mortality pattern. All 4 companies from different countries survey put egg yolk peritonitis as most frequent PM finding followed by leg problem, prolapse, calcium tetany, ascites etc. Egg yolk peritonitis, prolapse and calcium tetany are all closely related with metabolic disorder in broiler breeders. Egg yolk peritonitis without primary respiratory sign and prolapse are closely related with energy intake and body weight control. Calcium tetany is due to inadequate level of calcium in the blood. Leg problem is various from ruptured tendon to trauma and secondary staphylococcus infection after any kind of stress and intestinal health issues. Ascites could be caused by liver damage such as amyloidosis.

Egg Yolk Peritonitis (EYP)
Egg yolk peritonitis is characterized by acute mortality with fibrin or albumen-like material with a cooked appearance among the abdominal viscera. All 4 companies’ PM findings from different countries(USA, Korea, Japan and UK) showed EYP is most frequent finding in 2015. This finding is not so much different over years.

Table 1 Top 4 Mortality survey in 4 different companies in 2015

<table>
<thead>
<tr>
<th></th>
<th>Company A(USA)</th>
<th>Company B(Korea)</th>
<th>Company C(Japan)</th>
<th>Company D(UK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Egg yolk peritonitis</td>
<td>Egg yolk peritonitis</td>
<td>Egg yolk peritonitis</td>
<td>Egg yolk peritonitis</td>
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<tr>
<td>2</td>
<td>Prolapse/peck-out</td>
<td>Liver lesion</td>
<td>Ascites</td>
<td>Ruptured tendon</td>
</tr>
<tr>
<td>3</td>
<td>Leg problem</td>
<td>Leg problem</td>
<td>Prolapse/peck-out</td>
<td>Calcium Tetany</td>
</tr>
<tr>
<td>4</td>
<td>Calcium Tetany</td>
<td>Ascites</td>
<td>Ruptured Tendon</td>
<td>Histomoniasis</td>
</tr>
</tbody>
</table>

E. coli can be isolated from EYP together with other bacteria such as Pasteurella, Salmonella, etc. Birds die of bacterial infection but primary cause of EYP is reverse movement of albumin and yolk in oviduct by retrograde ciliary action to transfer sperm from sperm storage tubule in uterovaginal junction to infundibulum. In normal ovary hierarchy, one ovary should ovulate in oviduct. But if there are 2 ovaries, for example one in shell gland and the other in magnum, ovary in magnum will be reversed due to retrograde ciliary action which transfers sperm from sperm storage tubule after the egg in shell gland is laid. So, uneven hierarchy of ovary and/or over developed ovaries with less than 24hours development interval will reverse ovary in oviduct to abdominal cavity. Then bacterial infection could be made from air sac and/or oviduct.
To control incidence of EYP, management of body weight, uniformity of flock, non-excessive energy intake which can over-develop ovaries are the best preventive strategies.

Prolapse
During oviposition there is a tendency for the distal section of the reproductive tract to prolapse. Cloacal prolapse may involve the intestines, reproductive tract and ureter. The prolapsed tissue has a smooth surface and is shiny and congested. In laying hens, cloacal prolapse may result from egg laying (1). If prolapse of shell gland is not withdrawn from the vent into the body cavity, hens will be vulnerable to continuous pecking that can lead to cannibalism (2). Over-fed heavy hens with excessive fat pads could have more prolapse and peck-out in old hens. If there is an increased exposure time of the oviduct to the outside when the hen is laying, the chance for peck-out will be increased. Otherwise, prolapse will be increased in heavy young hens as their first egg size is bigger and more double yolks, which oviduct can’t afford to push egg out. Body weight, uniformity control in rearing period and non-excessive energy intake from light stimulation are also the best preventive strategies.

Calcium Tetany
Calcium tetany in broiler breeder is characterized by muscle weakness or paralysis and is caused by inadequate levels of calcium in the blood (3). The risk factors associated in the development of calcium tetany are poor uniformity, flocks coming into production quickly, high calcium feed(>1.2%) being fed before the onset of production and small particle sized calcium. It is similar to cage layer fatigue in commercial layers, milk fever in dairy cattle, and eclampsia in small animals. Although calcium tetany was first recognized as a significant cause of mortality over 20 years ago, it is a poorly defined disease of broiler breeder hens that results from acute hypocalcemia. The most common signs of calcium tetany are muscle weakness and paralysis which is usually most obvious during the early morning period or after feeding. Birds can be found twitching but this is rare. Mortality is often blamed on male aggressiveness but the underlying problem here is that affected hens cannot get away from the males. Typical post-mortem findings include an active, congested ovary, a partially or fully formed egg in the oviduct and possibly damage to the back of the birds from male abuse. These findings in an otherwise healthy bird with no leg problems or other explanations for the death are considered calcium tetany suspects. Calcium tetany should be confirmed by finding significantly decreased levels of ionized calcium in the blood, as diagnosis based on clinical presentation and necropsy results can be inaccurate (4).

Calcium can be supplemented in the form of oyster shell or large particle lime-stone at 2-5g/bird/day for three consecutive days followed by three days’ rest together with vitamin D to increase absorption of dietary calcium. Continuous high levels of supplementation can negatively affect calcium regulation and do more harm than good. This program can be used until the mortality is under control which may take several cycles. For prevention of calcium tetany, it is important that the majority of birds reach sexual maturity at the same time. Birds receiving high calcium feed in advance of sexual maturity are at a higher risk of developing calcium tetany. Waiting until 5% production to change to the breeder feed will ensure that most birds have reached sexual maturity, although many may not have started to lay eggs. Large particle calcium stays the intestinal tract longer than small particle calcium, increasing the time that it is available for absorption. As the eggshell is typically deposited well after the majority of the feed has passed from the intestine, the increased time of availability may be beneficial in maintaining blood calcium level during this period of demand. Avoid heat stress to manage blood CO2 and blood pH changes will be also helpful.

Leg problem
Broiler breeder leg health is a key to achieving optimum flock production and welfare.
Bumble foot is often associated with walking on hard, rough or sharp surfaces, which often occur, for example, where the litter is wet(5). A high percentage of slatted area (>30%) could also increase the risk. Feed affected with bumble foot are commonly infected with Staphylococcus. Good litter management in combination
with good environmental control is important to prevent bumble foot. The main cause of Foot Pad Dermatitis (FPD) in broiler breeder is wet litter. Litter type, moisture, stickiness and composition have all been identified as contributing factors. In the long term FPD can lead to infections in the foot and to more permanent leg pathology such as bumble foot. Ruptured tendon (RT) has been diagnosed in broiler breeders for many years, with cases reported as far back as the 1950’s. It is now recognized that several predisposing factors may contribute to RT, and it is when these factors are sufficient in number and/or severity, that one or both gastrocnemius tendon rupture. It is also important to note that although RT most commonly occurs during early to mid-lay, tendon damage will most likely have occurred earlier in the bird’s life. Feed restriction has been also suggested as a predisposing factor for the rupture of gastrocnemius tendon (6). A disease process may predispose the tendon to rupture after minor trauma later in life (7). Height of slat suggested a cause of damage of the gastrocnemius tendon (8). Prior damage to the tendon might have predisposed to its rupture when the birds were forced to jump from the slats onto the ground or over the feeders and waterers to avoid the aggressive males (8).

**Keywords:** Peritonitis, EODES, Calcium Tetany, Prolapse, Broiler Breeder

**References**

Diagnostics and Update on IBV Types Around the World

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Summary

Avian coronavirus infectious bronchitis virus (IBV) causes a highly contagious upper-respiratory tract disease in poultry. Clinical signs are mild but can lead to secondary infections that cause severe economic loss. The multitude of different IBV types that for the most part do not cross-protect make diagnosis difficult but extremely important. Diagnostic tests for IBV have gone from slow labor intensive virus isolation and neutralization testing to rapid and highly specific molecular tests that employ the polymerase chain reaction and nucleotide sequencing. Consequently, an enormous amount of sequence data is now available for comparison of viruses around the world. This paper will examine the diagnostic tests currently used for IBV and give an overview of currently circulating and important IBV strains around the world.

Introduction

Avian coronavirus infectious bronchitis virus (IBV) is an enveloped positive-sense single stranded RNA virus that causes a highly contagious upper-respiratory tract disease in poultry [1]. Coronaviruses have the largest (28Kb to 32Kb) single stranded positive sense RNA genome known, which codes for the viral polymerase (1a and 1b), 4 structural proteins (Spike [S], Membrane [M], Envelope [E], and Nucleocapsid [N]) and numerous regulatory proteins. The most important structural protein found in coronaviruses is the S glycoprotein. The S glycoprotein forms club shaped projections on the surface of the virus particles [2]. It is anchored in the envelope of the virus and consists of two subunits designated S1 and S2. Spike mediates host cell attachment and is for the most part responsible for host cell specificity. In addition neutralizing antibodies are directed against the S1 subunit [3]. For this reason, spike is the target of most diagnostic tests for IBV.

Clinical signs are a mild upper-respiratory disease characterized by watery eyes, mucus in the nares and trachea, mouth breathing and tracheal rales. Some strains of the virus can cause a nephritis and since IBV infects epithelial cells, it can also affect the reproductive tract of hens resulting in decreased production and poor quality eggs [1]. The virus is worldwide in distribution and extremely difficult to control because different types of the virus that causes the disease do not cross-protect. Different types of IBV include multiple serotypes and variants of the virus, which arise due to mutations and recombination events during replication [4]. Complicating this situation is the ability of IBV to rapidly change and adapt to the host. Thus, it is extremely important not only to rapidly identify the presence of the virus but also to identify the IBV type causing disease.

Traditional diagnostic tests

Serology: The enzyme linked immunosorbent assay (ELISA) is used to detect antibodies against IBV. The test detects circulating IgG and can be positive at approximately 14 days following infection. The ELISA does not differentiate antibodies against different serotypes of the virus and thus, it is used largely
as a screening tool to monitor vaccination programs and to inform poultry veterinarians when an outbreak has occurred.

The hemagglutination inhibition (HI) test can also be used to detect antibodies against IBV. The IB virus treated with neuraminidase will hemagglutinate chicken red blood cells. Antibodies against that virus will block hemagglutination indicating a positive test. And, although this test can be serotype specific, it suffers from cross reactivity especially in birds vaccinated multiple times with several different IBV types.

**Virus detection and serotyping:** IBV grows well in 9 to 11 day of incubation embryonated eggs [5]. The chorioallantoic sac is inoculated and embryos are examined 7 days later for typical lesions including stunting, hemorrhagic embryos, club down and sometimes urates in the kidneys. The gold standard for typing IBV is the virus-neutralization (VN) test performed in embryonated eggs. Generally, a two-way neutralization test is performed, first with known antisera and the unknown virus and then with known viruses and antisera prepared against the unknown virus. The VN titers are used to calculate a relatedness value that correlates quite well with protection in vivo.

**Molecular diagnostic tests**

Molecular typing methods for IBV were first used in 1993 and quickly became popular because they are extremely rapid compared to the two-way VN test. Those methods utilized the reverse transcriptase-polymerase chain reaction (RT-PCR) test to amplify the spike glycoprotein gene. Subsequently the type of the virus is identified by restriction fragment length polymorphism testing on the amplified spike gene or by specific RT-PCR primers against spike. It was soon discovered that the sequence of the spike gene correlates with the serotype of the virus. So, now RT-PCR amplification of the S1 glycoprotein gene (or a hypervariable portion of the S1 gene) followed by nucleic acid sequencing and analysis is used to type IBV [6].

Sequence analysis can identify unknown viruses using the Basic Local Alignment Search Tool (BLAST) available online at GenBank (www.ncbi.nlm.nih.gov) which screens the database for like sequences. The analysis includes sequence alignments that determine the relatedness of unknown viruses with specific strains in the database. Genetic typing of IBV makes it possible to identify and compare many virus isolates in a short period of time. A tremendous amount of information can be obtained from spike gene sequence data. The GenBank database is extensive and allows for immediate comparison of sequence data from IBV types around the world. Phylogenetic analysis of sequence alignment data shows family groups and genetic distances between strains of IBV, which can be used to infer which vaccines may be efficacious. This technology has transformed diagnosis and control of this virus.

Recently, real time RT-PCR has been used to directly detect IBV in a clinical sample [7]. Real time RT-PCR benefits from being a very rapid high throughput test, it is quantitative and it is relatively inexpensive. Primers and a probe to highly conserved regions in the viral genome are used in a pan IBV test that detects all serotypes but does not differentiate. A further development of that test is type specific IBV real time RT-PCR tests. Primers that flank a hypervariable region in spike and a probe designed to anneal to a type specific IBV sequence are used to detect different IBV types.

**Common IBV types around the world**

Thanks to genetic typing of IBV isolates, there is now an enormous amount of sequence data available for IBV types worldwide [8]. An examination of that data shows a plethora of different IBV genetic types but those types tend to be geographically restricted and they can be digested down to a limited number of important viruses causing disease in commercial poultry. Following is a listing of viruses recently identified and/or currently circulating in commercial poultry and Figure 1 below shows a phylogenetic tree for these viruses where full length S1 sequence is available.

- **USA:** Mass, Ark, DE, GA98, CAL99, CA1737/04, GA07, GA08, GA13, DMV/1639
- **Canada:** Mass, Qu_mv, Conn, CA1737/04, PA/Wolg/98, 4/91
- **China:** Mass, QX (LX4 type), Q1 (LDL type), LDT3, LHLJ, BJ, N1/62, LSC, 793B
Keywords: Infectious Bronchitis Virus, Diagnostics, Types Worldwide

References
Figure 1  Phylogenetic tree of full length S1 amino acid sequences. The sequences were aligned in MegAlign Pro and the phylogenetic tree was created using MUSCLE (DNASTAR, Madison Wisc.)
Control of IBV: Attenuated Live Vaccine Application Challenges

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Summary
Avian infectious bronchitis virus (IBV) is a coronavirus that causes a highly contagious upper-respiratory tract disease in poultry. The best strategy to control IBV is the use of attenuated live vaccines in broilers and a combination of live and killed vaccines in layers and breeders. Killed vaccines are injected and relatively straight forward in their application. However, live attenuated vaccines can be a challenge to mass administer to poultry effectively. This overview will focus on the administration of live attenuated IBV vaccines in the hatchery and in the poultry house. Proper administration of live IBV vaccines requires careful attention to vaccine storage and handling, proper working order of vaccination equipment and vaccine application according to the manufacturers’ instructions including delivering a full dose of vaccine.

Introduction
Avian infectious bronchitis virus (IBV) causes a highly contagious upper-respiratory tract disease in chickens. The disease is global and costs the poultry industry millions of dollars annually. Currently, the best strategy to control IBV is the use of attenuated live vaccines [1]. In the USA, live vaccines are given to broilers a one-day of age in the hatchery and in the field at 14 to 18-days of age. However, complete protection is difficult to establish because different IBV types do not cross-protect. In addition, application of live vaccines, either by spray cabinet in the hatchery or by water or spray in the poultry house is difficult to do properly. Equipment failures, mishandling of the vaccine and poor technique can lead to flocks that are not adequately protected. In addition, IBV is a relatively fragile virus that is easily killed. It is critical that the vaccine is properly handled according to the manufacturers’ recommendations and giving less than a full dose is never recommended. Killed IBV vaccines are used in long lived birds (breeders and layers) and must be injected. This is a relatively straight forward procedure with the main concern being missed birds. This overview will focus on the challenges associated with application of live vaccines.

Typically, two IBV vaccinations (which can contain two or three different IBV types) are given to broiler chickens. The first vaccination is given in the hatchery at one-day of age and the second is given in the field as a booster vaccination at 14 to 18-days of age. The birds develop some immunity from the initial “priming” vaccination in the hatchery, then the field boost vaccines are given to induce a strong local and systemic immunity. Using live vaccines induces a mucosal immune response that essentially blocks infection in the upper-respiratory tract. IgA is involved in protection from infection [2, 3]. Cell mediated immunity is also induced and is responsible for recovery from the disease [4]. Maternal antibodies (IgY), which does not appear to interfere with vaccination, can protect chicks from 1 to 2 weeks of age depending on antibody titers in the chicks. And although some cross-protection can be realized with different combinations of vaccine types, careful vaccine application remains key to inducing adequate protection.
Vaccine storage and preparation

Most live IBV vaccines are lyophilized and are usually stored a 4C. There are a few IBV types that are frozen products and they are typically stored in liquid nitrogen. Regardless of the vaccine form, one should always store IBV vaccines according to the manufacturers recommendations. IBV is an enveloped virus that contains a fragile RNA genome, and is easily inactivated by elevated temperature and chemicals such as chlorine, which is in many municipal water sources. Therefore, it is critical to prepare the vaccine working solution with cold pure (preferably distilled and deionized) water or diluent supplied by the manufacturer. Studies in our laboratory and by others showed that vaccine mixed with room temperature water was only viable for about 30 minutes (Jordan, et al. University of Georgia unpublished results). Mixing the vaccine with cold water will ensure viability until the vaccine can be completely distributed to chicks.

Hatchery spray cabinet

Spray vaccination in the hatchery is a cost-effective way of delivering IBV vaccine to many chicks in a short period of time. Typically, vaccines are delivered to a moving tray containing 100 chicks by a syringe system that pushes the vaccine through a spray nozzle. Disposable syringes used in the spray cabinet are designed for one use; however, they are typically used hundreds of times often leading to failures. In addition, it is easy to get air in the vaccine lines or the syringe body itself, resulting in less than a full dose of vaccine being delivered to the chicks.

As mentioned above, IBV is a fragile virus but shearing forces associated with movement of vaccine under pressure through one-way valves and in and out of the syringes was found for the most part to not result in a significant loss of vaccine titer [5]. However, spray cabinet parameters including the volume of vaccine reaching the chicks, spray pattern uniformity and droplet size are important for vaccine performance. Research has shown that increased volumes up to 21ml of vaccine delivered to 100 chicks by spray cabinet increases the total amount of sprayed vaccine reaching the chicks and improves efficacy. In addition, a larger droplet size also allows for more vaccine to reach the chicks increasing vaccine performance. Spray pattern and coverage across the entire chick box is another important parameter to achieve adequate vaccination, and here timing of chick box movement and syringe deployment are critical. Syringes are actuated by air pressure and the only way to affect the timing of vaccine delivery is to increase or decrease the air pressure used to actuate the syringes. Research on variable pressures used in the syringe system impacting the spray pattern and droplet size, and poor syringe performance and failures has led to the development of positive pressure hatchery cabinet vaccine delivery systems. Those systems are designed to deliver a constant pressure of vaccine through the nozzles, which are rated for droplet size and spray angle at that specific pressure, which ensures an even and consistent spray pattern. The constant pressure spray cabinets are also designed to keep the vaccine mixed and cold ensuring that high tittered vaccine virus is delivered to the chicks.

Using a gel to deliver vaccine to chicks in the hatchery has gained some popularity recently. Gels of different viscosity are available and are delivered using a bar with ports where droplets of the gel are dispensed under positive pressure. Gel systems were originally developed for coccidia vaccines but research in our laboratory has shown that gel is equivalent to spray with regard to IBV vaccination. Issues to consider with gel are that even mixing is critical and can be a challenge with very thick gels. In addition, gel bars need to be cleaned and maintained so they do not clog. Systems using gel to deliver vaccine should be monitored for the volume of vaccine applied and even gel distribution across the chick box.

Vaccine application in the field

Vaccines are applied in the field using a backpack sprayer or by water administration. Backpack spray vaccination is through vaccine delivered to a column of forced air (leaf blower) or by positive pressure forcing vaccine through a nozzle to deliver the vaccine. The main challenge to delivering vaccine by spray in the poultry house is making sure all chicks are covered. This involves at least 2 and usually 3 technicians spraying vaccine in the house. In addition, the timing should be such that all vaccine is delivered by the
time the technicians walk through the entire house and returning to the starting point. Research has shown that dimming the lights and turning off the fans are key to success [6]. Spray application of vaccine in the poultry house also requires careful mixing of vaccine with distilled deionized water or diluent, spray equipment sanitation and careful checks of equipment performance.

Water application although seemingly straight forward, also has many challenges. Vaccine can be applied by a proportioner which pumps concentrated vaccine into the water lines or by mixing the vaccine in a ‘tub’ and pumping it through the water lines. Here getting the vaccine concentration correct is essential to deliver a full dose to all birds and adding dye to the vaccine mixture makes it easy to verify that it is distributed throughout the water lines. Regardless of the method, it is critical to treat the water lines to insure they are clean and free of contaminants. Clean water without chlorine needs to be used, and the vaccine should be mixed with a recommended diluent or stabilizer. The birds must be adequately water starved prior to application and all birds should have access to the water containing vaccine. This last point involves walking the flock and making sure that reluctant birds get up and drink. Finally, since the vaccine is temperature sensitive, all the vaccine must be consumed in a reasonable amount of time, usually less than 1 hour.

**Characteristics of IBV vaccines**

Infectious bronchitis virus is highly infectious and although attenuated vaccines appear to be equally infectious, successful vaccination requires delivering sufficient vaccine to the chicks. Manufacturers do a lot of research to determine the proper dose that should be given and it is important to always follow the manufacturers recommendations and deliver a full dose of vaccine to each chick.

Typically, live IBV vaccines go through a period of replication in the upper-respiratory tract peaking at about 7 to 10 days post-vaccination. And, most vaccines are cleared from the upper-respiratory tract by 21 to 28 days of age, but some vaccines can be found in broiler chickens even up to the time they go to slaughter. Previously, we documented that Arkansas (Ark) type IBV vaccines were persisting in broiler flocks. More importantly, we found that spray vaccinated birds removed from the field were only partially protected against Arkansas challenge; whereas sound protection was observed in birds vaccinated and challenged with other IBV types (Mass and Delaware viruses) [7]. Experimentally, it was confirmed that there was no interference between Ark and other IBV vaccine types, and when Ark vaccine was given by eye-drop, sound protection was observed. However, Ark vaccine was not efficacious when administered by a commercial hatchery spray cabinet. We examined the spray cabinet dose of ArkDPI vaccine required to provide protection similar to eye-drop and found that a 100X dose was needed. Further analysis showed that a minor virus population in the ArkDPI vaccine was infecting the chicks and that a sufficient amount of that minor population (100X dose of vaccine) was needed to adequately infect and protect the chicks. Although subpopulations also exist in other IBV vaccine types, they appear to infect chicks equally and are not an issue like the subpopulations in ArkDPI type vaccines.

**Summary**

Although different serotypes of IBV can be a challenge to control with vaccines due to limited cross-protection, it remains that it is extremely important to properly apply live attenuated IBV vaccines to achieve full protection in poultry. Hatchery spray cabinets and field spray and water vaccination methods are excellent ways of mass delivering live vaccines to commercial poultry, but proper storage and handling of the vaccine, and well maintained and functioning equipment are keys to success.

**Keywords:** Infectious Bronchitis Virus, Vaccination, Live Attenuated Vaccines, Vaccine Application
References


Characterisation of the Korean Field Strains of Mycoplasma Synoviae

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Summary

Mycoplasma synoviae (MS) is a poultry pathogen that causes respiratory disease, infectious synovitis and egg shell apex abnormality in chickens. To reduce the economic losses caused by MS infection in the poultry industry, a live attenuated vaccine developed in Australia has been recently registered in Korea. After introduction of a live attenuated vaccine into the field, reliable diagnostic tools to discriminate vaccine from field strains were required. In previous study, 88.6% of tested broiler breeder flocks showed MS specific antibody in Korea. However, the Korean field strains of MS have not been characterized well. In this study, we isolated and characterized the Korean field strains of MS. Minimal inhibitory concentrations (MICs) of 8 antibiotics for the Korean field strains of MS have been determined. For molecular epidemiology of MS, phylogenetic tree analyses were performed using the vlaA gene and concatenated MLST target sequences. These results would be a first step to characterize the Korean field strain of MS and provide valuable information for development of reliable diagnostic tools for molecular epidemiology in Korea.

Mycoplasma synoviae (MS) is a poultry pathogen that causes respiratory disease, infectious synovitis and egg shell apex abnormality in chickens. MS infection most frequently occurs as subclinical, however MS may contribute to airsacculitis and infectious synovitis in mixed infections with other respiratory pathogens. MS can be transmitted either horizontally via direct or indirect contact or be transmitted vertically. To reduce the economic losses caused by MS infection in the poultry industry, a live attenuated vaccine (Vaxsafe® MS containing temperature-sensitive MS-H strain) developed in Australia, has been recently registered in Korea. After introduction of a live attenuated vaccine into the field, reliable diagnostic tools to discriminate vaccine from field strains were required.

In previous study, 88.6% of tested broiler breeder flocks showed MS specific antibody in Korea. However, the Korean field strains of MS have not been characterized well. In this study, we isolated Korean field strains of MS. To obtain pure MS isolates, three times of filtering and colony picking were performed until no contaminated organisms including Mycoplasma gallinarum detected in the MS cultures. Minimal inhibitory concentrations (MICs) of 8 antibiotics for the Korean field strains of MS have been determined. The Korea field strains were highly resistant to enrofloxacin (Figure 1).
Sequence analyses of the conserved region of the vlhA gene, encoding haemagglutinin, could be a useful tool for detecting and initial typing of MS strains. Conserved region of the vlhA gene of the Korean field strains was sequenced and phylogenetic tree analysis was performed. In phylogenetic tree analysis, nucleotide sequences of the vlhA gene between Korean field strains and vaccine strain were clustered into three genotypes (Figure 2).

Furthermore, discriminating power of sequence analysis of the vlhA gene was compared with that of MLST analysis for MS. The Korean field strains were clustered together in the vlhA gene tree, while they were separated into two different groups in the MLST tree. These results suggested that the MLST tree has better discriminating power than single target sequence analysis like vlhA gene.
Figure 3 Phylogenetic tree analysis of the vlhA gene and concatenated MLST target sequences between field strains and vaccine strain

These results would be a first step to characterize the Korean field strain of MS and provide valuable information for development of reliable diagnostic tools for molecular epidemiology in Korea.
What We Have Learned from Comparative Genome Analysis of Infectious Laryngotracheitis Virus

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Summary
Infectious laryngotracheitis virus (ILTV) is a gallid herpesvirus I causing a major respiratory disease in chickens. Advancement of sequencing technology facilitate to determine complete genome of ILTV and 31 of ILTV complete genomes were uploaded in the GenBank up to date. In genome alignment and phylogenetic tree analysis, ILTV strains were clustered into two large groups, and both of the genotypes possibly divided into two sub-genotypes. Natural recombinants between two genotypes have been identified in genome analysis. Herpesvirus has a double stranded DNA genome and lower mutation rate than RNA viruses. Complete genome sequence of experimentally passaged strains showed only few of amino acid changes throughout the entire genome compared to that of the non-passaged strains. After comparative genome analysis, new genotyping method, which would have better discriminating power than current PCR-RFLP, for ILTV was developed using allelic variations from multiple genomic regions. Complete genome sequencing will be easier and cheaper in the future, and the increasing genetic information would allow further understanding of molecular epidemiology and pathogenesis of ILTV.

Infectious laryngotracheitis virus (ILTV) is an alphaherpesvirus that causes acute respiratory disease in chickens. This disease causes economic loss in poultry industries worldwide (1). The virus contains a linear, double-stranded DNA genome. The genome arrangement consists of a unique long region and a unique short region flanked by identical internal and terminal repeat sequences. In previous studies, a full genomic sequence of ILTV used as a NCBI reference sequence (GenBank accession no., NC_006623) was assembled by concatenating partial sequences of six different ILTV strains (2). After introduction of high-throughput sequencing technology, the first complete genome of a single strain of ILTV has been reported in 2011 (3). Advancement of sequencing technology facilitate to determine complete genome of ILTV and until now 31 of ILTV complete genomes were updated. In genome alignment and phylogenetic tree analysis, ILTV strains were clustered into two large groups. Genotype I includes most of chicken embryo origin (CEO) vaccines used globally, a tissue culture origin (TCO) vaccine, and genetically related field strains to these vaccines, while genotype II includes mainly Australian origin CEO vaccines and field strains. Both of genotypes possibly divided into two sub-genotypes (Figure 1).
Herpesvirus has a double-stranded DNA genome having significantly lower mutation rate than RNA viruses. Complete genome sequences of vaccine strains passaged with different times showed a few number of amino acid changes throughout the entire genome (Figure 2). Furthermore, natural recombinants between two genotypes have been identified through comparative genome analysis and these recombinants tends to be highly pathogenic (Figure 3) (4).
The CEO vaccines have been using worldwide to help control of the disease outbreak. Unfortunately, the CEO vaccines can acquire virulence through bird-to-bird passages. In fact, virulent field strains of ILTV suspected to be derived from the CEO vaccines have been reported in many countries. To differentiate vaccine strains from field strains, PCR and restriction fragment length polymorphism analysis using single or multiple targets have been developed and widely used. After comparative genome analysis, new genotyping method for ILTV was developed using allelic variations from multiple genomic regions (Table 1) (5).

Table 1 Genotypes of strains of ILTV according to patterns of PCR-RFLP or multi-allelic PCR-sequencing

<table>
<thead>
<tr>
<th>Strains</th>
<th>Accession number</th>
<th>PCR-RFLP&lt;sup&gt;a&lt;/sup&gt; USA</th>
<th>Patterns (genotypes)</th>
<th>PCR-RFLP&lt;sup&gt;b&lt;/sup&gt; Australia</th>
<th>this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laryngo Vac</td>
<td>JQ083494</td>
<td>ABBBCBAAAAA (IV)</td>
<td>ABDCB (7)</td>
<td>AAAAAA (1)</td>
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<tr>
<td>Serva</td>
<td>HQ630064</td>
<td>ABBBCBAAAAA (IV)</td>
<td>ABDCB (7)</td>
<td>AAAAAB (2)</td>
<td></td>
</tr>
<tr>
<td>CEO TRVX</td>
<td>JN580313</td>
<td>ABBBCBAAAAA (IV)</td>
<td>ABDCB (7)</td>
<td>BAAAB (3)</td>
<td></td>
</tr>
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<td>LT Blen</td>
<td>JQ083493</td>
<td>ABBBCBAAAAA (IV)</td>
<td>ABDCB (7)</td>
<td>BAAAB (3)</td>
<td></td>
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<tr>
<td>TCO IVAX</td>
<td>JN580312</td>
<td>AABAAAAAAA (II)</td>
<td>ABDCB (7)</td>
<td>ABAAA (4)</td>
<td></td>
</tr>
<tr>
<td>81658</td>
<td>JN542535</td>
<td>AABAAbAAAAAA (III)</td>
<td>ABDCB (7)</td>
<td>ABAAA (4)</td>
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<tr>
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<td>ABBBCBAAAAA (V)</td>
<td>ABDCB (7)</td>
<td>AEEAAA (5)</td>
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<td>ACCBCAbBBBAB (VI)</td>
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<td>V1-99</td>
<td>JX646898</td>
<td>AABBBBbABBB</td>
<td>BBBBB (2)</td>
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<td>AAAA (1)</td>
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<tr>
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<td>AAAA (1)</td>
<td>DCFBEG (13)</td>
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</table>

<sup>a</sup> Results of in silico PCR-RFLP patterns were denoted using italic

<sup>b</sup> Indicate inconsistency of the results of PCR-RFLP between previous reports and in silico pattern

New sequencing technology would be developed continuously, and therefore complete genome sequencing will be easier and cheaper in the future. The increasing genetic information of ILTV collected worldwide would allow further understanding of molecular epidemiology and pathogenesis of ILTV.

Keywords: Infectious Laryngotracheitis Virus, Genome Analysis, Genotyping

References

Pathological Review of Clinical Cases in Chickens Focused on Liver Lesion

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Summary
It is known that infectious pathogens such as bacteria, viruses and parasite and non- infectious factors like toxicosis, nutritional problems and mis-management can affect the severity of liver lesions in chickens. Thus, the nature of the liver makes it the organ which is the most frequently collected and sampled for laboratory tests, especially, for histopathology. In this study, different clinical cases submitted for diagnosis will be discussed focused on the histopathological characteristics of liver. From a total of 207 diagnostic cases submitted to the Chungbuk National University, College of Veterinary Medicine, Avian Disease Laboratory during the first half of 2016, 92 cases with histological lesions in the liver were selected for discussion. Conventional Laboratory methods such as agent identification and isolation, serology and molecular analysis were also employed for diagnosis of the submitted cases. Cases which showed microscopic lesions, especially liver lesions during the histopathology evaluation after the preliminary diagnosis was selected for this study, Cases which were confirmed to show liver lesions were initially categorized based on the association with or without infectious diseases factors (Table 1). The cases were further categorized based on the specific criteria such as hemorrhage, lipidosis, hepatocyte degeneration and necrosis, inflammatory cellular infiltration, changes in portal tracts and granulomatous reactions. Based on this criteria of histopathological lesions, the cases were classified into 5 major categories (Table 2, Fig 1 – Fig 3) but various liver lesions which did not fit specifically in the criteria were also observed. Particularly, lesions associated with infection with Avian hepatitis E were confirmed, in which the existence of the virus itself were confirmed by RT-PCR and Nested PCR(Fig.4). The overall results and details of the study will be presented at the 2017 WVC, with additional cases from the last half of 2016, and including cases before 2016.

Table 1 Classification of infection type of the 92 cases submitted for diagnosis to Avian Disease Laboratory during first half of 2016

<table>
<thead>
<tr>
<th>Type of infection</th>
<th>Pathogen</th>
<th>Number of case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>Bacteria</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Virus</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Parasite</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
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<td>22</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>Virus + parasite</td>
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Table 2: Histological classification of liver lesions

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<tr>
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</table>

*: Number of positive / Number of examined

Figure 1. The appearance of lesion belonging to A TYPE. a: vacuole of hepatocyte (black arrow), b: hepatocyte vacuolization (black arrow) with hemorrhage.

Figure 2. The appearance of lesion belonging to B TYPE. a: piecemeal necrosis (black arrow), b: piecemeal necrosis with intralesional bacterial colony (black arrow).

Figure 3. Liver of ADL16 0900 case showed enlargement and subcapsular hemorrhage (black arrow) with right lobular rupture (white arrow).
Figure 4. Results of Nested PCR using primers of Hepatitis E virus (KaHEV-F1, KaHEV-R1, KaHEV-F2, KaHEV-R2). PCR products with a band length of 330bp were obtained. Lane A, ADL16 0900; Lane B, ADL16 1364; Lane C, ADL16 1365; Lane M, 1kb plus ladder; Lane D, negative control

**Keywords:** Histopathology, Infectious Disease, Non-infectious Disease

**References**

The Significance of Salmonella in Poultry Production and Public Health

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Avian Disease Division, Animal and Plant Quarantine Agency, Gimcheon-si, Gyeongsangbuk-do, Korea

Summary
Salmonella infections in poultry cause significant economic losses to the poultry industry and can be a potential source of Salmonella food-borne disease in humans. Control measures for Salmonella in poultry are accompanied by periodic monitoring of Salmonella by serological and bacteriological testing. Chickens and flocks positive for host-adapted Salmonella enterica serovars Gallinarum and Pullorum and other important paratyphoid serovars need to be eliminated. However, in many countries, vaccines and antimicrobial drugs are commonly used to prevent and treat commercial layer or broiler chickens. A live vaccine based on an attenuated serovar Gallinarum 9R (SG 9R) strain is most commonly used to prevent fowl typhoid worldwide, but there has been continued concern about the potential reversion to virulence of the strain in chickens. Our recent study showed that virulence attenuation of SG 9R might be associated with a large combination of impaired multiple virulence factors in addition to the LPS defect. Also, S. enterica serovar Enteritidis is the most frequent serovar causing human Salmonella infections in many countries including Korea, and the most common source of this organism is considered poultry meat and eggs. We recently demonstrated the presence of major genotypes in both human and chicken isolates of serovar Enteritidis in Korea by PFGE or MLVA, indicating the high potential for transmission of serovar Enteritidis of major genotypes between humans and chickens.

Salmonella infections in poultry can be grouped mainly into two categories, including pullorum disease and fowl typhoid and paratyphoid infections. Pullorum disease and fowl typhoid are septicemic diseases affecting primarily chickens and turkeys. Pullorum disease is caused by Salmonella enterica serovar Pullorum and fowl typhoid is caused by S. enterica serovar Gallinarum. These two organisms are nonmotile and host-specific for avian species. Both of these diseases have been responsible for serious economic losses to the poultry industry in some parts of the world (2). The second category, paratyphoid infections are caused by other motile Salmonella serovars such as S. enterica serovars Enteritidis and Typhimurium. These motile Salmonella serovars are nonhost-adapted, so they can infect a variety of animals and also cause food-borne disease in humans (1).

The main control strategy for host-adapted Salmonella is to establish breeding flocks free of the serovars. In spite of many efforts to control host-adapted Salmonella, fowl typhoid still remains of considerable economic importance to the poultry industry in some countries including Korea. Vaccines against fowl typhoid have been developed for use mainly in chicken farms and a live vaccine based on an attenuated serovar Gallinarum 9R (SG 9R) strain is the most commonly used vaccine worldwide. However, the only documented mechanism associated with attenuation of SG 9R is the lipopolysaccharide (LPS) defect and there has been continued concern about the potential reversion to virulence of the strain in chickens. Therefore, we compared the proteome and transcriptome of SG 9R with those of two wild-type strains to examine the genes associated with changes in virulence. As a result, we identified a virulence-associated protein only found in wild-type...
strains. In addition, there were many more differences in gene expression, not only the roughness, and many of them can explain the in vivo attenuation (Table 1). Overall data produced suggested that virulence attenuation of SG 9R might be associated with a large combination of impaired multiple virulence factors rather than the LPS defect alone (3).

*S. enterica* serovar Enteritidis is the most frequent serotype causing human *Salmonella* infections in many countries including Korea. Although outbreaks in humans caused by serovar Enteritidis are associated with multiple sources, the most common source of this organism is contaminated poultry meat and eggs. Therefore, many countries have national programs to monitor and control *Salmonella* in poultry. Hazard analysis and critical control point (HACCP) programs have also been implemented on poultry farms and slaughterhouses in many countries including Korea to reduce *Salmonella* contamination in poultry and poultry products. Nevertheless, there is still a risk of the transmission of *Salmonella* from poultry and poultry products to humans in most countries. It has been reported that similar subtypes of serovar Enteritidis were commonly isolated from humans and chickens, indicating their public health significance and clonal dissemination between animals and humans. We compared the pulsed-field gel electrophoresis (PFGE) patterns and multilocus variable-number tandem-repeat analysis (MLVA) profiles of serovar Enteritidis isolated from chickens and humans in Korea, and used a DNA microarray to characterize the genetic variability of representative isolates of different genotypes. In this study, the presence of major genotypes in both human and chicken isolates of serovar Enteritidis was demonstrated by PFGE or MLVA (Figure 1). DNA microarray analysis revealed high variability in the virulence gene content among different isolates of serovar Enteritidis even from the same source and of the same genotype. Overall findings suggested the high potential for transmission of serovar Enteritidis of major genotypes between humans and chickens (4).

*Salmonella* can be transmitted vertically and horizontally in poultry production systems. If *Salmonella* is introduced to a breeding flock, it can infect poultry in other units via hatcheries. Infected progeny can subsequently cause *Salmonella* contamination of eggs and poultry carcasses. Experience in the EU and other developed countries has shown that the best way to control *Salmonella* in the poultry production food chain from farm to table is to control *Salmonella* on the farm and prevent it from entering the processing plant (5). Consequently, stringent *Salmonella* control programs applied to at least breeding flocks and the poultry farm HACCP system will allow significant improvements on on-farm *Salmonella* intervention and poultry production food safety.

<table>
<thead>
<tr>
<th>Functional category</th>
<th>No. of genes</th>
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<td></td>
<td>Up-regulated</td>
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<tr>
<td>A. Metabolism</td>
<td></td>
</tr>
<tr>
<td>a. Energy</td>
<td>1</td>
</tr>
<tr>
<td>b. Amino acid</td>
<td>3</td>
</tr>
<tr>
<td>c. Carbohydrate</td>
<td>8</td>
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<tr>
<td>d. Lipid</td>
<td>4</td>
</tr>
<tr>
<td>e. Nucleotide</td>
<td>1</td>
</tr>
<tr>
<td>B. Adaptation</td>
<td>1</td>
</tr>
<tr>
<td>C. Defense</td>
<td>2</td>
</tr>
<tr>
<td>D. Cell wall/membrane biogenesis</td>
<td>3</td>
</tr>
<tr>
<td>E. Membrane and exported</td>
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</tr>
<tr>
<td>F. Signal transduction</td>
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<tr>
<td>G. Transcription</td>
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<td>H. Virulence</td>
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<tr>
<td>I. Multifunctional</td>
<td>4</td>
</tr>
<tr>
<td>J. Function unknown</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1 Genes up- or down-regulated in SG 9R but not in wild type 06Q110 compared to reference strain 287/91.
Figure 1 Genetic relationships between S. enterica serovar Enteritidis isolates from chickens and humans based on MLVA profiles. The dotted circle indicates MLVA types, including human isolates, of which MSE07, MSE09, MSE10, and MSE13 contained both human and chicken isolates.

Keywords: Salmonella, Gallinarum, Enteritidis, Genotype, Chicken, Human

References
Global Threats Regarding Foot-and-Mouth Disease: Recent Outbreaks and Patterns of Virus Spread

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1WRLFMD, The Pirbright Institute, Surrey, United Kingdom, on behalf of the OIE/FAO Laboratory Network for FMD

Summary

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. The disease is caused by a virus (FMDV) of the genus Aphthovirus, family Picornaviridae that exists as seven serotypes (O, A, C, Southern African Territories (SAT) 1, SAT 2, SAT 3, and Asia 1). The greatest burden of FMD remains in many African and Asian countries, where the disease impacts upon livelihoods within rural communities. The circulation of the virus in these endemic regions poses a continuous threat of onward transmission via uncontrolled movement of animal products, and contaminated persons or objects (fomites). Sporadic and sustained FMD outbreaks in previously FMD-free countries (such as those that have recently occurred in the Republic of Korea) reinforce concerns about how readily the virus can cross international borders, and highlight why coordinated global surveillance is necessary to identify these threats so that appropriate diagnostic tools and vaccines can be deployed. These activities are undertaken by the partners and affiliates of the OIE/FAO FMD Laboratory Network which aims to (i) understand global FMD virus distribution and inform vaccine recommendations, and (ii) harmonise and improve the quality of laboratory testing carried out by FMD reference laboratories.

The OIE/FAO FMD Laboratory Network has recently detected and monitored the spread of a number of viral lineages that have emerged from their established endemic pools to cause outbreaks in geographically distant locations. Particular attention has focussed on two virus lineages that normally circulate only within the Indian subcontinent (O/ME-SA/Ind-2001d and A/ASIA/G-VII [G-18]). FMD outbreaks due to the O/ME-SA/Ind-2001d lineage have been detected in the Middle East (UAE, Saudi Arabia and Bahrain), and have spread in a westerly direction across North Africa from Libya into Tunisia, Algeria and Morocco and on the islands of Mauritius in the Indian Ocean. During 2015/16, this viral lineage also spread east into Southeast Asia (Laos, Vietnam, Thailand and Myanmar), and has recently been identified as causing FMD outbreaks in the eastern part of Russia, China and the Republic of Korea. During 2015, another FMD viral lineage (named A/ASIA/G-VII [G-18]) also emerged from the Indian subcontinent to rapidly spread in some countries of the Middle East (Saudi Arabia, Iran, Armenia and Turkey). Importantly, in vitro vaccine-matching data indicates that established international and local vaccines that are used in the West Eurasia region may not be adequately matched against this viral lineage. These unexpected events highlight the ease by which FMDV can cross international boundaries and emphasize the importance of the work undertaken by OIE/FAO FMD Laboratory Network to continuously monitor the global epidemiology of FMD. There is probably no single factor that underpins
these recent events, although these dynamic patterns and transboundary movements of FMD viruses are probably influenced by the migration of people in North Africa and the Middle East due to the escalation of regional political crises, as well as new trading patterns and demand for animal protein that arise due to increased prosperity in Asian countries.

Body Text

Foot-and-mouth disease (FMD) is endemic in many countries in Africa and Asia where its presence impacts upon rural livelihoods and restricts trade opportunities, and from where it poses a constant threat to those countries that are free of the disease. Global surveillance is necessary to identify these threats, so that appropriate diagnostic tools and vaccines are available for detection and control. This requires sustained effort directed towards investigation of FMD outbreaks along with collection and characterisation of FMD viruses from field outbreaks. These activities are coordinated through the partners and affiliates of the OIE/FAO FMD Laboratory Network which aims to (i) understand global virus distribution patterns and use these data to inform vaccine recommendations, and (ii) harmonise and improve the quality of laboratory testing carried out by international and national reference laboratories (see http://www.wrffmd.org/ref_labs/fmd_ref_lab_reports.htm for latest annual report). This presentation summarises the global situation regarding FMD, and highlights recent significant changes in the epidemiology of the disease.

The seven serotypes of FMD virus (FMDV) are not evenly distributed throughout the world reflecting factors such as livestock density and species mix, patterns of husbandry, animal movement and trade, wildlife reservoirs and incentives and capacities for disease control. Despite the opportunities for spread of FMDV into new regions, viruses tend to recur in the same parts of the world, presumably reflecting some degree of either ecological isolation or adaptation. On this basis, the global pool of FMD viruses are subdivided into seven ‘regional pools’:

<table>
<thead>
<tr>
<th>Pool 1</th>
<th>Southeast Asia with spill over into Eastern Asia</th>
</tr>
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<tbody>
<tr>
<td>Pool 2</td>
<td>Southern Asia</td>
</tr>
<tr>
<td>Pool 3</td>
<td>EurAsia including the Middle East</td>
</tr>
<tr>
<td>Pool 4</td>
<td>Eastern Africa</td>
</tr>
<tr>
<td>Pool 5</td>
<td>Western Africa</td>
</tr>
<tr>
<td>Pool 6</td>
<td>Southern Africa</td>
</tr>
<tr>
<td>Pool 7</td>
<td>South America</td>
</tr>
</tbody>
</table>

**Recent long distance FMD viral movements:** Viral sequences can be used to reliably reconstruct the relationship between viruses recovered from different locations, or at different times (1). In addition to monitoring the epidemiology of viruses within the endemic pools, these sequences can be used to recognise FMD virus lineages in unexpected locations. The OIE/FAO FMD Laboratory Network has recently detected a number of viral lineages that have emerged from their established endemic pools to cause field outbreaks in geographically distant locations.

**From Pool 1 (Southeast/East Asia):** Three FMD viral lineages (O/SEA/Mya-98, O/ME-SA/PanAsia and A/ASIA/Sea-97) have spread northwards from mainland Southeast Asia into countries in East Asia (2). This pattern has been evident since at least 2009-10, when the geographical range of O/SEA/Mya-98 and A/ASIA/Sea-97 expanded to cause outbreaks in PR China, Japan, Mongolia, the Russian Federation, the Republic of Korea and the Democratic People’s Republic of Korea. The challenges posed by FMD control in the region are highlighted by continued FMD outbreaks in the Republic of Korea, where multiple virus introductions have affected the country since 2009; providing an indication of the extent to which FMD viruses are still circulating in the neighbourhood. Sequences generated for samples collected during 2015
from FMD outbreaks in Israel and the Palestinian Autonomous Territories show that they are also closely related to those from Southeast and East Asia within the O/ME-SA/PanAsia lineage, further highlighting the unpredictable nature of these long-distance movements of FMDV.

**From Pool 2 (Southern Asia):** Within the Indian sub-continent, specific lineages of three FMD virus serotypes (O, A and Asia-1) normally circulate. However, serotype O is now dominant in the region accounting for >95% of the total specimen submissions into the FAO Indian FMD Reference Laboratory (PD-FMD, Mukteswar) over the past three years. Almost all of these samples comprise representatives of the O/ME-SA/Ind-2001d lineage, which has recently displaced the O/ME-SA/PanAsia strain that was previously found in this endemic pool. This pattern is mirrored elsewhere in the region (based on reports in Nepal [2012-15] and Bhutan [2013]), and in Sri Lanka where this lineage has been detected for the first time in during 2014. During 2013-15, viruses from the O/ME-SA/Ind-2001d lineage have been detected in UAE, Saudi Arabia and Bahrain, and have also spread in westerly direction across North Africa from Libya into Tunisia, Algeria and Morocco (3, 4, 5). The cases of FMD in Algeria, Tunisia and Morocco were the first since 1999. More recently this viral lineage has also spread in an easterly direction into Southeast Asia (Laos PDR, Thailand, Myanmar and Vietnam), and onwards into East Asian countries during 2016/17 (Republic of Korea, Russia and China).

During 2015, another FMD viral lineage named A/ASIA/G-VII [G-18]] has also emerged from the Indian sub-continent and is now rapidly spreading in the Middle East (Saudi Arabia, Iran, Armenia and Turkey) (6). Importantly, vaccine-matching data using in-vitro tests indicates that field isolates are not well-matched against any of the established vaccines that are supplied by international and local manufacturers for use in the West Eurasia region (or are held in reserve by international vaccine banks). New tailored vaccines (such as those rapidly produced in Turkey) are now being developed and deployed to address this situation.

**From Pool 5 (East Africa):** The epidemiology of FMD in Africa is complicated by the co-circulation of endemic FMD viruses, as well as sporadic incursions of exotic viral strains from the Middle East and sub-Saharan Africa. Since 2012, **serotype SAT 2 (topotype VII)** FMD viruses have expanded into North Africa and the Middle East (7). This lineage caused extensive FMD outbreaks in Egypt, representing the first cases due to this serotype for over 50 years. At the same time sequence data was used to show that separate introductions of this virus lineage were experienced in Libya. Since these cases, OIE/FAO FMD Reference laboratories together with local National Reference Laboratories have monitored the continued expansion of this lineage in West Africa (Mauritania in 2014) and the Middle East (Oman in 2015). Similar long distance movements also appear to be evident for the O/EA-3 lineage in Egypt and another SAT 2 topotype (IV) in Bahrain.

**Vaccine Matching:** Laboratories within the OIE/FAO FMD Network provide recommendations regarding the suitability of vaccines for use against different circulating viral lineages. These guidelines consider the degree of heterologous protection that can be provided by vaccines produced by international suppliers, as well as those products tailored for local-use in discrete endemic settings. Detailed information is published in the Annual Report of the OIE/FAO FMD Laboratory Network. Generic recommendations for FMD virus strains to be included in FMDV antigen banks are updated on a quarterly basis. As previously discussed, recent in-vitro data for serotype A viruses from Saudi Arabia and Iran (A/ASIA/G-VII [G-18] lineage) highlight an apparent gap in vaccine coverage. Work is now urgently required to evaluate whether there is adequate in-vitro match with Indian vaccine strains (A/IND/40/2000) or whether in-vivo protection may be provided by established high-potency international vaccines. As an immediate alternative, tailor-made vaccines have already been generated by vaccine maufactures in Turkey and Russia.

**Conclusion:** There is probably no single factor that underpins these recent changes, although these dynamic patterns and transboundary movements of FMD viruses are probably influenced by the migration of people in North Africa and the Middle East due to the escalation of regional political crises, as well as new trading
patterns and demand for animal protein that arise due to increased prosperity in Asian countries. These unexpected events highlight the ease by which FMDV can cross international boundaries and emphasize the importance of the work undertaken by FMD Reference Laboratories to continuously monitor the global epidemiology of FMD.

**Keywords:** Foot-and-Mouth Disease; Epidemiology; Vaccines

**References**

FMD Outbreaks and Control Measures in South Korea

Jonghyeon PARK\textsuperscript{1}, Kwang Nyeong LEE\textsuperscript{1}, Young Joon KO\textsuperscript{1}, Jaejo KIM\textsuperscript{1}, AhYoung KIM\textsuperscript{1}, Jida CHOI\textsuperscript{1}, Bok Kyung KU\textsuperscript{1}, Soyoon RYOO\textsuperscript{1}, SungHwan WEE\textsuperscript{1}, Byoungchan KIM\textsuperscript{1}, Bong-Kyun PARK\textsuperscript{1}

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Summary

Foot-and-mouth disease (FMD) has occurred in South Korea since 2000. These outbreaks consisted of eight of type O and two of type A (with simultaneous outbreaks of two different serotypes). We think that nine outbreaks were caused by viruses introduced from abroad, Southeast Asia or East Asia, and the type O outbreak in January 2016 was caused by viruses that had circulated after the December 2014 outbreak. Vehicles and humans were identified as the most likely carriers of the FMD viruses that were spread into domestic regions. South Korea has carried out its own vaccination policies since the first vaccinations in December 2010. After the FMD outbreak in December 2014 over a five-month period, persistent outbreak pattern changed to steady mode. Accordingly, the country’s control policy has become more flexible in terms of destroying infected animals, vaccinating animals in the farms neighboring the infected animals and destroying additional animals only in epidemiological relations. During the surprising simultaneous type O and A outbreaks in cattle in February 2017, the government carried out strong control measures, such as standstills and additional nationwide vaccinations to cattle, which successfully controlled the outbreaks. The continuous implementation of vaccination programs in South Korea following the widespread FMD outbreak in 2011 created an internal demand for local production of vaccine production in Korea. Accordingly, we have strived for the development of local FMD vaccines in Korea and will so be ready for commercialization of Korean FMD vaccine in near future.

Foot-and-mouth disease (FMD) is a highly contagious viral disease that infects livestock, such as swine, sheep, goats, and cattle. The common FMD control policies are standstill, culling, and vaccination. During an FMD outbreak, the standstill measure should be performed, and most FMD-free countries also implement the policy of culling infected animals. In addition, FMD-free countries that use vaccination and culling all or a portion of susceptible farm animals in the farms that have been directly affected by an FMD outbreak. While the culling of animals is a highly effective method of controlling infectious diseases, it becomes burdensome as the number of affected farms increases. If a country encounters sporadic FMD outbreaks, vaccination of livestock against FMD may be useful way to reduce virus discharge from the FMD infected farms. Therefore, culling of infected animals could be minimized. Korea wants to be FMD free country with vaccination in near future.

The situation of FMD outbreaks in South Korea and neighboring countries

FMD viruses categorized as type O, A, or Asia1 occur in the region around South Korea. Type O and A viruses account for most of the FMD viruses and type Asia1 has rarely occurred since the outbreak in 2009. The trend of food-and-mouth disease (FMD) infections in South Korea has depended on the nature
of FMD outbreaks in neighboring countries. Genetic analyses have shown that FMD viruses in South Korea originated from South Korea's neighboring countries. FMD outbreaks of nine times have occurred thus far in South Korea beginning with the 2000 outbreak, which firstly occurred 66 years after the last outbreak in 1934 (once in 2000, once in 2002, three times in 2010, two times in 2014, once in 2016, and once (simultaneous outbreaks of two different serotypes) in 2017. These serotype consisted of eight O and two A types. Of these outbreaks, most cases were determined to have been introduced from abroad, and the genetic analysis of the type O FMDV in 2016 suggested that the outbreak was the re-occurrence of the FMD introduced in December 2014.

The cause of outbreaks and the genetic lineages of FMD viruses

Most FMD outbreaks in South Korea have been introduced from overseas. They have mostly likely been introduced from East Asia and Southeast Asia. In addition, vehicles and humans have been identified as the primary carriers of the FMD viruses that have been brought and spread within South Korea. In terms of the serotype and topotype of the viruses, these outbreaks consisted of three cases of type O ME-SA (two times of PanAsia and once of Ind2001d), five times of type O SEA (Mya-98), and two times of type A ASIA (Sea-97, Gl/ G2). In other words, the topotype SEA of serotype O was the most frequently occurring FMD virus, which was followed by the topotype ME-SA of serotype O and topotype ASIA of serotype A, respectively. Of the FMD viruses that occur in South Korea’s neighboring countries, types Asia1 and O (topotype Cathay) have not occurred in South Korea.

FMD eradication policies and vaccinations

South Korea has implemented FMD vaccination policies with its first vaccination program in December 2010 and has conducted thorough vaccinations to maintain the protective effects of the vaccines. Since 2014, however, FMD outbreaks have frequently occurred in some of the pig farms in which vaccinations were inadequately conducted. While South Korea culled 3.5 million animals during the November 2010 outbreak, the country has since carried out a partial culling policy of only FMD infected animals that show clinical symptoms. The focus of South Korea’s FMD eradication policy changed from culling to vaccination at the end of 2010. Since then, the animal number of non-structural protein (NSP) antibodies detected, which is used as an indicator of recurrent FMD infections, decreased over time and eventually showed no evidence of recurrence. Consequently, the country was recognized as an FMD-free country in May 2014 from OIE. However, while the FMD outbreak in July 2014, which consisted of only three pig farm cases, was successfully controlled, the outbreak in large scale pig farms in December 2014 led to continuous outbreaks due to the partial culling policy that was implemented to reduce the burden of culling all potentially affected animals. The government carried out a flexible control policy that included culling all animals in the farm that showed the first FMD outbreak in a county, vaccinating animals additionally in the neighboring farms, and culling additional animals depending on the situation. In 2017, the nearly simultaneous outbreaks of type O and A FMD viruses within a week occurred unexpectedly, but the government successfully eradicated them by carrying out strong control measures, such as standstills and additional nationwide vaccinations.

South Korea’s direction for the eradication of FMD

As long as FMD outbreaks do not end in South Korea’s neighboring countries, FMD outbreaks in South Korea are likely to continue. Therefore, the maintenance of thorough FMD vaccination policies is desired. With the continuous implementation of vaccination programs in South Korea following the widespread FMD outbreak in 2011, the country acquired the status of an FMD-free country through the use of vaccinations in 2014. The introduction of FMD viruses since then has caused sporadic FMD outbreaks, and the country has inevitably had to rely on proper vaccinations. For this reason, South Korean research teams have strived to produce FMD local vaccines, have acquired most fundamental technologies. We are now ready for establishing the conditions of FMD mass production. The operation of vaccine production facilities for commercialization in Korea is planned to begin around 2020.
Keywords: FMD, Control, South Korea, Vaccination, Culling

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(3) Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 19, No. 4, April 2013
New Tools for the Detection and Characterisation of Transboundary Diseases

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Summary

Accurate and rapid diagnostic tests are an essential component of initiatives to control and eradicate transboundary viral diseases such as foot-and-mouth disease (FMD). These tests are deployed to confirm initial cases of diseases, monitor the spread of the virus, characterise the causative viral strain, and to provide evidence of disease freedom after outbreaks. Diagnostic testing in sophisticated state-of-the-art laboratories can be supplemented by point-of-care (POC) technologies (e.g. lateral-flow devices [LFDs], mobile PCR and isothermal tests) that can be deployed into scenarios where swift confirmation of clinical suspicion is required. Although index case diagnosis is always likely to occur in National Reference Laboratories (NRLs), these simple-to-use tests hold great promise to allow control decisions to be taken at the earliest possible time point in the midst of an outbreak; allowing new FMD cases to be rapidly identified. This presentation reviews progress to develop new diagnostic tools that can be used to detect and characterise viruses that cause important livestock diseases. Together with European partners (via the Rapidia-Field project: http://rapidia.eu), we have evaluated sample preparation protocols and new equipment that utilizes lyophilized reagents to allow real-time RT-PCR or isothermal RT-LAMP assays to be performed in simple laboratories or in field settings in under 60 minutes. The analytical sensitivity of some of these molecular tests is similar to the established real-time RT-PCR assays employed for front-line diagnosis in NRLs. Therefore, these tests may provide an approach to rapidly negate suspect cases of FMD, with the prospect to reduce immediate risk-based slaughter of uninfected animals. Using field-tests (such as antigen lateral-flow devices), this work has also explored novel cost-effective approaches that can be used to triage, and ship specimens to FMD Reference Laboratories. Once FMDV has been detected, nucleotide sequencing is used to compare field strains with reference viruses and to allow phylogenetic trees to be constructed. The MiniON (Oxford Nanopore) is a portable, real-time device for DNA and RNA sequencing that identifies nucleotides by measuring the changes in electrical conductivity generated as DNA strands pass through a biological nano-sized pore. As part of a second European collaborative project (www.episeq.eu), we have shown that this platform can be used to generate >4 kb FMDV capsid sequence data. A similar approach has been used to characterise Ebola viruses collected during the recent epidemic in West Africa and Zika virus in Brazil. The impetus to develop these technologies has been largely influenced by the desire for more rapid diagnostic capability within Europe (and other developed countries); however, results from recent studies in East Africa (Kenya, Tanzania and Ethiopia) support the deployment of the POC technologies to enhance the surveillance for transboundary diseases in endemic settings.
Body Text

This talk describes new tools that can be used to diagnose livestock diseases, with a particular focus on research that has been recently undertaken as part of two EU-funded projects (http://rapidia.eu and www.episeq.eu) to develop rapid and simple assays to detect and characterise foot-and-mouth disease virus (FMDV). FMDV is a picornavirus (genus: Aphanovirus) with a positive-sense RNA genome of approximately 8300 nucleotides in length. FMDV exists as seven genetically discrete serotypes, and causes an acute disease in cloven-hoofed livestock (cattle, sheep, goats and pigs) that is associated with the development of vesicles on epithelial surfaces of the mouth and feet. FMDV infection also generates a transient viraemia in infected animals that typically lasts for approximately 5 days. Tests that exploit clinical windows form the basis of laboratory approaches currently used to diagnose FMD. These assays aim to detect FMDV in epithelium and fluid from vesicles, as well as in blood and swabs from mucosal surfaces (oral and nasal swabs). Virological assays utilise three different strategies: propagation of FMDV, detection of viral antigenic proteins, or use of molecular assays to amplify specific RNA sequences. FMDV-specific antibody responses can also be detected using serological assays.

In addition to use in state-of-the-art centralised laboratories, there are opportunities to locate diagnostic technologies close to the animals with suspected clinical signs (1):

Field tests to detect FMD antigen: Lateral-flow devices (LFDs, also referred to as immuno-chromatographic strip tests) have been developed for the detection of FMD viral antigen. These simple-to-use and rapid tests utilise FMDV specific antibody reagents (normally characterised monoclonal antibodies) in a format similar to the sandwich capture ELISA used for laboratory diagnosis. Positive test signal is generated by the diffusion of coloured, antibody-coated latex beads or colloidal gold particles through a membrane towards an immobilising band of trapping antibody. As an example, an LFD has been developed for the detection of all seven FMDV serotypes which uses a pan-serotypic monoclonal antibody (2). Sample preparation in field conditions can be achieved using simple disposable tissue homogenizers for preparing epithelial suspensions. In terms of diagnostic sensitivity and specificity, the overall performance of this LFD is similar to laboratory-based antigen ELISA, although the diagnostic sensitivity of the current test is lower for SAT 2 field strains (2). Recent data from the field illustrates the potential for the LFD to be used in locations close to animals to provide rapid support to veterinarians in their clinical assessment of suspected FMD cases. The simplicity and stability of the LFD may be important features for diagnosis in FMD endemic countries in sub-Saharan African and Asian countries. Furthermore, the ability to rapidly recognise FMDV in clinical material may improve the triage of diagnostic samples that are shipped to reference laboratories for subsequent isolation and/or strain characterisation. In addition to testing in situ, recent results have also indicated that it is possible to recover RNA from FMDV positive LFDs (3); providing a new more cost-effective method to ship FMD-positive samples to international reference laboratories for down-stream real-time RT-PCR (rRT-PCR) assays and sequencing studies.

Mobile RT-PCR systems: there are opportunities to deploy rRT-PCR technologies close to the animals with suspect clinical signs. These test formats may be particularly suitable for use in FMD-endemic areas where the time taken to collect and dispatch samples to a laboratory for disease investigation can be protracted. Work in this area has explored the use of new hardware platforms to allow PCR testing to be deployed into the field for use by non-specialists. The focus of current work is the development of hardware platforms incorporating a simple-to-use and robust template extraction process such that all the steps of the assay can be performed without user intervention (4). We have recently deployed these assays into challenging laboratory and field settings within East Africa, where they proved to be reliable in their ability to detect FMDV in a range of clinical samples from acutely infected as well as convalescent cattle (5). The design of the current rRT-PCR formats has been largely influenced by the priorities of developed countries with FMD-free (without vaccination) status. These assays are pan-serotypic and are designed to have broad
sensitivity across all seven FMDV serotypes - in order to recognise incursions of new virus lineages into disease free regions. In FMD-endemic settings, the priorities for diagnosis can be quite different: rather than pan-serotypic tests for FMD virus detection, tests that provide data to characterise the serotype (and genetic lineage) of FMD viruses in field samples is often a more important consideration, since this information is used for vaccine selection and for tracing the sources of field outbreaks. Therefore, future work for field-based rRT-PCR assays may include the transfer serotype-specific assays (6, 7) on to these platforms.

**Isothermal assays:** The recent development of portable equipment for PCR has made molecular diagnosis of FMD in the field an achievable goal. However, this approach relies on precision thermocycling requiring instrumentation which can be fragile, prohibitively expensive and that will require decontamination when transferred from one site to another. As an alternative to PCR, isothermal (single temperature) amplification methods for the detection of FMDV have been developed. Since the specific amplification step occurs at a constant temperature, there is less reliance upon expensive equipment and there is obvious potential to use of these assays as the basis of an inexpensive (or even disposable) molecular test. RT-LAMP is an isothermal autocycling strand-displacement DNA synthesis technique which utilises four specific primers to recognise six regions of the target genome. Pan-serotypic RT-LAMP assays have been designed for FMDV (for an example see 8). Validation data indicates that RT-LAMP has equivalent analytical sensitivity to rRT-PCR and may be less sensitive to inhibition by problematic sample matrices such as OP fluids and faecal samples. RT-LAMP products are generated in abundance and can be detected using equipment to monitor turbidity, agarose gels or real-time PCR machines. Furthermore, it is also possible to visualise dual-labelled LAMP amplicons using novel lateral flow devices (9). Together with simple methods to prepare template RNA, these simple readouts for RT-LAMP have been evaluated in East Africa and shown to be reliable in field settings (5).

**Next-generation sequencing platforms:** Once FMDV has been detected, nucleotide sequencing is a useful tool used to compare field strains with reference viruses and allows important phenotypic characteristics, such as antigenic determinants, to be elucidated. Genetic characterisation of FMDV routinely uses VP1 sequence data generated by RT-PCR. This region of the FMDV genome is approximately 630 nucleotides in length and encodes a protein (1D) that comprises an important component of the FMD viral capsid. These sequences are used to categorise field strains into discrete sub-groups (or topotypes) which frequently show geographical clustering based on the historical distribution of the virus. These analyses provide evidence for the transboundary movements of FMDV and provide support to regional and country-level programmes to control FMD. Next-generation sequencing methods are now being routinely applied for FMDV and other viruses of veterinary interest (10, 11). Portable sequencing devices (such a MinION, Oxford Nanopore) are now available that utilize disposable flow cells. These devices plug directly into a standard USB3 port, run flow cells containing protein nanopores embedded in an electrically resistant polymer membrane that generates long single-molecule reads of sequence data. These nanopores are able to process both DNA and RNA template. Our preliminary data collected shows that this platform and in-house bioinformatics workflows can be used to generate capsid sequence data >4 kb in length, equivalent to the amplicon length of a pan-capsid RT-PCR for FMDV. A similar approach has used this technology to characterise Ebola viruses collected during the recent outbreak in West Africa and Zika virus in Brazil.

**Conclusion:** These new diagnostic tools can play a critical role in our ability to detect and monitor the spread of FMD in endemic regions of the world. However, it is also important to recognise that effective monitoring and control of FMD is reliant upon adequate resources, these are principally financial but also include availability of trained field personnel and a strong supporting laboratory infrastructure. In addition, international cooperation, transparency between different countries, sharing of epidemiological data and ownership of disease are also key factors in the control of important trans-boundary disease such as FMD.
Keywords: Transboundary Diseases; Rapid Diagnostics; Sequencing

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Swine Enteric Coronaviruses (PEDV, PDCoV, TGEV) – Pathogenesis, Impact, Prevention and Control

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Summary
Coronavirus is one of economically significant viral pathogens causing enteric disease in many animal species. In swine, three coronaviruses have been implicated in enteric disease, named transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and porcine deltacoronavirus (PDCoV). Because clinical manifestations and lesions associated with infection by these viruses are similar, the viruses have been grouped into ‘Swine Enteric Coronaviruses’ (SECoV) even though these viruses are genetically and antigenically distinct. As implied from the name, all three viruses are able to alter the morphology and function of the gut barrier and tight junction leading to malabsorption and increased osmotic secretion which results in diarrhea clinically. PDCoV appears to be less virulent than the other two viruses. In general, younger pigs get more severe disease than older pigs. Mortality associated with SECoV infection is usually limited to neonates or pre-weaning pigs. Other factors such as strain, challenge dose, immunity and other conditions on farms influence the outcome of disease. For prevention and control, biosecurity, particularly concerning external source of virus, is a key component. Once an outbreak occurs, environmental contamination of virus is a significant issue to manage via thorough cleaning and disinfection since infected pigs, particularly naïve ones shed a large amount of the virus. Feedback (i.e., intentional and controlled oral exposure of pigs to infectious material) is an effective tool to increase the herd immunity on positive farms but should be used in careful manner. Vaccines are available for TGEV and PEDV but often show limited effectiveness due to various reasons. No vaccine is currently available for PDCoV. Development of effective mucosal and lactogenic immunity against SECoV is an area to be further studied for better prevention and control.

Introduction
Coronaviruses belong to the family Coronaviridae in the order Nidovirales and have been important pathogens in humans and many animals (e.g., cattle, swine, horse, cat, dog, mouse, chicken, bat). There are four genera in the Coronaviridae family: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. Members of Alphacoronavirus, Betacoronavirus and Deltacoronavirus have been implicated in swine diseases. While porcine respiratory coronavirus (alphacoronavirus) and hemagglutinating encephalomyelitis virus (betacoronavirus) infect the epithelial cells of lungs, other alphacoronaviruses, such as transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV), and porcine deltacoronavirus (PDCoV) infect the enterocytes lining the gastrointestinal track leading to diarrhea. While TGEV has been historically a well-known significant viral enteric pathogen of swine, PEDV and PDCoV were more recently recognized in conjunction to the swine enteric diseases since 1971 and 2012, respectively. These three enteric pathogens are genetically and antigenically distinct. Yet, clinical manifestations of their infection are often indistinguishable without laboratory confirmation. The term, ‘swine enteric coronaviruses’ or SECoV in short was coined to recognize such a similarity.
Pathogenesis and clinical manifestations

As SECoV infect intestinal enterocytes, atrophic enteritis is a hallmark microscopic lesion throughout the small intestine. Virus infection alters the cellular morphology and function of gut barrier (i.e., epithelium) and tight junction in small intestine, leading to malabsorption and increased osmotic secretion. Due to such alterations, thin-walled intestine filled with watery material is commonly observed in affected pigs on necropsy. Colitis is not a feature with SECoV infection although scattered virus-infected cells can be found in the colon.

It appears that SECoV do not affect organs not associated with the gut as no lesion are evident although transient virus infection in non-gut tissues may occur likely due to short-term viremia at low level. Interestingly, experimental studies have shown that mesenteric lymph can support SECoV replication. Pathobiological and immunological significance of such observations are unknown at present.

The main clinical sign of SECoV infection is watery diarrhea (1-3 days post exposure) with occasional vomiting in all ages, leading to dehydration and malnutrition. The severity and outcome of disease in the field, however, can vary depending upon age as well as other factors such as strain, challenge dose, immunity and other conditions on farms. Clinically TGE and PED tend to be more severe than PDCoV infection. There are PEDV strains with deletions/insertion in its spike protein, which is less virulent. In general, neonatal and pre-weaning piglets get more severe clinical disease than post-weaning and adult pigs. Affected neonates start to die within 2-3 days once diarrhea starts. The mortality rate is often 30-50% in neonates and can reach 100% in piglets born to negative dams. Disease outbreak in naïve herds can result 4 to 12 weeks of production loss. Older pigs, particularly after weaning, generally do not die of SECoV infection even if immunologically naïve unless complicated. Yet, weight gain is impacted by the disease, particularly PED and TGE. Wean-to-finish farms should expect 2- to 3-week delay in the time to market after outbreak. Fecal shedding of SECoV from exposed pigs can starts as early as 24 hours post infection. Although the number of diarrheic pigs sharply declines after a week, a large number of subclinical pigs still shed the virus in feces for a long time. This should be taken into consideration for pig movement and herd management.

Prevention and control

As affected pigs shed SECoV in feces, fecal-to-oral route is the main mode of SECoV transmission. Any strategies to block or minimize exposing to virus source is a start for prevention and control. In this sense, biosecurity plays a critical role in prevention and control of SECoV infection as it includes control and management of pig and semen sources, feed supply, people entry, facility, and transportation. During outbreaks, people flow, thorough cleaning and disinfection, and early weaning should be practiced to remove the virus from barns, lower the virus amount in barns and reduce fomite or aerosol transmission. While several commercially available disinfectants have shown to be effective against SECoV, their efficacy can be affected by sample matrices and environmental factors including weather.

As for immune-mediated prevention and control of SECoV, ‘feedback’ (i.e., intentional and controlled oral exposure of pigs to infectious material) is commonly practiced. When feedback is used as a way to boost the herd immunity, a care must be taken with respect to diagnostic confirmation of causative agent, selection of material to give (e.g., piglets versus older pigs; feces versus intestine), and frequency and interval of feedback. It should be kept in mind that virus-infected pigs can shed a high level in feces for a longer period of time, which can be easily spread to neighboring pigs or farms by various sources or methods. Furthermore, a high dose of virus challenge or continuous viral challenge from dams or environment could outweigh the maternal immunity of piglets. Strict and enhanced biosecurity, including herd closure and thorough disinfection practice, should be used in parallel to feedback in order to obtain the maximum intervention effect.

Vaccination can also be utilized for prevention. The goal of vaccination is to induce good lactogenic immunity which can be passed to newborn piglets while protecting sows from disease and significantly reducing virus shedding. Because of such a goal, vaccination is generally done on pregnant sows. There are commercial vaccines for TGEV and PEDV but not for PDCoV. Field observations suggest that killed vaccines are able...
to reduce the impact of subsequent TGEV and PEDV infection when vaccination is used on positive farms (i.e., after outbreak). The efficacy of killed vaccines on naïve pigs is, however, in question although multiple vaccinations or use of attenuated live virus vaccine have been suggested to circumvent this concern. The literature indicates that antibody in blood circulation may have a limited effectiveness against SECoV, raising the need to find a convenient measurable correlate with mucosal immunity. Since it is generally believed for enteric viral diseases that efficacious immunity by vaccination requires priming, it is foreseeable that veterinarians and producers may need to use both live virus exposure (i.e., feedback or attenuated live virus vaccine) and killed vaccine in combination and in a certain order for effective SECoV prevention and control.

**Keywords:** Pigs, PED Virus, TGE Virus, Deltacoronavirus, Pathogenesis, Control
Oral Fluid Sampling and Testing – A Paradigm Shifting for Disease Monitoring in Pig Populations

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Summary

In modern swine production, the average number of pigs per farm has become larger, and frequent animal movement between farms or from farms to slaughter plants is an integral part of pig production. Consequently, introduction of an undesirable disease can be detrimental to not only animal health but also livelihood of producers. Continuous surveillance and monitoring is an essential tool for early detection and timely control of diseases in swine farms. Although blood-based testing has been commonly practiced for disease surveillance or monitoring, statistically randomized individual animal blood sampling often could not meet the desire of modern swine production for rapid and reliable determination of disease-free status. Pen-based oral fluid sampling can be an alternative for prognostic profiling of herds for specific pathogens of interest because: a) oral fluid is a mixture of internal (saliva, upper respiratory secretions, oral mucosal transudate and host cells) and external (food debris, environment) components besides microorganisms and drugs; b) oral fluids are easy to collect and represent a larger number of pigs on farms; and c) pen-based oral fluid sampling provides a significantly higher probability of detection with fewer samples than traditional blood sampling. Oral fluids have been used to detect many pathogens and/or antibodies with a growing list. It should be noted though that oral fluid sampling is for population-based monitoring for diseases, not for disease diagnosis. Hence, positive test results reflect active circulation of detected pathogen(s) in the population, which should be interpreted in context with clinical and production history.

Introduction

Surveillance and monitoring is an essential tool for early detection and timely control of diseases in production animals including pigs. Historically, bloods have been the most common sample of choice for disease surveillance or monitoring via agent detection and/or antibody detection. Occasionally other bodily fluids, such as nasal discharge/secretion and urine, and feces have also been used. As traditional sampling for surveillance or monitoring has been limited to a certain number of animals in a population regardless of disease prevalence and population size, a concern on the accuracy of measurements was raised from time to time. Sampling, particularly blood collection can be an animal welfare issue and sometimes impose a safety risk to collectors. Therefore, the need for convenient sampling which can provide a reliable assessment of disease status in animal population raised. Recently, collection and testing of oral fluids has been suggested as alternative for disease surveillance and monitoring purpose in swine.

Oral fluids and collection

Oral fluid is the liquid present in the oral cavity of animal which is composed of saliva, oral mucosal transudate, upper respiratory secretions, host cells, food debris and microorganisms. Oral mucosal transudate is serum-like biological fluid which enters the mount through the buccal mucosa from blood circulation (mainly capillaries), whereas saliva is a mixture of salivary gland secretions (2,3).
To collect oral fluids from pigs, cotton rope [1/2” to 5/8” (= approximately 1.3 cm to 1.6 cm) in diameter depending upon size of pigs] is highly recommended. Ropes made of non-absorbing materials, such as nylon, should not be used. Cotton ropes are hung in accessible locations (e.g., rail or gate) of selected pens at the shoulder height to the pigs as illustrated in the figure. One rope is shown to be sufficient for a pen of 25-30 pigs. It is advised not to place ropes in extremely dirty areas of a pen or in a close proximity to water or feeder to minimize environmental contamination in oral fluids. It has been reported that placing ropes for approximately 30 minutes is sufficient to get a rope saturated with oral fluids as more than 75% of pigs in the pen access and chew it. It appears that placing ropes in the morning generates better collection when pigs are more active. Oral fluid can be extracted from each rope by squeezing it into a plastic bag, which is then drained to a 50 ml plastic tube for submission to a testing laboratory. The fluid can be dispensed into smaller tubes if desired. Oral fluid samples should be treated like serum samples with respect to submission to a testing laboratory.

It is highly recommended that all ropes should be removed from the facility after each sampling and should not be re-used. Visual illustration (photos or video footage) of oral fluid collection can be found at several websites: https://vetmed.iastate.edu/vdpam/research/disease-topics/swine/oral-fluids; https://www.prrs.com/en/prrs/diagnostics/oral-fluids/collection-oral-fluids; and http://www.cfsph.iastate.edu/video.php?link=oral-fluid-collection-in-pigs.

Utility of oral fluid samples for disease monitoring

Oral fluids are known to contain microorganisms, antibodies and even drugs besides other components mentioned above. In human medicine, oral fluids/saliva have been used to test for a variety of infections, most notably sexually transmitted diseases such as human immunodeficiency virus. In pigs, oral fluids have been successfully used to detect infectious agents and/or their specific antibodies, such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), influenza A virus, porcine epidemic diarrhea virus, hemagglutinating encephalomyelitis virus, classical swine fever virus, foot-and-mouth disease virus, Seneca Valley virus, Mycoplasma hyopneumoniae, and some bacterial agents. It is anticipated that the list will grow. Nucleic acid based tests, such as polymerase chain reaction-based assay and sequencing, have been most commonly used with oral fluids for agent detection, although virus isolation has also been attempted. For antibody detection, enzyme-linked immunosorbent assay has been shown to be the most suitable platform.

The best use of oral fluid sampling is for population-based monitoring for diseases, not for disease diagnosis, since test-positive results reflect active circulation of detected pathogen(s) in the population. As such, a strong merit that the pen-based oral fluid sampling provides over random individual blood sampling is a significantly high probability of detection with fewer samples. Therefore, periodic oral fluid sampling can be used for prognostic profiling for specific pathogens of interest in swine production facilities. The detection of pathogen circulation can be higher and more reliable if sampling pens spaced throughout the facility and the same pens repeatedly over time. Although sample size calculations for specific pathogens remain to be determined for optimal detection, sampling 6 to 8 pens at 2-week intervals appears to provide a reliable estimate of farm status for several viral pathogens, such as PRRSV, swine influenza virus, and PCV2, in a modern production facility.

For best test results, oral fluid samples should not be pooled as each oral fluid represents pigs in a pen, and should be kept cold or frozen to preserve sample integrity. Test procedures should be optimized and validated for oral fluid samples at the lab providing the test.

Keywords: Swine, Oral Fluids, Population-Based Testing, Disease Monitoring, Prognostic Profiling
Porcine Circovirus Type 2 Vaccination in a Changing Epidemiological Scenario

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Summary
Porcine circovirus type 2-systemic disease (PCV2-SD) is of multifactorial nature but the simple vaccination against the virus (PCV2) represented the most advantageous system to control the disease under farm conditions. Moreover, vaccination of non-clinically affected pigs demonstrated a significant improvement of average daily weight gain and, in consequence, the economic importance of the PCV2-subclinical infection. After more than 10 years of PCV2 vaccination worldwide, being nowadays the most used vaccine elsewhere in the world, the overall infectious pressure has decreased, fact that implied a change in the epidemiology of this viral infection as well as changes on PCV2 vaccination strategies.

Introduction

Four porcine circovirus type 2 (PCV2) vaccines for use in piglets and/or breeding stock are commercially available in most significant pig producing countries worldwide. The number of commercial PCV2 vaccines is even higher in some geographical areas like Southeast Asia. In addition, there are a number of pharmaceutical companies that nowadays are developing new PCV2 products, suggesting that these overall numbers will increase in the future.

Convenience of vaccinating piglets or breeding stock or both

Vaccination of sows might have two potential objectives: 1) to prevent porcine circovirus diseases (PCVDs) of the offspring, or 2) to protect against PCV2-reproductive disease (PCV2-RD). In the first case, vaccination should take place at late gestation, as it is recommended by the manufacturers of the PCV2 vaccines intended for sows. If the objective it to prevent PCV2-RD, vaccination might be applied before mating, being at the lactating period or at weaning for 1st parity or older sows, or during the acclimatization in gilts. In any case, the use of sow vaccination at the end of the gestation period in multiple cycles in the farm has been shown to provide protection against PCV2-RD. However, minimal data do exist regarding the benefits of the continuous vaccination of the farm and its effects on PCV2-RD.

A second possibility would be to select piglet vaccination as the way to control PCVDs in the farm; in fact, this is the most common practice by far. It is known that control of PCV2-systemic disease (PCV2-SD) in affected farms is quicker if piglet (instead of sow) vaccination is used, observing a positive effect in the very first vaccinated batch. The main reason is that vaccine applied in pigs is able to elicit protective immune responses in the animal that subsequently suffer from the disease. Non-published field data indicate that sow vaccination helps controlling clinical disease in growing-finishing pigs, but needs 6 months to 1 year of continuous
sow vaccination to generate comparable effects to piglet vaccination in a single batch.

A third option is to vaccinate both sows and piglets. There are several reports on the benefits of this schedule at productive and virological levels. It presumably joints the benefits of controlling PCVD in a “continuous protection” fashion since it provides strong herd immunity by vaccination sows/gilts, and protects piglets against the development of PCV2-SD and ameliorates the outcome of PCV2-SI. Repeated sow vaccination by cycle should also potentially benefit the reproductive outcome. In this double vaccination scenario is important to take into account the putative interference of MDI upon PCV2 vaccine efficacy in piglets, since colostrum intake provides higher amounts of PCV2 antibodies. It is true that the levels of maternally derived antibodies (MDA) must be very high in order to jeopardize the effects of PCV2 vaccination in piglets, at least with the so far tested vaccines. It would be interesting to assess if this is true for all vaccines in the market. This situation is obviously linked with the timing of piglet vaccination.

**Timing of piglet vaccination**

It is known that the effect of MDI (measured as MDA) is significant, to the point that it may protect against PCV2 experimental challenge and influence the humoral response developed after vaccination. On the other hand, a significant negative correlation between MDA at the timing of vaccination and the increment of antibody titers to PCV2 4 weeks post vaccination was confirmed. In other words, the higher the antibody titer at vaccination (generally at weaning), the lower the degree of seroconversion some weeks later. This effect has been observed for all vaccines tested.

Based on these observations, optimal vaccination strategies must balance the advantage of delayed vaccination in case of high antibody titers at weaning age with the need to induce immunity prior to exposure to pathogens under field conditions. In consequence, a “vaccination window” has been proposed, defined as the range of antibody titers at which piglets should be vaccinated to minimize interference with MDA and, at the same time, ensure the development of protective immunity before PCV2 exposure.

This “vaccination window” defines the age at piglet vaccination. Product label indicates vaccination from 2-3 weeks of age onwards, although one vaccine is licensed for an earlier age if a double dosage is used (see previous chapter). In fact, and taking into account that MDA interference with vaccine seroconversion has been demonstrated, the important point is to ascertain if this is paralleled with interference with vaccine efficacy. A first study using a particular vaccine indicated that efficacy is not jeopardized by MDA, with independence of antibody titers at vaccination. This implies that most of the pigs in a batch overcome MDI on a practical basis. In the worst case scenario, such putative interference would happen in the proportion of pigs with relatively high or very high antibody titres. Such proportion might be different from batch to batch, but it is probably low or very low in most of the cases. A recent study concluded that PCV2 vaccination in the presence of high MDA levels is efficacious when used in 3 week-old but not in 1 week-old pigs; however, for the same farm, MDA levels at the first week of age are systematically much higher than those at the third week of life.

For most of the cases, vaccination around weaning (3-4 weeks of age) will do the right job of protecting pigs against PCVDs. However, there are a number of scenarios that may modify this general practice:

- Vaccination of piglets that are already infected by PCV2
- Vaccination of piglets that are infected by porcine reproductive and respiratory syndrome virus (PRRSV)
- Vaccination of piglets with very high PCV2 MDA
- Vaccination of piglets with very low PCV2 MDA
PCV2 vaccination in a changing epidemiological scenario

During last years, the high vaccination pressure exerted in the world pig population has implied a change in the PCV2 infection epidemiology. It has been observed that after such repeated vaccination, viral loads diminish over time, to the point, in some cases, with no detection of circulation evidence in pigs. This may imply certain batches of pigs reaching seronegative at slaughter age. In principle, is very positive since we are almost eliminating the effects of the virus on growth, but the situation may be different for those animals that will be selected as replacements (gilts and boars).

Although with low prevalence, PCV2 circulates in the breeding stock, and the introduction of naïve gilts into the system increases the likelihood of infection of these animals and the perpetuation of PCV2 within the sow-herd. Under such scenario, the probability of infection during gestation (mainly of gilts) is higher, as well as the proportion of viremic-born piglets and early infection in the offspring. In turn, it may happen that we vaccinate already infected animals. Although from an experimental point of view, PCV2 viremic gilts vaccinated against PCV2 are able to cope with the infection, and able decrease viremia and histopathological lesions compared to a viremic non-vaccinated group, efficacy under field conditions may be variable. During last few years it has been noticed an increase of PCV2-SD diagnoses in farms with vaccinated piglets; the terminology “vaccination failure” has been used to designate those situations. What probably happens here is that vaccination at weaning might not provide sufficient time to develop vaccine-elicited immune response before natural infection and a proportion of animals may develop PCV2-SD and not just a PCV2-SI. Recommendations in this case are either 1) perform sow vaccination, trying to delay natural PCV2 infection, or 2) earlier PCV2 vaccination (i.e., at 10-15 days of life). This latter option should be coupled with serological analyses indicating low antibody values at the time of vaccination.

Overall, it is nowadays believed that “vaccination failure” scenarios (i.e., unequivocal diagnosis of PCV2-SD in vaccinated pigs) are mostly associated with an inadequate management of the vaccine (conservation, dose applied, etc.) and timing of application (too early – potential interference with maternally derived immunity or at the time of early infections, too late – too close to natural infection, or in diseased animals – i.e., PRRSV viremia). Looking at the major causes of the so-called “vaccination failure”, it is more a “human failure” rather a vaccine efficacy problem. If putative “vaccination failure” will occur in the future due to PCV2 escape mutants is still to be determined.

Keywords: Porcine Circovirus Type 2; Vaccination; Epidemiology
Emerging Infectious Diseases of Pigs: What’s Next?

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Summary
The number of new diseases affecting swine has increased substantially in the last 30 years. Some of them have been the product of new pathogens which were apparently not present in the pig population, but most of them are caused by infectious agents which were already present, even for a number of years. Moreover, there are an increasing number of microbes, mainly viruses, which have been discovered by means of modern research and diagnostic techniques such as high throughput sequencing; the real meaning of these novel agents in the context of disease is rather unknown for most of them. Therefore, this presentation aims to discuss about new swine diseases or novel presentations of already known diseases, as well as newly recognized infections with a not well-defined pathogenic effect in pigs.

Introduction
Emerging infectious diseases (EID) can be defined as those which incidence is increasing following its first introduction into a new host population or in an existing one as a result of long-term changes in its underlying epidemiology. This concept can also include those diseases linked to pathogens expanding into an area in which it was not previously reported, or due to pathogens that changed significantly its clinico-pathological presentation. During last 10 years, much emphasis has been focused on human EID caused by pathogens of animal origin. All these zoonotic threats and events have emphasized the need for a “One Health” approach, which integrates communication, collaboration and coordination between public health, animal health and other communities at multiple levels to prevent, detect and control emerging or re-emerging infectious diseases at the animal–human–environment interface.

The objective of the present review is to discuss about new swine diseases or novel presentations of already known diseases, as well as newly recognized infections with a not well-defined pathogenic effect in pigs.

Emerging diseases in pigs
The number of novel conditions in swine included under the concept of emerging and re-emerging diseases has increased importantly during last 20-30 years. Most of them are infectious diseases, being those of viral origin of great importance. Their transmissibility and maintenance into a population is favoured by a number of phenomena, including intensive rearing practices and globalized/international trading.

Besides those novel or re-emerging pathogens able to cause overt disease, there are a number of newly discovered agents for which no evidence of associated disease does exist. For example, from 1985 to 2010, novel pathogen species were identified at an average annual rate of 3 in pigs (including all types.
of pathogens). The advent of modern diagnostic and research methodologies, sometimes without the need of previous knowledge about the putative pathogen (i.e., high throughput sequencing), has increased significantly the number of microorganisms that are infecting animals. In consequence, a complex scenario with novel infectious agents which importance is rather unknown is being faced nowadays by researchers and veterinarians. Such scenario implies to play with certainties and uncertainties, since last 30 years taught us about:

- The emergence of global diseases for which there is still not a clear definitive solution (i.e., porcine reproductive and respiratory syndrome, PRRS)
- The emergence of global diseases for which the pathogen existed long before, but overt disease was only recognized recently (i.e., porcine circovirus type 2-systemic disease, PCV2-SD)
- The emergence of global diseases for which the pathogen has apparently varied in virulence (i.e., porcine epidemic diarrhoea, PED)
- The recognition of putative novel viruses for old diseases (i.e., atypical porcine pestivirus as cause of congenital tremors type AII)
- The discovery of viruses that were not novel but considered potential causes of zoonosis (i.e., hepatitis E virus)
- The discovery of viruses that were not novel with unknown outcome related with its infection, although considered to be harmless (i.e., torque teno sus viruses)
- The discovery of bacteria able to cause similar diseases to those already existing, without the right tools to detect them (i.e., Brachyspira hampsonii)

The list of new recognitions, identifications and discoveries is much longer and will definitively increase in the future. Moreover, who can predict the impact of these new pathogens? Are the today’s considered harmless pathogens the potential pathogenic agents of tomorrow? Are some of these agents part of the „normal” microbiome of the pig? Are we able to stop the entrance of some of these agents in our country or region? Do we really know if these agents are already in our region or country? Are we selecting pig genetics that are more susceptible to agents that currently do not cause overt disease? There are still many questions that remain to be answered in the field of swine diseases and the impact of novel pathogens.

Conclusions
It is hard to predict what will come next in the swine industry in terms of diseases, but the advent of PRRS by late 80s and beginning of 90s, PCV2-SD by late 90s, the pandemic influenza A/H1N1 by 2009 and PED in North America and Europe in 2013/14 implies to have new disease emergences every 7-8 years. Therefore, the risk of emerging and re-emerging diseases with significant economic losses and infections with unknown impact on production as well as on the human population is ensured, and deserves preparedness and proper basic and applied research. Pig production is a good example of a globalised industry, and swine veterinarians and researchers in conjunction with producers, consumers, and stakeholders should join efforts for more global, collaborative, and action-oriented approaches towards logical and practical solutions.

**Keywords:** Emerging Infectious Diseases; Pig; Preparedness
Influenza A Virus at the Interface Between Swine and Human – Current Understanding of Epidemiology and Disease Control

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Summary
Swine influenza, caused by influenza A virus (IAV), is an economically important viral respiratory disease in pigs of all ages. As IAV contains a segmented RNA genome, the virus is already naturally designed for rapid mutation and gene swapping. On top of that, external factors, such as larger herd size, constant influx of immunologically naïve pigs to herds due to replacement animals, and closer interaction with humans at a higher frequency than before, can also contribute to virus diversity. As a result, swine IAV can perpetuate in a herd and continue to evolve through accumulation of point mutations, reassortment and interspecies jump, which makes it harder to prevent and control the disease by traditional means. Systemic monitoring for IAV in swine populations is necessary for detection of subclinical virus circulation and emergence of new strains. It is highly desired to develop a technology for producing vaccines conferring good cross protection in a very timely manner.

Introduction
Influenza A virus (IAV) belongs to the family Orthomyxoviridae along with influenza B, C and D viruses and is of a major human and animal health significance. The virus predominantly causes febrile respiratory disease in pigs of all ages with occasional reproductive loss in sows due to elevated body temperature. Suboptimal weight gain is of concern when grow-finishing pigs are exposed to IAV, which causes significant economic loss to swine producers. Non-influenza A viruses are rare in pigs although there have been reports of identifying influenza C and D virus infection/exposure.

Swine influenza is ranked the second for respiratory diseases diagnosed among submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL). Despite of extensive vaccinations and other intervention measure, effective disease control has been proven to be difficult due to frequent emergence of new strains. With substantial increase in the average number of pigs per farm, the existence of immunologically naïve or subclinically infected subpopulation within a herd appear to also contribute to virus perpetuation in the farm and the difficulty with prevention and control of swine influenza.

Viral and host factors for IAV diversity
The virus contains a negative-sense, single-stranded segmented RNA genome which can drives virus diversity through mutation and reassortment. Based on the antigenicity and sequence of two viral surface proteins known as hemagglutinin (HA or H) and neuraminidase (NA or N), influenza A viruses can be classified into any combinations of 18 HA and 11 NA subtypes to date. Yet, H1N1, H1N2 and H3N2 have been the most common subtypes of IAV implicated in swine respiratory disease although other subtypes have been seldom identified in pigs.

Pigs are known to have receptors for both mammalian and avian influenza A viruses in their respiratory track, which has contributed to the speculation that pigs may serve as a ‘mixing vessel’ for the emergence of a new IAV strain. While swine is not a necessary host in the mixing vessel theory, influenza A viruses...
have been shared between pigs, human and turkeys in the US. According ISUVDL diagnostic data, co-infection of more than one IAV subtype is not a common event although mixed infection does occur. It appears that there has been an increased spillover of human viruses into pigs since 2009.

Ecology of swine influenza virus
The species barrier has built lineages among influenza A viruses, namely human, swine and avian lineages with distinct sequence profiles which can be used to track virus spillover event between species. In the US, human IAV has been frequently spilled over into swine and some become established as swine IAV (e.g., H3N2, H1Δ, H1N1pdm09). Avian IAV has been seldom found in pigs but has not become established in US swine. Geographical separation of pig production has also created lineages known as North American European, and Asian lineages. Each of these lineages has distinct genetic signature (i.e., sequence profile) allowing molecular tracking of the virus movement between continents. IAV continues to evolve genetically and antigenically within each lineage. While IAV with Eurasian signature has not been found in US swine, intercontinental spreading of IAV has been documented particularly in Asia more likely due to pigs trade and people travel.

A high rate of mutations has created so many clades or clusters within each subtype. In US swine, 9 clusters (α, β, γ1, γ2, δ1, δ1a, δ1b, δ2, H1N1pdm09) have been identified among H1 viruses to date while 10 clades (I, II, III, IV, IV(A), IV(B), IV(C), IV(D), IV(E), IV(F)) have been identified among H3 viruses. It is anticipated that the number of clusters/clades will continue to grow.

Control
Herd management including biosecurity and vaccination are major tools for prevention and control of IAV in swine. Herd management is accompanied with surveillance or monitoring for infection/exposure in the herd. Oral fluid testing is increasingly utilized in prognostic profiling for IAV. PCR testing followed by sequencing for cluster/clade identification is commonly practiced as no good cross protection is anticipated between clusters/clades. Many times this information is taken into consideration for vaccine selection too. Phylogenetic analysis is also commonly used by swine practitioners for molecular epidemiology concerning introduction of a new strain into the herd.

In the US, commercially available vaccines are inactivated whole virus vaccines with oil adjuvant and contain multiple strains to confer a broad coverage of subtypes and clusters/clades. Autogenous vaccines using either whole virus or subunit are also available. All vaccines are given intramuscularly. Vaccination is primarily used on pregnant sows pre-farrow to alleviate IAV-associated abortion and build maternal antibody which can be passed to offspring. Killed vaccines generally provide adequate homologous protection and induce a measurable robust hemagglutination inhibition antibody response. Killed vaccines tend to induce poor cross protection against heterologous IAV. Under experimental conditions, mismatch between vaccine strains and challenge strain resulted in exacerbation of disease. There is a concern that vaccination might drive virus evolution due to the selective pressure by vaccine-induced immunity. To overcome shortcomings of killed vaccines, attenuated live virus vaccines or other administration route of killed vaccines are being considered to induce better mucosal immunity.

Keywords: Swine, Influenza A Virus, Diversity, Ecology
Impact of Maternally Derived Immunity on Active Immunization Against Swine Pathogens: The Example of Porcine Circovirus Type 2 (PCV2)

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Summary

Maternally derived immunity (MDI) is crucial for the post-natal development of piglets. Besides the obvious benefits such passively transferred immunity exerts against peri- and post-natal infections, it also affects the timing and magnitude of subsequent piglet immune responses against these pathogens as well as vaccines applied in the first 10 weeks of age. Importantly, vaccine efficacy is dependent on the ability of vaccines to overcome MDI and generate sufficient immune priming to fight against the pathogens of interest. This document revises the most significant aspects to be taken into account when designing a proper vaccination schedule, using porcine circovirus type 2 (PCV2) as a model.

Introduction

Swine has an epithelio-chorial and diffuse placenta with the fetal trophoblasts in direct contact with endometrial epithelial cells. Such fact has traditionally been interpreted in the line that there is no transplacental transfer of immunoglobulins from the sow to piglet during gestation. However, it has recently been hypothesized that small amounts of antibodies (demonstrated for porcine circovirus type 2, PCV2) are able to cross the placenta in some fetuses coming from sows with very high serological titers, probably related with small damage to the placental barrier during gestation. In any case, such leakage of antibodies from sow to fetuses is probably of negligible impact when interpreting results of maternally derived antibodies (MDA) in piglets that have been suckled under conventional conditions. Altogether implies that piglets are born virtually agammaglobulinemic in absence of intrauterine infections after their immunocompetence. In consequence, piglet protection against the highly infectious pressure of the farm environment depends on the intake of colostrum. Moreover, the non-completed development/maturation of the immune system at birth also implies that passively acquired maternally derived immunity (MDI) is key for their survival during the very early period of life.

Colostrum is, however, a source of antibodies but also of other components that help protecting the piglets against pathogenic agents, such as lactoferrin, lysozyme, cytokines and immune cells like neutrophils and lymphocytes. It has been demonstrated that colostral cells cross the neonatal intestinal epithelium and are found in the newborn’s circulation as well as in lymphoid tissues. Curiously, heat-killed cells and cells from a source other than the piglet’s own dam do not cross the intestinal barrier. This is in contrast with immunoglobulins, which cross the neonatal intestinal epithelium and enter the circulation, independently whether immunoglobulins are from another dam or even from another species, although only shortly after birth.

In summary, the newborn piglet is usually protected against the pathogens present in a herd by means
Vaccination risk in infectious and efficacy of antibodies

This is probably the main reason by which monitoring duration and decay of MDI has been traditionally based on antibody detection by different serological tests.

Maternally derived antibodies and early life vaccination

Most of the pigs worldwide are vaccinated against different pathogens relatively early in life, usually within the first 4 weeks of age. Good examples would be porcine reproductive and respiratory syndrome virus (PRRSV), PCV2 and Mycoplasma hyopneumoniae, which account for the most frequent vaccinations. Sometimes, vaccination against other pathogens also take place during this early period, such as the case of Haemophilus parasuis, toxigenic Escherichia coli (edema disease), swine influenza virus (SIV) or Classical swine fever virus (CSFV) in some countries. For most if not all these pathogens, vaccination is taking place in face of certain (variable) degree of detectable maternally derived antibodies (MDA) by different serological techniques. Although MDI neutralization of vaccine antigens has been unequivocally demonstrated for certain pathogens at early life, such in the case of Aujeszky's disease (pseudorabies) virus, porcine parvovirus and erysipelas, most of the abovementioned vaccines are able to overcome MDI. In other words, most of these vaccines are able to elicit the corresponding immune response protecting the pigs against development of clinical disease in presence of MDA. However, this does not preclude that pre-existing immunity of maternal origin does not affect at all the vaccine uptake and subsequent humoral immune response; MDI may interfere with vaccination elicited-seroconversion as observed for a number of pathogens including PCV2, M. hyopneumoniae, SIV and CSFV. Curiously, minimal information is available regarding PRRSV vaccination and its relationship with MDI. The main reason is probably related with the relatively short duration of MDA against this virus, which is usually considered around 3-6 weeks after birth anyway, such duration might depend on the vaccination status of sows. A different scenario would be a much earlier vaccination within the two first weeks of life; studies regarding potential MDI interference on PRRSV vaccine efficacy are lacking in the literature.

It is important to highlight, however, that immunity elicited by vaccines is not only based on antibodies but also cellular immunity. Therefore, it is paramount to assess if the existence of MDI, basically measured by MDA, is able to counteract the cellular immunity generated by vaccines applied at early ages. Although this issue has been minimally tackled in the literature, it has been demonstrated that vaccination of neonatal pigs against M. hyopneumoniae in face of MDI resulted in cell mediated immunity (CMI) priming and anamnestic CMI responses following subsequent vaccination against M. hyopneumoniae; conversely, interference with antibody response to vaccination was suggested. A similar scenario has been also indicated for PCV2.

Obviously, within the complex scenario of vaccination in face of MDI, the major question is posed on the efficacy of vaccination. Taking into account all abovementioned factors, efficacy of vaccines applied at early ages is based on the overall balance of overcoming MDI, putative interference with vaccine immune response and subsequent duration of immunity. Such vaccine efficacy can be measured by a number of parameters including prevention or ameliorating of clinical signs, increase in average daily weight gain, decrease of infectious pressure (lesser pathogen load in systemic or mucosal locations as well as shedding), lower risk of co-infections and/or lesser use of antibiotics.

Vaccination of piglets against PCV2 and potential interference with MDI

PCV2 vaccination probably represents one of the most revolutionary successes of the pig industry in the last 30 years. Definitively, PCV2 vaccines are the most used ones worldwide, and can be applied to piglets (usually around weaning time) and/or sows (Segalés, 2015). Taking into account that PCV2 is a ubiquitous
virus, this means that both sows and piglets are vaccinated in presence of serum/mucosal antibodies. The objective to vaccinate sows is to provide homogeneous high levels of specific antibodies that will prevent disease in piglets by means of extended MDI as well as, probably, to protect the undesirable effects of PCV2 on reproduction. The objective to vaccinate pigs is basically to clinically protect the animal population that develops the PCV2-systemic disease as well as PCV2-subclinical infection. Therefore, in a systematic way, piglets are vaccinated against PCV2 in face of MDI.

It is known that MDI (measured as MDA) may protect against PCV2 challenge and influence the humoral response developed after vaccination. In these later reports, a significant negative correlation between MDA at the day of vaccination and the increment of antibody titers to PCV2 4 weeks post vaccination was confirmed. Therefore, it was unequivocally demonstrated that the higher the antibody titer at vaccination (generally at weaning), the lower the degree of vaccine-elicited seroconversion 4 weeks later. Moreover, one of these studies compared a protocol of both sow and piglet double vaccination, with application of the product in pigs at 3 and 7 weeks of age. Evident MDA interference with vaccine seroconversion was observed in the 3-week-old piglet vaccination schedule; however, such interference was not significantly noted with the vaccine-elicited cellular immune response. The latter result has also been demonstrated by others.

Again, the issue of PCV2 MDI interference must be tackled in relation to vaccine efficacy. Existing reports suggest that while interference of MDI on vaccine seroconversion is a fact, interference with vaccination efficacy might be negligible. Anyway, some results might be considered controversial, perhaps related to the features of the different vaccines applied. Earlier works concluded that MDA levels did not affect the efficacy of the vaccine measured as ADWG by comparing what they called high (>1:1000 Indirect Fluorescence Antibody Titration [IFAT] titers) and low (<1:1000 IFA titers). Others did not find statistically significant differences in terms of ADWG between 4-week-old vaccinated piglets derived from vaccinated and non-vaccinated sows. In this study, the best vaccination schedule performance included vaccination of both sows and piglets, but the correlation between initial MDA and ADWG of this group showed a negative slope, suggesting a potentially higher negative effect on efficacy when higher MDA titer was present at vaccination age; however, such result was not statistically significant. In a relatively recent study, it was suggested that age but not antibody titer at the time of PCV2 vaccination may affect vaccine efficacy (taking into account ADWG). However, results were not entirely clear since piglets vaccinated at 3 weeks of age had higher ADWG than those vaccinated at 1 week of age, but antibody titers of pigs at 1 week of age were higher than those at 3 weeks of age. Finally, another work did a study selecting on purpose piglets with low and high antibody values at vaccination, but no evident MDA interference with ADWG was observed. These authors postulated that perhaps animals with very high antibody titers at vaccine application may have true interference problems with efficacy. However, the same authors concluded that such proportion of piglets at-risk is probably very low in most conventional farms.

Based on all these observations, optimal vaccination strategies must balance the advantage of delayed vaccination in case of high antibody titers at weaning age with the need to induce immunity prior to exposure to pathogens under field conditions. In consequence, a “vaccination window” has been proposed, defined as the range of antibody titers at which piglets should be vaccinated to minimize potential interference with MDA and, at the same time, ensure the development of protective immunity before PCV2 exposure. Of course, this concept is applicable not only to PCV2, but also to the rest of pathogens infecting swine.

**Keywords:** Maternally Derived Immunity; Vaccination; Interference
21st Century, Rationally Designed and DIVA Compatible Vaccine for Rift Valley Fever

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Summary

Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic pathogen that causes serious morbidity and mortality in livestock and humans in Africa and the Arabian Peninsula. The virus has great potential for transboundary spread due to the presence of competent vectors in non-endemic areas. There is currently no fully licensed vaccine suitable for use in livestock or humans outside endemic areas. Here we report the evaluation of the efficacy of a recombinant subunit vaccine based on the RVFV Gn and Gc glycoproteins. RVFV structural proteins, amino-terminus glycoprotein Gn and carboxyterminus glycoprotein Gc, were expressed using a recombinant baculovirus expression system. The vaccine elicited strong virus neutralizing antibody responses in sheep and was DIVA (differentiating naturally infected from vaccinated animals) compatible. In a sheep efficacy study, animals were vaccinated subcutaneously with the glycoprotein-based subunit vaccine candidate and then subjected to heterologous challenge with the virulent Kenya-128B-15 RVFV strain. The vaccine elicited high virus neutralizing antibody titers and conferred complete protection in all vaccinated sheep, as evidenced by prevention of viremia, fever and absence of RVFV-associated histopathological lesions. We conclude that the subunit vaccine platform represents a promising strategy for the prevention and control of RVFV infections in susceptible hosts.

Keywords: Rift Valley Fever Virus, Glycoproteins, DIVA Subunit Vaccine
ASFV: Novel Approaches Towards Vaccines and Diagnostics

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Summary
The purpose of this study was to evaluate the immune response of pigs to various ASFV antigens including recombinant proteins and cDNA constructs, using a heterologous prime-boost vaccination approach. The ASFV genes encoding the structural proteins p15, p35, p54, CD2v (CD2-like), p30, p72 and p17 were synthesized based on the ASFV isolate Georgia/2007 and the respective recombinant proteins were expressed in a baculovirus or E.coli expression system. The cDNAs were cloned into pcDNA3.1 expression vector and included the p72, p32, CP312R and CD2v genes. Three-week old piglets were used for the immunogenicity study. The vaccination groups consisted of a combination of different recombinant proteins and plasmid DNAs. The piglets were inoculated intramuscularly with 100μg of recombinant protein mixed with ISA25 adjuvant and 100μg of plasmid DNA. Piglets were inoculated three times at two-week intervals and euthanized one week after the last immunization. Blood collection was carried out on the day of vaccination and at the time of euthanasia. ASFV-specific antibody responses in serum of immunized pigs were evaluated using ELISA, western blot and virus neutralization tests. The results of ELISA and western blot showed that antibodies were induced against each recombinant protein. In virus neutralization assays, neutralizing activity was found mainly in sera from pigs immunized with structural ASFV proteins; in some cases the neutralizing activity was increased by combination with cDNA plasmids especially cDNA for p72 and CD2. Pigs immunized three times with ASFV p15, p35 and p54 proteins in combination with cDNA plasmids CD2v, p72 and p32 were selected for challenge with virulent Armenia 2007 virus. Challenge was done in BSL-3Ag biocontainment and 5 age controlled animals served as challenge controls. Neither the vaccinated nor the mock-vaccinated animals were protected against virulent ASFV challenge. These results will guide us in our efforts to develop a potential vaccine against ASF.

Keywords: African Swine Fever Virus, Heterologous Prime-Boost, Subunit Vaccine
Residual Virulence of the MLV (LOM) and Genetic Characteristics of Field Strain of the Current CSFV

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Summary
Typical clinical manifestation of classical swine fever (CSF) was observed in naïve pigs infected by a current vaccine strain (LOM) in Jeju Island. Along with clinical signs, vertical and horizontal transmission of the vaccine strain were identified by the CSFV specific RT-PCR and sequence analysis. Otherwise, a novel outbreak by the field strain of CSFV which belongs to genotype 2.1 also occurred in a vaccinated commercial farrow-to-finish farm located in mainland of Korea. This study described the severe clinical manifestation related to CSFV vaccine strain in naïve pig herds and a novel CSF outbreak in a vaccinated pig farm.

Classical swine fever (CSF) is a highly contagious disease of global importance caused by classical swine fever virus (CSFV) which belonged to the genus Pestivirus within the family Flaviviridae. Due to its devastating impact on a country’s swine industry, enormous efforts, especially vaccination, to control and eradicate CSF have been prompted and eventually paid off in some parts of the world. In case of South Korea, mandatory vaccination using LOM strain for the establishment of herd immunity has been performed in the mainland after nationwide CSF outbreaks in 2003, whereas Jeju Island has maintained CSFV-free area without vaccination since 1998. Consequently, in the mainland of Korea, downward trend in the occurrence rate had been observed until 2009, and thereafter, except for one outbreak case on a non-vaccinated farm in 2013, there has been no official report about CSF outbreak over 6 years. However, in September 2016, an acute form of CSF outbreak by a field CSFV strain occurred on a vaccinated pig farm in Gyeonggi-do. Otherwise, in Jeju Island, unexpected outbreaks of CSF by a MLV vaccination (LOM strain) have occurred sporadically in naïve pig herds with typical CSF clinical symptoms since 2014. The purpose of this study was to pose a question about safety of CSFV vaccine strain and to determine genetic characteristics of current CSFV strain.

Several clinical specimens including aborted fetuses, dead pigs, feces, blood, and tissue samples from Jeju Island or Gyeonggi-do were submitted for laboratory diagnosis. Systemic necropsies were performed on dead pigs and the tissue samples were fixed in 10% neutral formalin for histological examination. After pre-treatment procedure for each specimen, CSFV-specific PCR was performed for diagnostic purpose. Specifically, for aborted fetus from Jeju island, additional PCR tests for other viral agents including porcine reproductive and respiratory syndrome virus, Aujeszky’s disease virus, porcine parvovirus, Japanese encephalitis virus, and encephalomyocarditis virus known for reproductive failures were executed. After the
confirmation of CSFV antigen in the clinical samples, additional PCR steps for nucleotide sequence analysis were performed for further investigation. Phylogenetic analysis was performed with Neighbor-joining with Kimura 2-parameter model to construct phylogenetic tree.

Typical lesions for CSF such as cyanotic ear, intestinal button ulcer, petechial hemorrhage on the surface of internal organs were observed in naive pigs affected by vaccine strain. Meanwhile, the pathological lesions by field strain of CSFV were slightly different from diseased pigs by vaccine strain (Figure 1).

The vaccine strains clustered with CSF genotype 1.1 isolates were identified in aborted fetus and placenta from Jeju with no other detection of tested viral agents (Figure 2). Furthermore, the CSFV was also detected in blood and fecal samples of suckling and weaned pigs. These two findings indicate the possibility of vertical and horizontal transmission of the CSF vaccine strain.

The field strain of CSFV (KU16Y) belonged to genotype 2.1 (Figure 2) was detected in various organs (tonsil, lymph node, lung, brain, and liver) from vaccinated growing pigs. The complete genome sequence of CSFV field strain (KU16Y) is 12,104 nucleotides (nt), including a 221-nt 5' NTR, a 11697-nt polyprotein coding region, and a 186-nt 5' NTR. The genome sequence has been deposited in GenBank under accession no. KX870109. Comparative analysis of KU16Y with a wild boar isolate (PC11WB) and three other vaccine strains including C, GPE-, and LOM strain was performed at the nucleotide and amino acid level. It showed 83.4% and 89.3% homology to MLV(LOM) in E2 coding nucleotide and putative amino acid sequences, respectively (Table 1).

As per the OIE terrestrial manual (3), CSFV vaccine strain should satisfy following criteria specified: 1) safety in young animals, 2) safety in pregnant sows, 3) non-transmissibility, and 4) no reversion to virulence. Given the outcomes of clinical inspection and molecular analysis, it is suspected that the vaccine strain caused chronic form of CSF and has virulence in young piglets and pregnant sows as well as vertical and horizontal transmissibility.

In China, large-scale outbreaks of CSF have rarely occurred since after the introduction of biannual vaccination policy using C-strain. However, growing numbers of CSF outbreaks has been reported in southeastern area since 2014 (4). According to Luo, Ji [5], the emerging CSFVs possessed altered antigenic characteristics escaping viral neutralization induced by C-strain vaccination. The Korean CSF strain KU16Y were grouped within a cluster characterized by vaccine escape mutants in China (Figure 2).

Considering these situation, further studies on the residual virulence of current MLV and characteristic of field strains seems to be necessary.

**Table 1** Comparison of sequence identity level between CSF KU16Y strain and other known CSFVs

<table>
<thead>
<tr>
<th>Region</th>
<th>Sequence identity (%)</th>
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<td></td>
<td>between KU16Y and other strains</td>
<td></td>
<td>between KU16Y and other strains</td>
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<td>PC11WB GPE - LOM C train</td>
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<td>PC11WB GPE - LOM C train</td>
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<td>Npro nt</td>
<td>97.8 87.43 87.23 85.43 NS2-3 nt</td>
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Figure 1 Comparison of gross lesions in kidney of CSF diseased pigs by the vaccine strain (a) and field strain (b).

Figure 2 Phylogenetic tree based on full-length E2 encoding sequences. Numbers at the nodes indicate the values of 1,000 bootstrap analysis. Tree was rooted at the distinct CSFV strain “Congenital Tremor”

Keywords: CSF MLV, Residual Virulence, Genetic Characteristic

References
CEEZAD After Seven Years of Existence: Mission, Scientific Projects and Success Stories

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Summary

The Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) at Kansas State University was established in June 2010 to help protect the nation's agricultural and public health sectors against high-consequence foreign animal, emerging and zoonotic disease threats. CEEZAD is headquartered at Kansas State University in Manhattan KS. The university is part of the Kansas City Animal Health Corridor and houses the College of Veterinary Medicine and the Biosecurity Research Institute. It is also adjacent to the site for the Department of Homeland Security's premier animal research facility, the National Bio and Agro-defense Facility, or NBAF, under construction. CEEZAD has four principal missions:

- Development of novel, safe, efficacious and DIVA-compatible vaccines - for prevention and control of high-impact emerging and zoonotic diseases - that can be manufactured in the U.S.
- Development and expansion of technologies and platforms for laboratory and point-of-need pathogen detection.
- Development of models to predict high-consequence disease behavior in the U.S. to aid prevention or outbreak control.
- Development of education and training programs for students, veterinarians, first responders and researchers in high-impact animal diseases and animal emergencies.

CEEZAD funds scientists specializing in animal health, public health, education, diagnostics, therapy and vaccinology. In this way, CEEZAD is enhancing the resilience of the U.S. pre-harvest agricultural system through investigator-directed research. CEEZAD-funded and coordinated research is conducted at more than 15 U.S. and international universities as well as governmental agencies and industry partners. Two projects, one on point of need (PON) diagnostics, the other one on the development of a recombinant Newcastle disease virus-vectored Highly Pathogenic Avian Influenza (HPAI) vaccine for poultry, will be discussed. The NDV-vectored vaccine, for possible use in future HPAI outbreaks, provides excellent protection in live and inactivated vaccine forms, and via practical mass application.

Keywords: CEEZAD, Mission, Projects, HPAIV
New Strategies for the Control of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Summary
The etiologic agent of porcine reproductive and respiratory syndrome (PRRS), PRRS virus (PRRSV), is a positive-stranded RNA virus whose genome serves as a messenger RNA. The viral genome contains 10 open reading frames (ORFs) coding for 14 nonstructural proteins (nsps) essential for viral genome replication and transcription, and 8 structural proteins required for particle assembly. Porcine alveolar macrophages (PAMs) in the lungs of pigs are primary targets for PRRSV infection and there are three stages of infection established in pigs: acute infection, persistent infection, and clearance. These three infection stages usually take the entire life duration of a pig. During infection, pigs generate poor anti-viral responses featured by a low level of type I interferons (IFNs-α/β). IFNs-α/β constitute the most important host innate immunity and is the first defense mechanism to protect pigs from virus infection. PRRSV interferes the both production and signaling pathways of IFNs-α/β. Six viral proteins have so far been identified as viral IFN antagonists. Nsp1α disrupts the formation of IFN enhanceosome to suppress IFN production. Nsp1β suppresses IFN production by blocking host mRNA nuclear export and translation. Nsp2 contains the de-ubiquitination (DUB) and de-ISGylation activities, which reduces IFN-mediated antiviral functions. Nsp4 cleaves some crucial IFN adaptors in the IFN production pathway, whereas nsp11 cleaves the mRNA for some adaptors to suppress the production of IFNs. Nucleocapsid (N) protein inhibits IRF3 phosphorylation and nuclear translocation that are vital for IFN production. This lecture will focus on the immune evasion mechanisms adopted by PRRSV to modulate host antiviral responses, and explore the potential new strategies for control of PRRSV.

The virus
It has been three decades since the emergence of porcine reproductive and respiratory syndrome virus (PRRS) in Europe and the US almost simultaneously but independently. The control measures however are unsatisfactory and PRRS is still considered one of the most economically significant swine diseases in many pork-producing countries worldwide. PRRS virus (PRRSV) is a positive-sense, single-stranded RNA virus contained in the viral envelope. The viral genome is 5'-capped and 3'-polyadenylated with the size of approximately 15 Kb, and thus functions as a messenger RNA. Ten open reading frames (ORFs) are found in the genome, coding for two large polyproteins (ORFs 1a and 1b) and eight structural proteins. Two large polyproteins are further processed by proteolytic cleavages and a total of 14 nonstructural proteins (nsps) are generated. For structural proteins, seven out of eight proteins are viral membrane-associated, and the remaining protein N (nucleocapsid) is associated with the viral genome to form an icosahedral nucleocapsid. While the structural proteins constitute the virion particle, nsps are found only in virus-infected cells and function for viral genome replication and transcription (1).
Host-virus interactions during PRRSV infection

PRRSV targets porcine alveolar macrophages (PAMs) in the lung, and thus PAMs are the main host cells in which PRRSV replicates. PRRSV also replicates in monocytes and monocyte-derived dendritic cells but does not replicate in lung-derived dendritic cells. Three stages are considered for PRRSV infection. The first stage is an acute infection during which viremia appears as early as 6 to 12 h post-infection. In this stage, PRRSV replicates rapidly in PAMs and dendritic cells in the lungs, and the viremia appears. Viremia lasts for 3 to 4 weeks and eventually disappears. The second stage is persistent infection. PRRSV is transported to the lymphoid tissues, largely tonsils and lymph nodes by macrophages and dendritic cells and the virus resides in those tissues with a limited replication. The viral persistence in the lymphoid tissues may last up to 180 days post-infection. The third stage is an extinction period when the virus is cleared from the host. In the modern operation of commercialized swine farms, pigs are held for 250 days before marketing, and thus PRRSV infection may be considered as life-long infection (2). During the PRRSV infection, pigs are unable to eliminate the invading virus during the acute stage, nor to eliminate persisting virus resided in the lymphoid tissues, suggesting that PRRSV may have a function to modulate the normal anti-viral function of the host.

Innate immunity is the first line of defense of host against viral infections, and type I interferons (IFNs-α/β) constitute the most important anti-viral immunity. During PRRSV infection of pigs, IFN-α level remains low in the serum, and no increase of IFNs is seen in the lungs where PRRSV actively replicates. In cells, PRRSV infection also suppresses type I IFN production upon stimulation. Both in vivo and in vitro data suggest that PRRSV may suppress the type I IFN production of host during infection. For IFN response, two critical steps are involved. The first step is the IFN production pathway. In this process, pathogen associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs) which are composed of two major receptors; the retinoic acid-inducible gene-like receptors (RLRs) in the cytoplasm and the toll-like receptors (TLRs) in the endosome. Cells turn on the signaling cascades in which various adaptor proteins are involved including IRF3. Once activated, IRF3 is translocated to the nucleus and binds to the IFN promoters initiating the IFN production. The second step in the type I IFN response is a signaling process. After production, IFNs are released from the cell and bind to the IFN receptors in an autocrine manner to its own cell or a paracrine manner to neighbor cells. The binding of IFNs to IFN receptors triggers activation of the signaling, called the JAK-STAT pathway, which further activates signal transducers and activator of transcription (STATs). Activated STATs then form the ISGF3 (IFN-stimulated gene factor 3) complex which is translocated to the nucleus, where it drives the expression of hundreds of IFN-stimulated genes (ISGs) (3). The ISGs are the major executors of cells to establish the antiviral status.

During the past several years, we have been investigating the viral strategies as to how PRRSV survives better in the host and undergoes a long-term persistence without being eliminated by the immune system. PRRSV indeed appears to suppress the type I IFNs production during infection, and six viral proteins have subsequently been identified as viral antagonists fighting against the IFN production of the host. They are nsp1α, nsp1β, nsp2, nsp4, nsp11, and the N protein. Among these, nsp1 in particular (both nsp1α and nsp1β subunits of nsp1) is the most potent viral antagonist able to suppress the IFN production.

nsp1α: PRRSV nsp1 is the first viral protein generated during infection of cell. It is generated by autocleavage from the polyprotein and is further processed to two subunits, nsp1α and nsp1β. The nsp1α subunit is distributed in the both nucleus and cytoplasm, suggesting multiple roles of this protein. Nsp1α has been found to carry the ability of IFN suppression in nsp1α-expressing cells. Further studies show that nsp1α degrades the CREB-binding protein. The CREB-binding protein is a critical transcription co-activator to drive the IFN gene expression in the nucleus. Thus, degradation of the CREB-binding protein by nsp1α causes the failure of formation of IFN enhanceosome, resulting in the prevention of IFN production (4).
nsp1β: The nsp1β subunit also inhibits the IFN production. Surprisingly, nsp1β has been found to block the transport of host mRNA from the nucleus to the cytoplasm. When the host mRNA nuclear export is blocked, host protein synthesis does not occur, and as a result, the host cell will not function properly to combat viral infection. In turn, virus replication may be enhanced since viral mRNAs are synthesized in the cytoplasm without the need of host cell nuclear function and thus viral replication will not be affected even without the host cell nuclear function. A specific motif referred to as SAP has been identified in nsp1β with a consensus sequence of LQxxLxxxGL. Single-amino acid mutations have been introduced in the SAP motif and a series of nsp1β mutants have been constructed. These mutants then have been examined for their subcellular distribution, their ability for the inhibition of host mRNA nuclear export, and the host protein syntheses. It appears that the mutants L126A (leucine at position 126 of nsp1β mutated to alanine), R128A, R129A, L130A, and L135A have lost their ability to block the host mRNA nuclear export (5). The mutant viruses have been examined in pigs for their virulence and appear to be attenuated.

nsp2: Nsp2 is the largest viral protein expressed by PRRSV. Mutations and deletions are frequent in the hypervariable regions of nsp2 while other regions are relatively conserved. The ovarian tumor (OTU) domain represents a superfamily of proteases found in eukaryotic, bacterial, and viral proteins, and some of these contain ubiquitin (Ub)-deconjugating activity (6). PRRSV nsp2 has a proteinase domain which belongs to the OTU superfamily and this domain of nsp2 has been shown to contain the de-ubiquitination (DUB) and de-ISGylation activities. Those activities have been shown to antagonize the antiviral effects of ISG15. These activities of nsp2 interfere the production and signaling of IFNs (7).

nsp4: Nsp4 is the main protease responsible for processing the remaining nonstructural proteins from the polyproteins, besides nsp1α, nsp1β and nsp2. The protease activity of nsp4 also cleaves some crucial IFN adaptors which play roles for IFN production (8). The nsp4-mediated IFN suppression is NF-κB dependent (9).

nsp11: Nsp11 is a component of the RNA-dependent RNA polymerase complex which is essential for viral RNA synthesis. Nsp11 contains an uridylate-specific endoribonuclease activity and this activity of nsp11 is unique for viruses in the order Nidovirales, designating as the ‘NendoU’ domain. The nsp11 NendoU activity has been shown to cleave mRNA of MAVS (also known as VISA and IPS-1) and down-regulates IFN expression (10).

Nucleocapsid (N): The N protein is the most abundant structural protein of PRRSV and constitutes the viral capsid protecting the RNA genome. N is the nucleocyttoplasmic protein distributed in the cytoplasm and nucleus, especially to the nucleolus. PRRSV N inhibits IFN production by inhibiting the IRF phosphorylation and its nuclear translocation (11). N has been shown to upregulate interleukin-10, suggesting the activation of NF-κB by N. Indeed, N has been shown to stimulate the NF-κB activity and subsequently been shown to upregulate the proinflammatory cytokine production. The viral mechanism for NF-κB upregulation by N has been determined and will be discussed.

Conclusion
Porcine reproductive and respiratory syndrome causes severe economic losses in the swine industry worldwide. PRRS virus preferably infects PAMs in the lungs and upper respiratory tract of pigs, and becomes latent in the lymphoid tissues. The virus is cleared eventually and this process usually takes long time. The delayed antiviral response is partially due to the suppressed innate immune responses by PRRSV. Understanding the viral strategies for host-immune evasion will help develop alternative control measures for PRRSV.

Keywords: PRRSV, Immune Evasion, Type I Interferons
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Interplay Between Porcine Epidemic Diarrhea Virus (PEDV) and Anti-PEDV Interferon Responses of Host

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Summary
Porcine epidemic diarrhea (PED) is a highly contagious acute and devastating enteric disease in sucking piglets with up to 90% mortality. It has emerged in the United States in 2013 and has spread rapidly to most pork-producing states causing significant economic losses to the US pork industry. Type I interferons (IFN-α/β) are the major components of the innate immunity of hosts and are crucial for the establishment of antiviral state during virus infection. Many viruses in turn have evolved to counteract the production of type I IFNs. We found that the IFN production was significantly suppressed during PEDV infection in cells. To identify viral components responsible for the suppression of IFNs, all viral coding sequences were individually cloned and expressed. Of 16 PEDV nonstructural proteins (nsps), nsp1, nsp3, nsp7, nsp14, nsp15, and nsp16 were found to inhibit the IFN-β production. The sole accessory protein ORF3, the envelope (E), membrane (M), and nucleocapsid (N) proteins were also shown to inhibit IFN production. PEDV nsp1 was the most potent IFN antagonist. PEDV nsp1 interrupted the enhanceosome assembly of IRF3 and CREB-binding protein (CBP) by specifically degrading CBP. We further found that CBP degradation by nsp1 was proteasome-dependent. PEDV nsp1 also suppressed the NF-κB-mediated IFN production. PEDV blocked NF-κB activation in virus-infected cells when stimulated with TNFα. Nsp1 was identified as a potent inhibitor of NF-κB, resulting in the inhibition of pro-inflammatory cytokines. To identify the motifs important for suppression of NF-κB, a panel of nsp1 mutants was made according to the predicted high-order structure. Interestingly, mutations in the conserved residues altered their cellular distributions and subverted their NF-κB suppression, suggesting that the conserved high-order structures were crucial for nsp1-mediated NF-κB suppression. Taken together, our study shows that PEDV suppresses the production of type I IFN, and nsp1 is a multifunctional antagonist for IFN and NF-κB.

Porcine epidemic diarrhea (PED) was first described in the UK in the early 1970s and has since become endemic in some parts of Europe. Since 2010, severe PED outbreaks have occurred in some countries in Asia. In the US, PEDV was first identified in 2013 and caused significant economic losses with high morbidity and mortality of up to 100% in some farms. PED is an acute and highly contagious enteric disease characterized by severe enteritis, vomiting, watery diarrhea accompanied by a high mortality rate in neonatal piglets (1,2,3). Porcine epidemic diarrhea virus (PEDV) belongs to the Coronaviridae family. The genome is a single-stranded, positive-sense RNA of ~28 kb in length with a 5′-cap and a 3′-polyadenylated tail. It encodes two polyproteins pp1a and pp1a/b, one accessory protein (ORF3), and four structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N). The pp1a and pp1a/b polyproteins are further processed into 16 nonstructural proteins (nsps) by proteinase activity of nsp3 and nsp5. Among all nsps, nsp1 is the most N-terminal product and is the first cleavage product. Upon virus infection, host cells react quickly to invading viruses by producing type I interferons (IFN-α/β) and establish an antiviral state, which provides a first line of defense against viral infections. Released viral nucleic acids are sensed by host
innate immune system by pattern-recognition receptors (PRRs), which leads to the activation of cytosolic kinases. This process promotes the activation of IRF3, IRF7, and NF-kB, and their subsequent translocation to the nucleus where they bind to their respective sequences for production of type I IFNs. Activated NF-kB also translocates to the nucleus and triggers IFN-β expression. IFNs are then secreted and bind to their receptors on both virus-infected cells as well as neighbor cells for activation of JAK/STAT pathway, leading to the production of hundreds of interferon-stimulating genes (ISGs) to restrict viral infections (Reviewed in 4).

To evaluate the innate immune regulation by PEDV infection, several different lines of mammalian cells were examined for PEDV replication. We found that PEDV efficiently replicated in MARC-145 cells and suppressed IFN-β production in infected cells. To identify the viral IFN antagonists, we cloned all of PEDV genes representing nsps 1 through 16, and structural genes for S, E, M, and N including the ORF3 accessory gene. All of the genes were individually expressed and examined for their ability for IFN suppression. Among the nsps, nsp1, nsp3, nsp7, nsp14, nsp15, and nsp16 were determined as the viral IFN antagonists. In addition, ORF3, E, M, and N were also determined as the viral IFN antagonists (5). PEDV nsp1 inhibited the IRF3-mediated IFN signaling. PEDV nsp1 was a nuclear-cytoplasm protein and was found to function in the nucleus. In the nucleus, IRF3 associates with the CREB-binding protein (CBP) to assemble the basal transcription complex to turn on the IFN gene expression. Thus, the assembly of enhanceosome is crucial for IFN expression. We found that PEDV degrades CBP in the nucleus in virus-infected cells. CBP was depleted by nsp1, demonstrating that nsp1 degrades CBP in the nucleus. CBP degradation by PEDV nsp1 was further confirmed by biochemical assays and western blot. For PEDV nsp1-expressing cells, approximately 90% cells showed more than 80% reduction of CBP, while no CBP reduction was observed in control cells. The CBP degradation was MG132 dependent, indicating the CBP degradation by nsp1 was proteasome-dependent.

PEDV N protein blocks NF-kB activation and inhibits IFN-β production and ISGs expression (6). PEDV nsp5 is a 3C-like proteinase and cleaves NEMO, which is an essential NF-kB modulator (7). This suggests that PEDV has the ability for NF-kB suppression. We found that in PEDV-infected cells, NF-kB was not activated up to 12 hpi. NF-kB however began to be activated from 18 to 24 hpi indicating that PEDV modulated NF-kB functions in a time-dependent manner. Of the 10 IFN antagonists, nsp1, nsp3, nsp14, nsp15, ORF3, E, and N protein were found to inhibit the NF-kB activity. Nsp1 was further found to block the NF-kB activation and inhibit the IkBα degradation. Using a series of nsp1 mutants, we found that the conserved high-order structures were crucial for nsp1-mediated NF-kB suppression. Together, our findings show that PEDV inhibited the production of type I IFN and nsp1 was a multifunctional IFN antagonist, which may facilitate the replication and pathogenesis of PEDV during infection.

**Keywords:** Porcine Epidemic Diarrhea Virus, Interferon Regulation, Nsp1, CREB-Binding Protein, IRF3, NF-kB

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Salmonella Reduction in Pig Herds as Contribution to the Overall Salmonella Control in the Food Chain

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Summary
Minimizing the contamination of food of animal origin has to be organized as a continuum from feed production and storing, feeding, animal husbandry with biosecurity and hygiene, animal transport, slaughter, processing, retail to the kitchens of the consumers. However, Salmonella control at farm level has a crucial importance for reducing the risk of Salmonella infections for consumers. The paper explains as an example for a systematic national Salmonella control programme the German Salmonella monitoring and reduction programme for the pig and pork production chain. It concludes with describing the relatively new focus on the internal biosecurity at farm level to reduce the “residual” Salmonella load of pig barns in pens and in the entire buildings before new pigs are introduced into the cleaned and disinfected pens as one of the most powerful measures to minimize the frequency of Salmonella-positive pigs sent to slaughter.

The European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) reported 151,995 human cases of salmonellosis in 2007 in the 27 Member States of the European Union (EU), being an incidence of 31.1 per 100,000 population (Anonymous, 2009). Humans can acquire the Salmonella infection through consumption of contaminated food (most cases) or drinking water. Products of animal origin, mainly eggs and meat (products) are known as the main food sources. Until recently the human cases of food-borne Salmonella infections in the EU were to about 60% attributed to eggs and poultry meat products, to about 30% to pig meat products and to about 10% to milk and bovine meat products. However, in recent years, the incidence of human cases of salmonellosis has considerably dropped, which the ECDC/EFSA Zoonosis Report attributes mostly to quite successful efforts in the poultry industry (vaccination of grand-parent and parent flocks as well strict biosecurity measures), and to an increased awareness of consumers and improved kitchen hygiene. Although there are national Salmonella control programmes in the pig production sector of several EU Member States (Denmark, Germany, UK, Austria, The Netherlands and Belgium), the reduction of the human cases is hardly due to these efforts. This means that the proportion of human cases due pig meat products is nowadays higher than before the successful Salmonella reduction in poultry production. This is a strong reason to increase the efforts to minimize the contamination rate of pork and pork products (Blaha, 2008; Anonymous, 2009).

It is well-known that the introduction of multiple Salmonella serovars into the food chain can occur at any point in the food production chain: in feed, in sow herds, in flat decks and finisher herds, during animal transport, at slaughter, during processing the meat, at the retail level and even in the consumers’ kitchens. However, the strongest determinants of the frequency of Salmonella contaminations of pork and pork products are a) the prevalence of Salmonella infected and contaminated pigs delivered to the slaughterhouse, and b) the hygiene status at slaughter that determines whether the contamination of the carcasses with Salmonella
is increasing along the slaughter line or decreased.

All in all, it is not only common sense that the primary determinant of the prevalence of Salmonella in pork and pork products is how many pigs sent to slaughter are infected or contaminated with Salmonella or are Salmonella carriers, but also the fact that the Salmonella serovars adapted to live pigs (S. Typhimurium, S. Derby, S. Infantis, etc.) are also predominant in pork and pork products. These two facts are a strong reason to intensify the Salmonella control at farm level.

However, successful control of Salmonella at the farm level is not straightforward. There are, although there are some Salmonella serovars that are more adapted to pigs, over 50 different serotypes that can be found in pig herds, Salmonella can survive for a long time in the environment, and infected pigs usually become long-term carriers that can shed the bacteria during periods of decreased immunity.

Like in Denmark and The Netherlands, a national Salmonella monitoring programme for finisher herds was also implemented in Germany. The basic principle of the programme is identifying those finisher herds that have the highest intra-herd Salmonella prevalence. The method of measuring the intra-herd prevalence is the systematic serological testing of meat juice randomly taken from the carcasses 60 pigs per herd evenly distributed over 12 months (Blaha, 2004, Merle et al., 2011). The herds that are categorized into the following categories: Cat. I (zero or low prevalence) are herds with less than 20% of pigs with Salmonella antibodies, Cat. II (medium prevalence) are herds with more than 20% and less than 40% of pigs with Salmonella antibodies, and Cat. III (high prevalence) are herds with more than 40% of pigs with Salmonella antibodies. This categorization is used a) to enable slaughter plants to plan a logistic slaughter by slaughtering the pigs from herds of Cat. I before slaughtering pigs of Cat. III, and b) to demand from the owners of pig herds in Cat. III to implement salmonella reducing measures in their pig herds. The most important precondition for developing reasonable and potentially successful farm-specific Salmonella reduction programmes is to know the causes and the dynamics of the occurrence of Salmonella in pig herds.

Theoretically, pig herds can get infected by:

a) the horizontal introduction of Salmonella serovars into a sow herd, a flat deck and/or a finisher barn (feed, rodents, humans, birds, contaminated materials etc.);

b) the vertical translation of Salmonella from sows to piglets to weaners and finally to the finishers;

c) the permanent Salmonella infection-contamination-infection cycle (i.e. the exchange of salmonella between Salmonella shedding pigs and their Salmonella contaminated environment.

In the last decades, the horizontal introduction of Salmonella into pig herds was regarded the major reason for Salmonella-positive pigs, and feed was thought to be the most important source. However, in many cases, the subsequent search for the introduction source by bacteriologically testing multiple feed samples from infected herds produced relatively seldom positive results. Consequently, researchers tested new hypotheses for explanations for the many positive pig herds, in which the search for the sources of the introduction of Salmonella were unsuccessful.

Gotter et al. (2012) carried out a case-control study about the influence of Salmonella contaminated environments (both the “direct environment” of pigs that pigs have contact with and the “indirect environment that pigs cannot reach, but Salmonella can be carried into the pens by e.g. humans, their tools, rodents and flies) by sampling both kinds of environments in herds of Cat. I and of herds of Cat. III.

The results about the frequency of positive samples in both groups, in short, are: In general, there is a considerably close correlation between the frequency of antibody-positive animals in a herd and the number of positive faeces and environmental samples.
(29% positive samples in Category III herds vs. only 15% in Category I herds. And: locations with the highest Salmonella contamination in the direct environment of pigs are: pen walls (28% Salmonella-positive), feeders and troughs (27% Salmonella-positive), drinking nipples (27% Salmonella-positive) and pig toys (21% Salmonella-positive); and locations with the highest Salmonella contamination in the indirect environment are: central hallway in barn (32% Salmonella-positive), shoes/boots of farmers (25% Salmonella-positive), anterooms (25% Salmonella-positive) and driving boards (23% Salmonella-positive).

The result of subtyping the Salmonella isolates of the case-control study by phage-typing is that in all herds the same serovars and even mostly the same subtypes of Salmonella were found in subsequent production cycles per farm, which proves that residual Salmonella from the previous production cycle are the main infection source of pigs in the next production cycle on the same farm.

Both results led to the conclusion that there is, of course, the possibility that salmonella is introduced horizontally into pig herds, and that there is, of course, a vertical translation from sows to piglets to weaners and to the finishers, but the importance of residual Salmonella in the direct and indirect environments of pigs has been greatly underestimated. This knowledge allows for optimistic expectations that the Salmonella load of pig herds can effectively lowered if the overall hygiene level is increased by such as cleaning and disinfecting areas and tools more intensively that have been identified as often still positive after cleaning and disinfection, and especially the internal biosecurity is taken into more account by such as changing boots and overalls before entering another compartment of the herd, using paper towels instead of cloth towels, separating “black” and “white” zones in the anterooms.

**Keywords:** Serological Salmonella Monitoring, Residual Salmonella in Pig Barn Buildings, Internal Biosecurity

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The Paradigm Change in Meat Inspection: From Inspecting Products to Optimizing the Production Process

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Summary

The emergence of new food safety risks such as BSE, Salmonella and dioxin contaminations in the late 20th century triggered a paradigm shift from the traditional inspecting animals prior to slaughter for their being fit for slaughter and carcasses and organs at slaughter for their being fit for consumption to a modern approach to food safety by taking the entire food chain into consideration. The focus is now not any longer on the identification of lesions in carcasses and organs, but on minimizing the microbiological, chemical and drug residue contaminations of meat and meat products. This “farm-to-table” approach is definitively not only as good as the traditional meat inspection, but it addresses much better the fact that most health risks for consumers of meat and meat products stem from mistakes and deficiencies in the production steps prior to slaughter, which need to be minimised in the sectors of feed production, animal husbandry, during slaughter, deboning, processing up to retail and the consumers’ kitchens.

The classical meat inspection mainly based on the scientific work of the German physician Rudolf Virchow and the German veterinarian Robert von Ostertag in the end of the 19th century focuses on inspecting slaughter animals directly prior to slaughter by a veterinarian to make sure the animals are fit for slaughter, and on inspecting the carcasses and their organs at the slaughter line by veterinarians and/or trained meat inspectors to make sure the meat and the organs are fit for consumption. This method has been more or less implemented in most countries all over the world. Until the end of the 20th century this inspection paradigm (= every animal is accepted to be slaughtered and veterinarians and meat inspectors sort out animals and carcasses from being sold for consumption) was a success story, since it made the consumption of meat and meat products very safe and it contributed to the eradication e.g. of tuberculosis and of tape worm infestations. Additionally, the inspection at the slaughter line contributes to removing parts of the slaughtered animals (abscesses, bleedings, bone fractures and organ lesions) that should not reach the consumer.

However in the late 80’s and the 90’s of the last century, animal diseases (e.g. BSE, latent Salmonella infections) and carcass contaminations (e.g. dioxins, drug residues) emerged that do not result in visible and palpable lesions in the carcasses and organs of slaughtered animal. The result was that there was an increasing feeling that the meat and meat products became more and more unsafe. Thus, the EU Commission issued the White Paper on Food Safety in 2000 (Anonymous, 2000), which acknowledged that the traditional inspection method was not any longer suitable to guarantee the safety of meat and meat products. After two years, the European Regulation (EC) 178/2002 (Anonymous, 2002), which has become the basic law that characterizes the paradigm shift from traditional meat inspection by only inspecting the end product of the food animal production and slaughter (carcasses and organs) to decide, whether the product is „fit for consumption”, or needs to be „condemned” to the risk-based meat inspection Monitoring and optimizing
the production process „from farm to table” to make the product „fit for consumption” by optimising the entire food production chain from feeding, raising and slaughtering the animals to the final food of animal origin.

The major aspects of the new paradigm are:

- the responsibility and the self-controls of the food producers are strengthened including also feed producers and food animal farmers (“food safety is the responsibility of the food producers and not the controllers”),
- food safety risks are to be addressed at all levels of the food chain (feed, animals, transport, slaughter, deboning, processing and retail),
- animals need to be healthy and drug residue-free to be accepted to slaughter (farmers have to guarantee this by a mandatory “Food Chain Information”)
- food producers have the duty to report any possible risk to the next production sector.

The Reg. (EU) No. 854/2004 that is describing the new way of meat inspection along the food chain specifies, which questions the “Food Chain Information” (FCI) have to be asked for guaranteeing that animals are fit for slaughter and consumption:

- Do the animals come from an “integrated system” and “controlled housing condition”?
- What is the animal health status of the holding of provenance and/or of the regional animal health status?
- Herd health status?
- Veterinary medicinal products or other treatments with a withdrawal period greater than zero?
- Diseases that may affect the safety of meat?
- Diagnostic results including monitoring and control of zoonoses and residues relevant to the protection of public health?
- Reports about previous ante- and post-mortem inspections?
- Production data that might indicate the presence of disease?
- Name and address of the private veterinarian?

Since the terms “integrated system” and “controlled housing condition” are not very clear and especially official veterinarians were not sure when to agree on the new risk-oriented and the food chain integrating modern meat inspection, the EU Commission issued another follow-up regulation which gave definitions on integrated systems and controlled housing conditions for pigs, the Reg. (EU) No. 1244/2007 on “Controlled housing conditions and integrated production systems” (Anonymous, 2007). The definitions are:

- Controlled feed (Reg. (EU) 183/2005) - or appropriate treatment to inactivate bacteria
- All-In/ All-Out production system and possibilities for quarantine
- Detailed food chain information (FCI) – see above
- In case of outdoor holding, exclusion of risks for disease outbreaks
- In case of bedding, exclusion of contamination of the bedding
- Compliance of the personnel with general hygiene regulations
- Access control of the animals premises
- In case of tourism and camping on the farm, exclusion of animal contact of the tourists
- No access of the animals to dumpsites or domestic wastes
- Existence of a rodent control plan
- In case of silage feeding, exclusion of food safety risks
- No access to sludge or sludge fertilization of feed plants.

In the first decade of the 21st century there were quite a lot of discussions about the reasonability of the paradigm shift, since there very many doubts whether the new strategy is as good as the traditional approach to guarantee the safety of meat and meat products. Therefore, the European Food Safety Agency (EFSA)
published the “Scientific Opinion on the public health hazards to be covered by inspection of meat” (EFSA, 2011). The conclusion of the paper was: “Finally, it was considered that palpation/incisions used in current post-mortem inspection should be omitted in pigs subjected to routine slaughter, because of the risk of microbial cross-contamination. These techniques should be limited to suspect pigs identified through FCL/ante-mortem inspection and/or post-mortem visual detection of relevant abnormalities and where it would lead to risk reduction. In such situations, palpation/incision should be performed separately from the slaughter line and accompanied by laboratory testing as required. In other (very generalized) words: “The meat inspection of R. von Ostertag (inspecting carcasses and removing pathological lesions) is ‘dead’, Identifying zoonotic contaminations and residues is the ‘meat inspection’ of the future”.

With this scientific opinion, the EFSA made clear that shifting from the traditional “lesion-oriented” inspection of carcasses and organs to the new risk-oriented meat inspection and efforts to optimize the quality and safety of the production processes along the food chain is not only “as good as” the traditional method, but it addresses much better the health risks of today, which are almost exclusively characterized by microbiological, chemical and drug residue contaminations which occur in the production steps prior to slaughter and cannot be taken out of the food chain by searching for lesions at slaughter.

It is common sense that, if efforts to reduce the Salmonella prevalence in the pig herds by improving the external and internal biosecurity and the hygiene level in the farms delivering slaughter pigs as described by Gotter et al. (2012) are implemented, the level of safety will even increase. Those improvement measures are:

- targeted cleaning and disinfecting areas and tools more intensively that have been identified as often Salmonella-positive after regular cleaning, and
- changing boots and overalls before entering another compartment of the herd, using paper towels instead of cloth towels, and separating “black” and “white” zones in the anterooms.

**Keywords:** Traditional Meat Inspection, Risk-Oriented Meat Inspection, Self-Controls of Food Producers, The “Farm-to-table” Concept

**References**

(6) Gotter, V.; Blaha, T.; Klein, G. (2012): A case-control study on the occurrence of Salmonella spp. in the environment of pigs. Epidemiology and Infection 140 (1) 150-156
Elimination of Virulent Porcine Epidemic Diarrhoea 2a Virus from Pig Farms

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Summary
The control of Porcine Epidemic Diarrhoea must be an elimination programme. This virus cannot be tolerated on production systems as the losses to the gilt farrowing system is too high. Porcine Epidemic Diarrhoea will sweep through an area and will infect farms irrespective of their biosecurity protocols. Air filtration systems did not stop infection on US farms. However, once a farm is positive, the virus must be eliminated and at this point strict adherence to prescribed biosecurity protocols must be followed. The disease can be stopped but in pig dense areas the risk of reinfection is always present.

Elimination template
The techniques would be valid for the PED type 1 (common in Europe) as well as the more virulent PED type 2 which has become a problem in Asia and North America. Having batching or multisite production systems would significantly help the control model as the viral pathogen can be confined to one unit while other units are cleaned up.

Porcine Epidemic Diarrhoea Virus science
Survives 7 days in warm climates but will survive for months in the cold
Pigs produce the virus for 10 days but some sick ones may be 30 days
Antibodies in the colostrum only last 14 days post consumption by the piglet
Vaccines may only work in previously positive pigs.
Immunity may only last in adults for 10-12 weeks.
Very small amounts are infective
Very large amounts are produced by a sick pig
It will take about 21 days for the virus to die out on the farm if no new cases occur

Stop moving PED around on people
Minimal staff in each department
No contractors
Separate farm staff for different departments
Complete separation between (breeding and gestation) and farrowing
Complete separation between Farrowing A (less than 10 days) and Farrowing B (more than 10 days).
This means separate dining and smoking area
All staff to wear gloves
Stop shaking hands
No staff meetings unless absolutely necessary
Boots not clogs to be worn – no laces
Foot baths and not pads
Passageways to be sprayed with H2O2 disinfectants 3x daily
Limewashed over night
No entering pens unless absolutely necessary

Phase 1. Controlling the outbreak. This can take 20 weeks in cases of PED type 2

Day one
1. Euthanasia of all piglets less than 10 days of age.
2. The euthanasia of all piglets for the next 3-4 weeks, at birth.
3. At the initial outbreak weaning of all piglets more than 10 days of age.
4. Removal of intestines from dead sick piglets and nursery pigs. This material is used to make an autogenous vaccine by feedback.
5. When making the autogenous vaccine ensure that the meat grinder does not get too hot which can kill the virus. Do not add chlorinated water to the ‘soup’ as this may destroy the virus.
6. The presence of the virus can be confirmed by a pig side lateral flow device.
7. It may be necessary to delay the euthanasia on day 1 for 1 day to allow for sufficient piglets to have died to provide material for a feed-back programme.

Over the next few weeks
8. Provide feedback material to all adult sows 10 ml of intestine contents per sow for 3 occasions 3 days apart.
9. Place sufficient intestine contents in a freezer to allow for a minimum of 18 weeks of feedback materials.
10. Repeat feedback programme in 3 weeks.
11. The feedback programme then continued for sows 6 and 3 weeks pre-farrowing.
12. Consider the use of commercial vaccines to adults who have been infected previously.
13. Use of charcoal to nursery, finishing and adult pigs to provide an antidiarrhoeal treatment.
14. Sows reproduction and cycling needs to be controlled using altrenogest and a rational breeding programming.
15. Staff and equipment movements around the farm need to be curtailed. All contact between different departments need to stop, especially once the farm starts producing piglets again.
16. Staff to wear clearly different clothing, boots and gloves between departments.
17. Disinfect and lime wash all passageways and any equipment.
18. Practice strict all-in/all-out and batch production.
19. Once piglet production starts again, stop all processing until 10 days of age, including iron injection
20. Do not enter the farrowing pen unless absolutely necessary and then disinfect boots.
21. Stop all cross-fostering
22. Collect colostrum from immunised sows, this can be frozen. Use 5ml to newborn piglets especially if repeat outbreak appears to occur.

Rationale
1. Produce a consistent anti PED colostrum from immunised sows. But note this may only last 20 weeks. Subpopulations are a problem.
2. Reduce viral load on the farm by euthanasing affected newborn piglets.
3. The virus alone will die out on the farm within a couple of weeks
4. Virus excretion by weak pigs may occur up to 35 days.

Piglet production
1. Provide colostrum to all gilt litters from colostrum taken from sows
2. Stop all processing until weaning including iron injection, tail docking and castration.
3. Do not enter the farrowing pen unless absolutely necessary and then disinfect boots.
4. Stop all cross-fostering
5. Three weeks after immunisation programme has started, collect colostrum from immunised sows, this can be frozen. Use 5ml to newborn piglets especially if repeat outbreak appears to occur.
Monitoring of the outbreak
Pig Flow impact of PEDv on a farm practicing weekly batching.
Breeding batch 0 was the start of the outbreak

Breeding line is longest, followed by the farrowing line and finally the weaner line

Long term
Once the farm has stabilised and are producing normal weaners, destroy the feedback material in the freezer, removing any remaining virus on the farm. Or repeat infections can occur.

Keywords: Coronavirus; Pathogen Elimination; Biosecurity
Transmission of Antimicrobial Resistance Between Animal and Human

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Antimicrobials have played an important role in maintaining the animal health and in producing the high quality food. The concern that the use of antimicrobials in animal and human can increase the risk of selection of antimicrobial resistant bacteria that may cause failure of treatment has led to international expert meeting and reports. Although the prevalence of zoonotic antimicrobial resistant bacteria in food animals is maintained still low, however, resistant genotypes similar to or identical with those of the human isolates were also found in non-human sources. Therefore, the risk management interventions should be urgently implemented. Furthermore, a “One health” approach to antimicrobial use and resistance is essential to minimize the antimicrobial resistance in humans and animals, because these are the responsibility of all three health communities: human health, animal health, and environmental health-communities.

Methicillin Resistant Staphylococcus aureus (MRSA)
Recent reports have documented MRSA detection in animal and human. Now it is considered as one of the most important zoonotic pathogens. In Korea, MRSA has been isolated from various non-human sources such as pet, live pigs, raw meat, and bovine milk. In a recent study, two different lineages of MRSA were identified namely human associated (HA) type (ST5, ST59, ST72) and livestock associated (LA) type (ST398, ST541, ST692) in non-human sources and slaughterhouse workers.

CTX-M producing Enterobacteriaceae
Extended-spectrum β-lactamase (ESBL)-mediated resistance is of considerable importance in both human and veterinary medicine. During the past couple of decades CTX-M type ESBLs or cefotaximases have been increasingly reported in many countries of the world. In a study done in Korea, CTX-M producing E. coli and Salmonella were detected in animals, raw meat, farm environment, and farm workers. Although a variety of CTX-M types was involved in the resistance against 3rd generation cephalosporins, blaCTX-M-14 and blaCTX-M-15 were the most prevalent in non-human sources. Furthermore, identical PFGE patterns and conjugative IncFII and IncI1-ly plasmids were detected in Non-Typhoidal Salmonella (NTS) from both human and animal source. These results suggest that a combination of clonal and horizontal transmission is spreading of CTX-M resistant NTS between animal and human sources.

Strengthening the monitoring systems on antimicrobial usage and resistance
Surveillance of antimicrobial usage and resistance provides important data for the identification of resistance problems and contributing factors for the development and spread of resistance at a national and local level. Antimicrobial resistance trends should be consistently monitored over time and across geographical areas and should be shared at the regional and global levels. Furthermore, harmonization and standardization are needed to compare the situations at the national and international levels.

Strict enforcement of prudent use guidelines for veterinarians and producers
International organizations have emphasized the importance of prudent and rational use of antimicrobials
in animals in order to minimize the possible impact of animal antimicrobial usage on public and animal health. A guiding principle with respect to antimicrobial usage should be “as little as possible, as much as necessary” since we owe it to both present and future generations to use these agents with care and discrimination. It is essential that all parties work together to ensure safe use and to minimize the development of resistance.

**Reduction of antimicrobial use in animal and human**

Prevention and control of infections is essential in fighting antimicrobial resistance. Thus, to minimize infections in animal and human and to decrease the volume of antimicrobials used, efforts should aim to improve animal and human health.

**Keywords:** Antimicrobial Resistance, Zoonotic, One Health

**References**


The Animal Welfare Concerns About Pig Husbandry in Europe

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Summary
The paper takes the explanation of the recent changes in the human-animal-relationship in Europe and the consensus on the “Five Freedoms” for all animals under human care as starting point to report about the European legislative regarding animal welfare.
This is followed by analyzing the major animal welfare concerns about pig husbandry in Europe: the still “permitted” routine tail docking in most EU Member States, the fact that male piglets are widely castrated without anaesthesia and/or analgesia, the fact that pigs in confinement do not have access to different climate zones, the fact that non-pregnant sows and sows in early pregnancy are kept in tight single crates as well as farrowing and suckling sows in cages to prevent the crashing of piglets by the sows, and the highly various frequency of lesions in carcasses due to the vastly varying health status of pig herds.

Concluding, the paper explains which measures to improve pig health and welfare in the EU Member States are and will be implemented.

It is an ethical achievement in our developed societies that molecular biology and modern ethology makes animals more “human” and humans more “animal”, which leads to the following moral obligation: Humans have a high responsibility for the welfare and wellbeing of the sentient animals they own or care for. Animals are no longer only things in the legal meaning of “property”, which can be treated as the owners want to, but also feeling creatures that have the right that their needs and interests are met by those that are responsible for the animals.

Although there is a large degree of consensus that animals should be kept in a decent way and that their needs have to be met, but due to the fact that urban people lose more and more contact to and knowledge about animal husbandry in agriculture, and measure their understanding of animal welfare from their relationship with their beloved pet animals, opinions about the quality of the lives of food producing animals are controversial and often quite emotionally disputed in the public discourse.

It is important that animal production professionals, veterinarians, ethologists carry the science and evidence-based knowledge about the different needs of the different species of and about the actual living conditions of food animals into the public discussions. Those that are responsible for guaranteeing a good animal welfare status of food animals and those that are responsible for controlling the verification of the compliance of animal owners and keepers are well advised to stick to the definition of animal welfare given by the Terrestrial Health Code (2014) of the World Animal Health Organisation (O.I.E):

“Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if it is healthy, comfortable, well-nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires
Adisease prevention and veterinary treatment, appropriate shelter, management and nutrition, humane handling and humane slaughter or killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.


The European Commission has been pro-active by having funded the FP6 Welfare Quality® Project (FOOD-CT-2004-506508), which highlights the “Five Freedoms” that describe the current European understanding of good animal welfare:

- Freedom from hunger and thirst, by ready access to fresh water and a diet to maintain full health and vigour.
- Freedom from discomfort, by providing an appropriate environment including shelter and a comfortable resting area.
- Freedom from pain, injury, and disease, by prevention or rapid diagnosis and treatment.
- Freedom to express normal behaviour, by providing sufficient space, proper facilities and company of the animal’s own kind.
- Freedom from fear and distress, by ensuring conditions and treatment that avoid mental suffering.

The European Commission has especially for pigs issued several legal acts: the most general is the Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs (Anonymous, 2008). The focus of this directive is on: improving the quality of the flooring surfaces; increasing the living space available for sows and gilts; introducing higher levels of training and competence on welfare issues for personnel; setting requirements for sufficient light and maximum noise levels; providing permanent access to fresh water and to materials for rooting and playing; and setting a minimum weaning age of four weeks.

However, despite these clear legal guidelines and demands, there are major concerns regarding pig production in Europe:

1) the Europe-wide (except of Sweden, Finland and Switzerland) still “permitted” routine tail docking, which is legally prohibited by European law: in most EU Member States the violation of this law is not prosecuted;
2) the fact that male piglets are widely castrated without anaesthesia and/or analgesia,
3) the fact that pigs in confinement do not have access to different climate zones
4) the fact that non-pregnant sows and sows in early pregnancy are kept in tight single crates as well as farrowing and suckling sows in cages to prevent the crushing of piglets by the sows; and
5) the highly various frequency of lesions in carcasses due to the vastly varying health status of pig herds.

Ad 1) Since in most EU Member States the violation of the legal prohibition of routine tail docking in most EU Member States is not prosecuted, the Commission Recommendation (EU) 2016/336 of 8 March 2016 on the application of Council Directive 2008/120/EC was issued, which is laying down minimum standards for the protection of pigs as regards measures to reduce the need for tail-docking. However, although there is a Commission Staff Working Document on best practices with a view to the prevention of routine tail-docking and the provision of enrichment materials to pigs (from March 8, 2016), in practice, abandoning tail docking in modern pig husbandry systems is not easy, since without heavy changes in the pen design and in the
animal care, and without providing rooting material, tail biting increases especially in the flat deck drastically. Therefore, e.g. in Germany, there are several pilot projects at regional level that support farmers and veterinarians in learning step by step what to do to be able to raise pigs with intact tails.

Ad 2) NGO's and growing proportions of the European consumers do not accept any longer that male piglets are surgically castrated without anaesthesia and analgesia, since this is regarded as cruelty in pig husbandry. Therefore, there is a European Declaration on alternatives to surgical castration of pigs, issued in 2012, which demands that surgical castration of pigs should be abandoned by 1 January, 2018. Right now, there are lots of debates in the EU Member States about which of the three accepted alternatives (1. local anaesthesia or a general narcisus and analgesia to redeem the post-operation pain, 2. finishing intact boars, 3. applying “Improvac” as immunocastration) is feasible. Animal welfare activists regard the immunocastration as the alternative that means the least stress for the animals, since non-vaccinated boars are often quite hyperactive and cause lots of injuries in their pen mates.

Ad 3) and 4) There are strong demands from NGO's to completely change the housing system for pigs regarding access to outdoor areas and to phase out the use of crates for pregnant sows and piglet protecting cages for farrowing sows. There is not yet any European law on these issues, but it indicates the next steps in the implementation of animal welfare demands in the European Union.

Ad 5) The European modern thinking about the intrinsic value of animals does not any longer allow animal owners to treat their animals “as they like”, but it expects farmers to treat the animals decently and with best management and animal care practices. Thus, animal-oriented animal welfare indicators such as slaughter check data and tools like the meat juice multi-serology (Meemken et al., 2014) will be implemented and used as animal welfare benchmarking systems, which allow for identifying pig herds with a poor animal health and welfare quality.

**Keywords:** Human-Animal-Relationship, EU Animal Welfare Legislation, Tail Docking, Pig Castration

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Biosecurity: Basis of Swine Health Control

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**Summary**

In terms of outbreak of disease, we need to understand that disease is related to number of pathogens, virulence of pathogen and immunity of herd. Focus of swine health control will be no entry of pathogens into the farm, reduction of number of pathogens such as disinfectant and to increase the level of immunity in the swine herd.

Biosecurity is the concept to control and prevent disease and defined as the rules and procedures implemented to prevent infectious agents from entering each stage or site or building of production. The goal of external biosecurity is to prevent the introduction of any pathogens from outside sources and the goal of internal biosecurity is to prevent the spread of pathogens within the farm.

Pathogens can be transmitted by direct porcine vectors such as pigs and semen. Indirect introduction of pathogen is carried out through people, livestock related vehicles, tools/supplies, feed and water, vectors such as wild animals, birds, rodents, flies/mosquitos, and air etc.

Biosecurity is a basic concept to rear pigs with healthy status against pathogens transmission.

**Introduction**

According to Terrestrial Animal Health Code of OIE, Biosecurity means a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestation to, from and within an animal population.

Swine viral and bacterial pathogens must be carried by something else not capable of locomotion. To understand the risk factors of outbreak of diseases, how pathogens enter swine farms and how to prevent pathogens from outside to farms are the keys on biosecurity.

**Risk factors and Transmission of Pathogens**

Strict control of the movement of pigs, personnel and vehicles is the key to successful biosecurity and prevention of infectious agent introduction or spread.

Pathogens are transmitted by direct porcine vector to pigs such as gilt, nursery pig and culling pig delivery. Infectious agents are also transmitted by semen as shown in Table 1.

Indirect transmission is carried out by human for instance employee, veterinarian, consultant, driver for livestock related vehicles, human for facilities repairing and others. Vehicle for transporting pigs is one of most important factors and vehicles for semen, manure; feed, animal health products and other vehicle to visit farms from outside are also risk factors to transmit pathogens.

Entry of wild animal, birds, rodents, insect, feed, supplies, water and air is also important risk factors to transmit infectious agents.
**Table 1** Pathogens capable of transmission by semen

<table>
<thead>
<tr>
<th>Viral Disease</th>
<th>Bacterial Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical swine fever virus</td>
<td>Burcelia spp.</td>
</tr>
<tr>
<td>Foot and mouth disease</td>
<td>Mycoplasma hyorhinis</td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>Leptospira pomona, bratislava.</td>
</tr>
<tr>
<td>Porcine circovirus</td>
<td>Chlamydia spp</td>
</tr>
<tr>
<td>Porcine enterovirus</td>
<td>Actinobacillus pleuropneumoniae</td>
</tr>
<tr>
<td>Porcine parvovirus</td>
<td>Streptococcus porcinus</td>
</tr>
<tr>
<td>Porcine reproductive and respiratory syndrome virus</td>
<td></td>
</tr>
<tr>
<td>Aujeszky's disease virus</td>
<td></td>
</tr>
<tr>
<td>Rubula virus</td>
<td></td>
</tr>
<tr>
<td>Swine vesicular disease virus</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Risk factors on Swine Farms

<table>
<thead>
<tr>
<th>Items</th>
<th>Details</th>
<th>Pathogens/Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine Movement</td>
<td>Introduction of gilt</td>
<td>Almost diseases</td>
</tr>
<tr>
<td></td>
<td>Marketing pigs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weaned and nursery pig</td>
<td></td>
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<tr>
<td></td>
<td>Culling pigs</td>
<td></td>
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<tr>
<td></td>
<td>Semen delivery</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>Live pigs</td>
<td>FMD, PED, TGE and others</td>
</tr>
<tr>
<td></td>
<td>Feed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fuel</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tools/supplies</td>
<td></td>
</tr>
<tr>
<td>People Movement</td>
<td>Entry of farm employees</td>
<td>PRRS, M. hyo, S. suis, Erysipelas</td>
</tr>
<tr>
<td></td>
<td>Repair inside/outside barn</td>
<td>Brucellosis, SIV</td>
</tr>
<tr>
<td></td>
<td>Entry of other visitors</td>
<td></td>
</tr>
<tr>
<td>Entry of Vectors</td>
<td>Wild animal</td>
<td>CSF, L. intracellularis</td>
</tr>
<tr>
<td></td>
<td>Birds</td>
<td>TGE, Erysipelas, PRRS</td>
</tr>
<tr>
<td></td>
<td>Rodents</td>
<td>B. hyodysenteriae, Salmonella, EMC</td>
</tr>
<tr>
<td></td>
<td>Flies</td>
<td>S. suis, B. hyodysenteriae</td>
</tr>
<tr>
<td>Entry of Airs</td>
<td>Wind</td>
<td>FMD, AD, PRRS, M. hyo, PED, App, Pm,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PRCV, H. parasuis, S. suis</td>
</tr>
</tbody>
</table>

Prevention of Introduction of pathogens from outside

Location and density of surrounding pig population are very important factors to protect pathogens from outside. The nearest farm should be at least 3km away and it is safer if nearest herd is small and closed herd producing weaners rather than an open herd such as finishing unit.

In order to prevent introduction of risk factors from outside, to divide farm area into 3 sectors as contaminated, buffer and clean area is needed and install fences all around farm area. Special action must be carried out when moving from contaminated area to clean area such as shower, change clothes and booth, foot bath and disinfection in personnel. People associated with livestock must stay at least overnight prior to entering the pig farm. Vehicle must go through the disinfection area after cleaning. All the materials such as equipment, supplies, electronics and stationary etc. for using inside of the farm must be carried in after clean and disinfection.

As mentioned in Table 1, some pathogens are transmitted by semen and require disease status of AI Station and introduce semen free of PRRSV and other important disease.

Loading and unloading shuts are some of the most likely modes of introducing disease into a pig farm. The key to prevention of disease introduction via this route is simply not to allow people, pigs, or materials present on a transport vehicle to enter the pig-rearing facility. A clear set of action steps must be taken immediately to assure through cleaning and sanitation at the point of entry of contaminant. To prepare conveyer belt at the end of the chute is useful tool not to contact pathogens between people and vehicle.
To prepare regular vector such as wild animal, birds, rodents, flies and mosquitos control program is important to not to introduction of pathogens shown as table 2. 
Diseases spread through the air by aerosol droplets and study on the speed and direction of wind. If you conduct loading and unloading work and introduction of bulk feed by vehicle, it is best time when the wind direction is from farm to outside. Air filtration of building is also alternative to prevent pathogens by air. Age segregation and all-in all-out can be nice tool to disconnect pathogens between the age.

**Keywords:** Biosecurity, Pathogens, Transmission

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The Basics to Achieve Antibiotic Free Farming in Modern Pig Production

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Summary
Farming without antibiotic medications is not difficult, but does take a steely determination by the whole health team to want to make it happen.

Before, embarking on an antibiotic free farm regime, a health team needs to be constructed. This needs to involve the owners, manager, stockpeople and the veterinarian. But it also needs the understanding of the genetic and nutrition suppliers. A degree of openness is required which is alien to many farms that are more used to a degree of silence about actual farm events. This takes trust and honesty. Each member of the farm health team has specific roles; the veterinarian plays the vital role of the “pig” speaking up for the animal’s biology and is the “honest broker” with robust checking systems.

Antibiotic free farming must not be at the expense of pig welfare and well-being.

Antibiotic free farming is about getting the management right:
- Management of pathogens
- Management of pig flow
- Management of immunity
- Management of the environment
- Management of any compromised pigs
- Management of people

Management of pathogens
It is hard enough to develop control programmes for the existing farm pathogens and opportunists as well as having to contend with new pathogens being introduced into the farm. The farm health team needs to develop active biosecurity protocols against all the natural routes of threat to the farm. Lack of discipline and carelessness are the major threats to a pig unit. This is largely associated with greed or a panic response to “improve”. If the farm has good health, do not throw it away on a pipe dream of a “better” greener pig on the other side of the fence.

Minimum requirements for an antibiotic free system
Location – The farm should be a minimum of 1 km away from adjacent pig farms, this includes the isolation area(s).

Dead animal disposal has to be away from the farm or utilise burial, incineration or, ideally, composting. Truck hygiene has to be well understood, especially trucks which take animals to the slaughterhouse. Vermin control has to be pro-active.

On-farm and off-farm concepts. It is imperative for all visitors and staff to wear on-farm clothing (especially outer clothing) and boots. The first thing a pig does when a “stranger” enters their pen is to lick and examine their boots and clothing in great detail and if these items come from another farm at this point pathogen
transfer occurs.

**Cleaning of batch areas**
Antibiotics are used to treat sick pigs or prevent pigs from getting sick and thus enhance their welfare. As this immediate fall-back position is not readily available, the antibiotic free farm needs to minimise contact with pathogens. Pathogen reduction is achieved by removing all faeces and allowing the room to dry.

**Reducing internal spread of pathogens – note flies and needles**
The concept of all-in/all-out is when a problem occurs during one batch; it does not spread to the next batch. However, while the farm designs and adopts a pig flow batch, the advantages can be lost by the lack of internal biosecurity.

**Partial depopulation and pathogen elimination**
Before, embarking on an antibiotic free regime, the elimination of certainly pathogens would make the enterprise more likely to succeed. The major pathogens which should be removed from a commercial farm would include: *Brachyspira hyodysenteriae* (Swine Dysentery), *Sarcopsec scabiei var suis* (Mange), *Mycoplasma hyopneumoniae* (Enzootic or Mycoplasma pneumonia), Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Aujeszky’s Disease. It is assumed that the farm is free of major OIE pathogens or active vaccine programmes are in place to control the pathogen.

**Management of pig flow**
It has been well proven that placing pigs in a clean environment which is dry and warm (appropriate temperature) will perform better than pigs placed in a dirty environment. Pigs stressed by a “clean damp cold” room will die or at least not perform.
Antibiotic free farming is about kg sold per batch, not achieving a “1000” sow unit. Take the “fun” out of pig farming. The farm must be routine, run as a business, batch on batch, year on year. Farms need to be “profit driven” not “production target driven”.

**Correct stocking densities assist health**
The purpose of instigating a pig flow model is to eliminate over and under-stocking of the farrowing house, nursery and finishing area. It is only when a room is empty of pigs can it be cleaned and as importantly maintained. It is impossible to fix broken drinkers and feed lines with pigs biting the back of the stockperson’s legs and getting under their feet.

**Records**
Records need to be organised around the batch of pigs. No producer batches monthly (28 to 31 days depending of the moon?) so the monthly recording system must be scraped. In addition, which day is day 1 of the batch? It is the day after weaning. It is essential to ensure that all gilts and returns which are mated on days 1 to 3 are included in the correct batch.

**Management of immunity**

**Innate Immunity**
The best immunity is using animals which have no receptor sites for the “pathogen. The selection of pigs and the use of DNA mapping will enhance our knowledge of pig’s natural resistance to pathogens. However, back to today’s reality:

**Healthy adult herd**

**New animal introduction**
A major risk to the herd’s health is the deliberate introduction of live animals into the herd. This risk can only be mitigated by time in isolation allowing any pathogen which the new animals are incubating, reveal themselves.
Of the major pathogens, which gilts and boars need to acquire on positive farms, the most important is Porcine Reproductive and Respiratory Syndrome virus. On positive farms, adopting an antibiotic free system, must ensure that gilts are positive to the native PRRSv variant and will not introduce “new” variants into the main herd. To allow for adequate time for the virus to infect the gilts and excretion stop and be available
for breeding at 220 days of age and 130 kg live-weight, the gilt needs to enter the isolation area at 60 kg or earlier.

**Parity profile**
A herd's natural immunity can be disturbed by the introduction of large numbers of gilts. This can be prevented by monitoring closely the parity distribution of a herd to a 3.2 parity average. The pig flow model will predict the number of gilts required allowing for careful planning of isolation. It is not unusual for “outbreaks” of Enzootic (Mycoplasma) pneumonia or Porcine Reproductive Disease Complex (PRDC) to be associated with a sudden increase in the proportion of finishers from gilt litters.

**Vaccine health**
To reduce the necessity of antibiotics, the health of the adult herd needs to be enhanced by the use of vaccines. Commercial vaccines to many pathogens are available. It is not necessary for every possible vaccine to be used to allow antibiotic free farming; however, a greater reliance on vaccines will be required.

**Administration process**
Irrespective of the vaccines chosen by the health team and their excellent storage, inappropriate administration, classically using too short a needle, depositing into fat tissue rather than vascularised muscle tissues, may negate the whole programme.
Note the pig’s actual age when vaccines are administered and consider any maternal interference in several vaccines. Erysipelat and Parvovirus vaccines are examples which cannot be administered at weaning. Do not unnecessarily raise maternal colostrum protection where possible; for example avoid PCV2 or Mycoplasma hyopneumoniae vaccines to pre-farrowing sows when piglet vaccination is going to be carried out as maternal immunity will affect the proficiency of the piglet vaccine process.
Ensure finishing Classical Swine Fever vaccination is not interfered with by maternal antibodies.

**Covering pathogens not available in vaccines**

**Feed-back programmes**
Gilts and boars, both purchased from outside or homebred need to be acustomised to the background pathogens, especially any reproductive pathogens (including pathogens resulting in congenital defects), before being bred. The easiest method of achieving this is to take faeces from nursery pigs (if the farm flow layout allows) and farrowing house materials – faeces from sows and piglets (especially any diarrhoea), stillborn and mummified materials. Bedding from farrowing paddocks or pasture raised pigs can be a good source of feed-back material.

The classical agents which are stabilised using these materials are Parvo virus and other SMEDI viruses (Enteroviruses and Circoviridae) and agents responsible for congenital tremor..

**The runt pig**
Piglets born less than 800g should be euthanased at birth before they have sucked any colostrum. Colostrum is limited gold and must be used wisely. Do not waste colostrum on piglets which have a 90% of dying. It is still possible to target 10.5+ weaned per litter with this policy. If piglet birthweights are low, review pre-farrowing feeding routines and management.

**Gilt litters**
Ideally all gilt litters should also receive some colostrum from an adjacent sow by spilt suckling. But note this has to be within 6 hours of birth. This can increase growth rates of the piglets by 60g a day and reduce the mortality by 50%. Sow colostrum can be obtained by milking the sow using a human breast pump, but this is very time consuming.

**Streaming – not all pigs are born equal**
The smaller 10% of pigs at weaning should be removed from the main group, given special attention and time to adapt to their circumstances. On many antibiotic free farms, these are actually removed from the system altogether and farmed along traditional lines with the use of conventional prophylactic treatment methods. This also allows for any fall-out pigs from the antibiotic free group/room/batch to be treated humanely.
Management of the environment
The environment can be broadly broken down into: Water, Food, Floor and the Air. Within each of these four factors, set parameters should be drawn up by the health team and ideally posted in a prominent position – for example on the entry door. The health team should be provided with suitable equipment to measure and adjust the environment of the pigs.

Management of compromised pigs
The compromised pig is a major health threat to the farm as well as creating enormous welfare problems. Farm staff must realise that euthanasia is a good welfare option and keeping crippled pigs alive is cruel.

Management of people
The success of an antibiotic free programme are the stockpeople. Training, encouragement and provision of confidence in the system is essential. Stockpeople need to realise that they cannot cheat the system. Antibiotics are easily revealed in the slaughterhouse and food processing laboratories. Destruction of the system is easy, just foster piglets, avoid all-in/all-out programmes, over- and under-breed, lie and mislead advisors and management staff. But in the end the pig will reveal all.

Keywords: Antibiotic Free; Health Management; Pig Management
Porcine Reproductive and Respiratory Syndrome
Reducing Contact Points by Batch Farrowing

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Summary
Reducing the transmission of Porcine Reproductive and Respiratory Syndrome virus (PRRSv) is a major battle in the control of one of the world’s major pig pathogens. The transmission of PRRSv can be considered in two scenarios: between farms and within farms. This paper considers the impact of multi-week batching options to achieve less PRRSv contact points (compared to a weekly farming option) and that multi-week batching options provide more opportunities to reduce the spread of the virus around an infected farm by allowed the virus to die on dry equipment and clothing.

Concept
Porcine Reproductive and Respiratory Syndrome virus (PRRSv) can be spread between farms in a number of different routes. The most significant being:

- Locality, although more than 1km the spread is generally limited. Locality spread is assisted by low wind speed, low temperature, high humidity and low sunlight.
- Introduction of positive animals – through gilts, boars or artificial insemination
- Introduction through trucking and vehicles – which are contaminated with infected material
- Introduction through vectors – such as infected clothing and faeces.

PRRVs can then be transmitted within the farm through contaminated equipment with oral fluids, faeces or blood from infected pigs via:

- Needles,
- Processing equipment and
- Biting insects.
- Clothing and boots

The source of the infection within a herd can be vertical transmission and comingling susceptible and infectious pigs.

PRRSv stability
The virus is remarkably unstable and is rapidly inactivated through dying. The virus generally survives less than a day in a day environment.

At 4°C the virus can remain stable for about 2 weeks (16 days).
However, the virus survives well below 0°C.

Batching on pig farms
Batching is a method where all-in/all-out is achieved on the farm by changing the work routine at weaning. Since the 1950’s weaning has generally occurred once a week. As farm sizes got bigger in the 1980’s
many farms, especially in North America moved to weaning multiple times a week so that breeding (through) natural boar methods occur happen with a reasonable number of animals being mated in one group/batch.

With the onset of artificial insemination larger numbers of females could be bred with relative ease. Two stockpeople can breed 50 to 70 sows an hour with boar control AI techniques. Whereas the same stockpeople would only manage to breed 12 sows an hour using natural mating techniques.

To attempt to break various pathogen infection cycles batching was reintroduced to European farms in the late 1990’s. (Batching was a well recognised pig flow concept before the 1950’s but on small family farms).

**Batching and its impact on PRRSv transmission risk inter-farm risk**

The batching concept can be applied specifically to various pathogens to determine the advantages to the system in pathogen control.

In this exercise we can examined batching and PRRSv contact points.

If we assume:

The risk of getting PRRSv on any one day is determined by one for four factors:

*Introduction of infected semen* from artificial insemination – one risk point. Thus with weekly batching systems there are 52 points where if infected AI entered a farm and the farm broke.

The *weaner movement* will occur as each batch moves from the farrowing area to the nursery.

The *slaughterhouse truck* could be an infected point. Batching does not really impact the timing of the slaughterhouse trucks. Irrespective of the batching system, pigs are sold on contract to the slaughterhouse every week. So all systems would have a risk 52 times a year (assuming pigs are sold once a week). *Breeding gilts* will move to the farm to complete the breeding target. Thus with weekly batching this is going to occur 52 times a year.

Locality is considered. But as each of the different populations would have the same number of animals and there is no real difference in the infectivity rate between a piglet or a sow become infected; batching is not considered to make any significant impact on pathogen transfer.

Using this simple model and these four factors, the use of any batching system – 3, 4 or 5 week models, reduces the inter-farm risk of transmission by about 50%.

**Batching and its impact on PRRSv transmission risk intra-farm risk**

Batching also would have an impact on the transmission around an infected farm.

If the virus dies out in 16 days above 4°C; any batching programme with an interval more than 16 days between batches would reduce the risk of transmission between one batch and the next.

<table>
<thead>
<tr>
<th>Batching time</th>
<th>0.5</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Week</th>
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<tbody>
<tr>
<td>Needles</td>
<td>-</td>
<td>-</td>
<td>++</td>
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<td>++</td>
<td></td>
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<tr>
<td>Processing equipment</td>
<td>-</td>
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<td>++</td>
<td>+++</td>
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<tr>
<td>Cleaning</td>
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<td>-</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Boots</td>
<td>-</td>
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<td>+</td>
<td>++</td>
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<td></td>
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<tr>
<td>Clothing</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
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</tr>
</tbody>
</table>
JC Personal assessment of risk of transmission: - = no impact. + = positive chance to reduce transmission.

Four and 5 week batching programmes are perceived to provide less risk in PRRSv transmission as distinct farrowing/weaning cohorts are produced. Thus movement of animals between batches is significantly less likely. With 3 week batching there are 2 cohorts and some leakage between batches is seen on most farms. With less than 3 week batching movement of animals occurs freely (despite advice to the opposite) on most commercial units.

As the PRRS virus can then be transmitted within the farm through contaminated equipment with oral fluids, faeces or blood from infected pigs, assuming these pieces of equipment are cleaned and allow to dry and are stored in a normal farm environment above 10°C; the virus will die within two weeks and thus the risk of transmission between multi-week batches are reduced.

**Keywords:** Batching, Health, Management, Pathogen, Control
Hypokalemia in Dairy Cows – Importance, Etiopathogenesis, Treatment

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Summary
Hypokalemia is a common finding in cows suffering from various disorders (abomasal displacement, ketosis) in the course of the transition period. The effect of oral supplementation of potassium was evaluated in cows admitted to the Clinic for Ruminants and Swine. Dairy cows that revealed either moderate (plasma potassium levels below 3.5 mmol/l) or severe (plasma potassium levels below 2.7 mmol/l) hypokalemia when admitted to the animal hospital were by chance assigned either to a treatment group (Group 1a: 19 cows) or to a control group (Group 1b: 17 cows), whereas all animals with severe hypokalemia (Group 2: 11 cows) were treated with potassium for ethical reasons. The animals had been referred to the clinic for various diseases including abomasal displacement to the left (LDA), ketosis, metritis and mastitis occurring as single entities or in combination. Cows enrolled in the treatment groups (1a and 2) were orally administered potassium in a bolus formulation by use of a balling gun while animals allotted to Group 1b served as controls. Blood samples were obtained for blood gas analysis and the determination of plasma levels of potassium, sodium, glucose, ionized calcium before and at 1, 2, 3, 12, 36, 60 and 84 hours following bolus or sham administration (Group 1b controls), respectively. ß-hydroxybutyrate, NEFA were determined at admission and at dismissal. A thorough clinical examination was performed each morning during the observational period of five days and at dismissal. Daily milk yield and feed intake were recorded. Diagnostic muscle biopsies were obtained to evaluate total body potassium status from a number of animals. Oral administration of potassium using a bolus formulation proved to be safe and easy. In cows with moderate hypokalemia (Group 1b) bolus administration resulted in reconstitution of mean and medium plasma potassium levels to normal (reference range 3.5 – 5.0 mmol/l) within one hour following administration, whereas the same was true for untreated controls (Group 1b) at 36 hrs after admission. In the group of severely hypokalemic cows (Group 2), where all animals received a treatment for ethical reasons, the mean potassium level passed the lower border of the reference range after 36hrs. Bolus administration in cows with moderate hypokalemia (Group 1b) did not result in significant differences compared to sham treated controls (Group 1b) with respect to plasma levels of NEFA, ß-hydroxybutyrate, glucose, sodium and calcium at day five following administration of the bolus. The variables rather reflected the stabilization of the metabolism within the recovery period for animals from all groups. Due to the importance of potassium for body functions and vital processes animals at risk such as anorectic high-yielding dairy cows should be treated orally with potassium as soon as possible in order to counteract unwanted side effects of hypokalemia such as muscle weakness or even recumbency.
Introduction

Hypokalemia (serum/plasma potassium concentrations <3.5 mmol/l) is frequently observed in high yielding dairy cows in early lactation. Although substantial amounts of potassium are drained via milk at the start of the lactation, healthy periparturient cows have been demonstrated to be in a slightly positive potassium balance of approximately +60g K (1). Excessive potassium is excreted via a highly efficient mechanism by the kidneys. Whenever deficient quality of the ration or diseases such as milk fever, ketosis, abomasal displacement (DA) or lameness lead to a reduction in feed intake or even turn the cow to an anorectic status, the renal mechanism is not able to compensate in time and the animal comes into a negative potassium balance and develops hypokalemia (2;3). Hypokalemic dairy cows show muscle weakness due to paralysis of striated and smooth muscles which can lead to recumbency and atony of the gastro-intestinal tract. Even cardiac functions are impaired by hypokalemia, resulting in atrial fibrillation in some cases. Moderate hypokalemia was observed in experiments following starvation in sheep (4), in metabolic alkalosis in cattle caused by abomasal outflow disorders (5) or as a side-effect following the use of mineral corticosteroids in the treatment of ketosis (2). In addition, alterations in the spectrum of fatty acids in milk were observed following oral administration of potassium as a consequence of changes in ruminal fermentation patterns (6). Potassium is thought to improve abomasal and uterine motility and to this end could contribute to a reduction in the incidence of DA and to the enhancement of the uterine mechanism of self-cleansing. In addition, several reports implicate a role of potassium in the immune defense.

Materials and Methods

Dairy cows that revealed either moderate (plasma potassium levels < 3.5 mmol/l) or severe (plasma potassium levels < 2.7 mmol/l) hypokalemia when admitted to the animal hospital were by chance assigned either to a treatment group (Group 1a: 19 cows) or to a control group (Group 1b: 17 cows), whereas all animals with severe hypokalemia (Group 2: 11 cows) were treated with potassium for ethical reasons. The animals had been referred to the clinic for various diseases including abomasal displacement to the left (LDA), ketosis, metritis and mastitis occurring as single entities or in combination. Cows enrolled in the treatment groups (1a and 2) were orally administered potassium in a bolus formulation by use of a balling gun while animals allotted to Group 1b served as controls. Blood samples were obtained for blood gas analysis and the determination of plasma levels of potassium, sodium, glucose, ionized calcium before and at 1, 2, 3, 12, 36, 60 and 84 hours following bolus or sham administration (Group 1b controls), respectively. ß-hydroxybutyrate, NEFA were determined at admission and at dismissal. A thorough clinical examination was performed each morning during the observational period of five days and at dismissal. Daily milk yield and feed intake were recorded.

Results

In the period from August 2012 to July 2013 in total 47 cows were enrolled in the study. Four animals were withdrawn as these met the exclusion criteria. 37 (78.7%) of the study animals were diagnosed with LDA at admission, 32 (68.1%) suffered from ketosis, two cows suffered from clinical mastitis (4.2% of all included animals) and 16 (34%) were diagnosed with metritis.

In four cows with LDA abomasal displacement was no longer present on day two. Two of these animals belonged to Group 2, one to Group 1a and one to the controls (Group 1b). Six cows out of 47 (12.8%) had to be euthanized, all but one after completion of the study. Four cows were excluded due to rescue treatment; data was nevertheless ascertained for analysis. Animals from all groups showed an increase in plasma potassium concentrations with time during the observational period. Mean and median plasma potassium levels in Group 1a (3.66 and 3.6 mmol/l) returned to levels within the reference range within one hour after bolus administration. The same was true for the control group (Group 1b) at 36 hrs. For Group 2 the median plasma potassium concentration was 3.6 mmol/l at 12 hrs (before administration of the second bolus) and mean potassium concentration was 3.93 mmol/l at 36 hrs following bolus administration. The overall change in plasma potassium concentrations was significantly different between treatments (p
< 0.01) and differed between treatments over time (p < 0.01). The time effect was significant (p < 0.01), i.e. the potassium level of the groups changed with time. Pairwise comparisons with Tukey-Kramer-Adjustment showed highly significant differences in the alterations in plasma potassium concentrations between Groups 1a and 1b (p < 0.01) and Group 1b versus Group 2 (p < 0.01).

Ruminal movements determined as the number of ruminal contractions audible within three minutes of auscultation increased in animals from all groups over the time but over the whole study period the increase did not differ between groups. Descriptive analysis shows, however, that animals belonging to group 2 had least ruminal contractions at 0 hrs (mean 1.18 contractions in 3 minutes) and a mean increase of 1.02 contractions per three minutes over the time span of 84 hours. However, Group 1a showed a mean increase of rumen frequency of 1.47 contractions per three minutes and group 1b showed an increase of 0.12 contractions within three minutes over the time span of 84 hours. There were no significant differences between treatment groups with respect to daily milk yield during the observation period.

Laboratory Analysis - Energy Metabolism

Non-esterified fatty acids (NEFA) concentrations decreased markedly but not significantly in all groups with time. Beta-hydroxybutyric acid (BHBA) levels decreased markedly with time following admission to the clinic, but were not significantly different for all groups. Plasma glucose levels decreased in animals from all groups following transport and hospitalization and reached the reference range (3.46-4.28 mmol/l) at 12 hrs. Alterations in plasma glucose levels did not show differences between groups. There were no significant changes observed for sodium concentrations neither with time, nor between different treatment groups. Mean concentrations of total calcium (mmol/l) and ionized calcium (Ca++; mmol/l) increased in all groups with time but there was no significant group or time effect. Mean pH, bicarbonate (HCO3- [mmol/l]) concentrations and Base Excess (BE [mmol/l]) did not show significant differences, neither with time, nor between different treatment groups. Analysis of hematocrit (HCT [%]), white blood cell counts (WBC [G/l]), red blood cell counts (RBC [T/l]) and platelet counts (PLT [G/l]) did not show significant differences between the groups. The amount of potassium contained in the boluses was sufficient to reconstitute normal potassium concentrations (reference range 3.5 mmol/l – 5.0 mmol/l) in cows with moderate hypokalemia (plasma potassium level < 3.5 mmol/l) that coincides with periparturient diseases such as LDA, ketosis, metritis and mastitis within a short period of time (one hour). In untreated controls the potassium pool was only replenished after return of the appetite following treatment of the underlying disease at approximately 36 hrs. To this end the bolus is indicated for high yielding cows that show a reduced appetite or even anorexia.

Conclusions

The oral potassium bolus formulation used in the present study has shown to be an effective and safe way to replenish potassium pools in hypokalemic dairy cows in a short period of time. Even repeated administration (up to three times on subsequent days) had no negative side effects on study animals. Cows with severe hypokalemia have a risk of succumbing as a consequence of the electrolyte alterations and for this reason might need a higher dosage and repeated treatments. However, these animals are only sporadically observed in the field and administration of potassium could rescue their lives. Feed intake, milk yield and ruminal movements did not significantly differ from untreated controls, however, the numbers of treated animals and controls might have been too low to make such differences evident. A tendency to lower glucose concentrations was observed in the control group. To this end the effect of the potassium bolus on milk yield in the recovery period of disease should be studied in a greater number of anorectic cows versus untreated controls. Since potassium plays a major role in all kinds of physiological processes in the body, the restitution of normal potassium concentration has to be considered of great importance for convalescence of hypokalemic individuals, especially those suffering from diseases that are known to influence energy metabolism and electrolyte concentrations. The animals used in the present study were sick dairy cows with severe diseases and disturbances in
energy metabolism, and many of them were undergoing abdominal surgery whereas the target animals in the farms (cows on the onset of lactation up to several weeks after calving) are likely to be in a better physical condition, not least because sick or weak animals can be detected earlier. The daily analysis of blood parameters is not possible for local practitioners and cow-side analysis of potassium concentration is yet to be developed. Therefore it is of great importance that possibly hypokalemic animals suffering from diseases that have been shown to be associated with hypokalemia can be treated quickly and safely without risk of overdosing healthy animals.

**Keywords:** Potassium, Dairy Cow, Abomasal Displacement, Oral Treatment

**References**


Pain Management for Abdominal Surgeries in Cattle

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All abdominal surgeries require the preemptive application of analgesics, such as non-steroidal anti-inflammatory drugs, prior to surgery. Their application continues for two to three days after surgery to control post-operative pain. Abdominal surgeries are most easily performed under general anesthesia with xylazine/ketamine or isoflurane inhalation anesthesia in lateral or dorsal recumbency, both in combination with local anesthesia of the surgical field (infiltration of nerve block anesthesia). For more practical reasons surgeries are performed mostly from the left or right flank in standing position in adult cattle. For desensitization of the flank L-blocks, paravertebral nerve blocks and infiltration of the incision line with local anesthetics are described. The combination of two techniques, e.g. distal paravertebral nerve blocks and infiltration of the incision line, appears more effective than the use of a single technique. Sedative and analgesic effects of low dose xylazine applications may complete the pain management protocol. To avoid surgical complications and thereby unnecessary pain and suffering or even death in the convalescence period sufficient knowledge and experience of the surgeon is indispensable and part of pain control in food animals.
Surgical Techniques for Treatment of Lameness in Dairy Cows

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Lameness in dairy cattle is mostly caused by claw horn lesions or lesions at the adjacent soft tissue. Defects at the sole or the wall are easily treated by therapeutic claw trimming according to Toussaint Raven. Loose horn is removed and a smooth transition to the healthy horn provided. Only the non-pain-sensitive horn in the area of the defect is supposed to be pared off, and the inflamed corium remains untouched. Afterward, the inflamed and sensitive corium is exposed and protected against mechanical irritation and pressure by a bandage and removing weight bearing from the afflicted claw through the application of a block to the opposing claw. However, although such lesions are frequent health disorder in dairy cows only few controlled studies on efficacy of such treatments are available. Therapeutic claw trimming is painful and requires pain control. In advanced stages, when inner structures of the horn shoe are involved, major surgeries such as claw amputation (AMP) or resection of the coffin joint (RCJ) are necessary. The advantage of RCJ compared to AMP is the conservation of the treated claw but post surgical convalescence, in particular lameness, is significantly longer. The productive life after RCJ is compared to AMP only little longer. Tip toe necrosis can be easily treated by resection of the toe and is almost always successful.
Techniques for Surgical Correction of Abomasal Displacement in Dairy Cows

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Today correction of abomasal displacement, which occurs mostly to the left side (LDA) and less frequently to the right side (RDA), is one of the most frequent surgical interventions in dairy cows. Open wound surgeries from the flank in the standing cow or from ventral in recumbent cows as well as transcutaneous, minimal invasive techniques are described. The approach from the right flank in the standing cow allow correction of LDA and RDA and is presumed as a universal technique with few complications. Widely used is also the transcutaneous fixation of the abomasum with toggle pins in LDA cases after rolling of the affected cow for correction of the abomasal dislocation according to Grymer and Sterner. Compared to the latter technique the advantage of the laparoscopic technique according to Janowitz is the fixation of the abomasum under visual control. However, also this technique bears slightly more risks of surgical complications than the right flank approach. A significant advantage of the minimal-invasive, transcutaneous techniques is a shorter convalescence period compared to the open wound surgeries. For successful surgeries, correction of electrolyte and alkalotic acid base disturbances and dehydration caused by abomasal reflux, in particular in RDA but also LDA cases, is necessary. Surgical complications and thereby unnecessary pain, suffering or even death in the convalescence period are avoided by sufficient knowledge and experience of the surgeon.
Insulin Resistance in Dairy Cows During the Transition Period

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Insulin is of pivotal importance to maintain adequate blood glucose levels because certain body cells (e.g. brain cells, blood cells) use glucose almost exclusively as energy substrate. In contrast to monogastrics ruminants, such as dairy cows, absorb little glucose from the intestinal tract. The majority of body glucose is produced during hepatic (and renal) gluconeogenesis with propionate from ruminal fermentation as one of the major precursors. The production of milk is depend on the production of lactose in the mammary gland. Thus, with onset of lactation mammary requirements for glucose as precursor for lactose production increase dramatically while at the same time dietary energy intake and subsequently propionate production from ruminal fermentation lacks behind. In consequence available body glucose is re-partitioned from use in muscular and adipose tissues towards the mammary gland. Therefore, after parturition blood insulin and adipose and muscular tissue sensitivity for insulin are reduced and thereby glucose uptake. Since glucose uptake by the mammary gland is almost insulin independent more glucose is available for milk production. Another effect of low insulin blood levels and reduced insulin sensitivity is enhanced lipomobilization and increased hepatic ketone body production. Both, fatty acids from adipose tissues and hepatic ketone bodies are used as alternative energy substrates instead of glucose by peripheral tissues. Low insulin sensitivity and low blood insulin level are risk factors for excessive fat mobilization and ketosis in dairy cows. The described mechanism is a physiological adaptation process in the transition period as long it is not driven to extremes. Various techniques test insulin sensitivity on body, organ or molecular level. Whole body insulin sensitivity can be tested by glucose-clamps, glucose tolerance tests or so called surrogate insulin sensitivity indices, which are calculated from blood concentrations of fatty acids, glucose and insulin. However, all techniques are laborious or not sufficiently validated for use in practice. High body condition is one of the major factors inducing low insulin sensitivity. In addition, inflammatory diseases reduce insulin sensitivity, likely to improve glucose availability as energy substrate to immune cells. Stress may reduce insulin sensitivity by activation of the hypothalamic-pituitary-adrenal axis with release of cortisol. A genetic disposition for low insulin sensitivity appears also to exist associated with the genetic merit for milk.
BM-07

Medical Treatment and Prevention of the Lipomobilization Syndrome in Dairy Cows

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Negative energy balance as consequence of high energy requirements and inadequate dietary energy intake is compensated in early lactation by mobilization of energy from body reserves, in particular fat from adipose tissues. Excessive lipomobilization and subsequently high availability of non-esterified fatty acids (NEFA) lead to subclinical or even clinical ketosis and fatty liver. Liver failure occurs only infrequently. However, even subclinical ketosis has economic and welfare impacts, since it is frequently associated reduced performance and increased health risks, such as metritis and mastitis or abomasal displacement. Glucose infusions, either as bolus or as continuous iv infusion, will induce the release of insulin and thereby reduction of fat mobilization. Full oxidation of NEFA improves and blood ketone body levels are reduced. Orally administered glucoplastic substances, such as propionate, appear on an iso-energetic level almost as effective as iv glucose infusions. Also low dose dexamethasone treatment showed to reduce blood ketone body levels and hepatic fat concentrations within a few days after treatment. Dexamethasone treatment in combination with glucose treatment appears to be more effective than dexamethasone treatment alone. However, the mechanism of action is not fully understood. Since high fat turnover is associated with oxidative stress adequate Vitamin E and selenium supply is important. An oral additive that proved clear effects in prevention of subclinical ketosis is monensin, approved as slow releasing capsule. The most important aspect in prevention of excessive lipomobilization is the avoidance of over-conditioning in dairy cows at parturition by adequate feeding strategies during late lactation and the dry period. Neuro-hormonal activation and activation of the HPA-axis by stressors with subsequent release of catecholamines and cortisol induce mobilization of fat. Thus, exposition to stressors should kept low by appropriate herd management. Prevention of milk fever will also reduce the risk of subsequent ketosis. Protocols for early detection and adequate treatment of any kind of disease, in particular inflammatory disease, should be in place, since they cause reduction in appetite and will enhance negative energy balance and stimulate insulin resistance and thereby fat mobilization.
Lesions at the horn shoe or the adjacent soft tissue are the major causes for lameness in cattle. However, also diseases of joints or synovial organs more proximal at the limb are often painful and induce lameness. Today, claw trimmer without anesthesia and analgesia often treat superficial claw lesions. However, although these manipulations are surgical interventions and painful they are trivialized and called "therapeutic claw trimming". This current practice should be reconsidered and adequate pain management for such cases standard procedure. Generally adequate pain management includes before surgery the application of analgesics, such as non-steroidal anti-inflammatory drugs, and local anesthesia for desensitization of the surgical area. Various techniques are available for local anesthesia leading to complete desensitization. For desensitization of the limbs intravenous regional anesthesia (IVRA) or nerve block anesthesia are widely used. However, onset of anesthesia occurs significantly faster after nerve blocks than after IVRA, which appears relevant when surgeries are performed in practice under time constraints. High caudal epidural anesthesia with xylazine also desensitize the pelvic limbs. The sedative and analgesic effects of systemic xylazine applications complete the pain management protocol. In addition, general anesthesia with xylazine/ketamine or isoflurane inhalation anesthesia in combination with analgesics allow surgeries at the locomotor system but for practical reasons they are only rarely used. For control of post-surgical pain the application of analgesics is continued for three days or as long as necessary.
Claw Health Atlas - International Standardization of Diagnoses in Lame Cows (ICAR Initiative)

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Summary
Lameness belongs to the most important health hazards of dairy cows. In most cases lameness is caused by disorders of the hoof and the skin adjacent to the coronary band. Insufficient housing conditions and deficient management practices are the main reasons for world-wide lameness prevalence ranging between 20% and > 50%. Furthermore, malformations of the distal limb in cattle as well as the disposition for laminitis and certain infectious diseases of the distal limb are more frequently observed in distinct cow families. To this end, breeders associations have started programs to identify and eradicate inherited disorders of the locomotor system in cattle. The latter programs, however, have to rely on a solid data base generated in the field by professional claw trimmers, veterinarians and farmers on the occurrence of digital diseases in individual animals. Besides hand-written protocols software applications became available that were developed by claw-trimming professionals together with software engineers. The International Committee for Animal Recording (ICAR) serves the improvement of animal identification, performance recording and evaluation in farm animal production. ICAR invited veterinary experts in the field of bovine orthopedics with the aim to harmonize the terminology and definitions of the most common claw disorders in cattle. As a result of these activities an illustrated claw health atlas is available, which includes concise descriptions of the most important disorders of the lower limb in cattle (ICAR Claw Health Atlas). In the meantime the atlas has been translated into 17 different languages with additional translations being in preparation. On basis of the ICAR Claw Health Atlas experts from Germany established a more detailed list of claw disorders. This list is already implemented in a software application (“Klaue”, dsp-Agrosoft, Paretz, Germany) for recording of claw disorders at claw trimming, that allows for following the disease history of individual animals, delivering herd data for farmers and their advisors including the breeding association as well as evaluating treatments.

Introduction
Lameness belongs to the most important debilitating disorders of dairy cows and for this reason form a welfare issue (1). Most cases of lameness are related to claw disorders, which are either traumatic or infectious by origin (2). Records of claw health data are needed for the establishment of efficient strategies to improve claw health on dairy farms. In recent years, the use of software applications by professional claw trimmers, veterinarians and farmers has become more and more popular (3). The latter data sets are also of importance for breeding associations that established programs for the improvement of animal health by consideration of the frequency of occurrence of distinct disorders in the offspring of distinct breeding bulls. The success of such programs relies on the validity of the datasets generated by claw trimmers, veterinarians and farmers. A valid correct diagnosis as well as a high agreement between different professionals with respect to the findings at claw trimming forms a prerequisite for a successful strategy to combat lameness on dairy farms. In this context international harmonization of recordings of data related to claw disorders is of crucial importance.
The International Committee for Animal Recording (ICAR; http://www.icar.org/) serves the improvement of animal identification, performance recording and evaluation in farm animal production. ICAR encourages the use of performance recording data for the purpose of assessing the value on the profitability of animal production.

Material and Methods

ICAR has taken the initiative and invited veterinary experts in the field of bovine orthopedics from all over the world in order to reach an international agreement with respect to the terminology and description of relevant claw disorders in cattle. These activities have resulted in the publication of an illustrated claw health atlas, which includes concise descriptions of the most important disorders of the lower limb in cattle (ICAR Claw Health Atlas) (4). Experts from Germany used the definitions of the different disorders summarized in the ICAR Claw Health Atlas as a basis for the establishment of a more detailed list of diagnoses. The latter diagnostic key does not only allow for assignment of a specific diagnosis to a distinct condition observed at claw trimming, but also includes a grading system for the severity of disease.

Results

The ICAR Claw Health Atlas is available online http://www.icar.org/documents/icar_claw_health_atlas.pdf. Originally published in English, the atlas has been translated into 17 different languages with additional translations being in preparation. As ICAR encourages spreading of the ICAR Claw Health Atlas all over the world, institutions from countries that are interested in making the atlas available in their native language should contact ICAR.

The diagnostic key for claw disorders as established on basis of the ICAR Claw Health Atlas has been included in the software application “Klaue” (dsp-Agosoft, Paretz, Germany). By use of the latter application the claw trimmer is able to call up and follow the “history” of the individual animal. He/she can prepare his/her invoice and distribute information on the prevalence and severity of the different claw disorders to the farmer and his advisors. In addition he/she is able to evaluate treatments of different claw disorders.

Conclusions

The ICAR Claw Health Atlas is an illustrated diagnostic guide for claw trimmers, veterinarians and farmers that includes concise descriptions of common claw disorders observed in cattle. It serves the worldwide harmonization of recordings of claw disorders at claw trimming. Valid data sets generated at claw trimming are of value for breeding associations that established programs for the improvement of animal health by consideration of the occurrence of distinct claw disorders in the offspring of certain breeding bulls.

Keywords: Lameness, Claw Health, Diagnosis, Electronic Recording, Herd Health Management

References:

Digital Dermatitis - Etiopathogenesis, Diagnosis, Treatment, Prevention

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Summary
Digital dermatitis (DD) is described as an inflammation of the interdigital skin located next to the coronary band of the bovine hoof which is characterized by five clinical stages with different outer appearance (M1 (early, subclinical), M2 (acute), M3 (healing), M4 (chronic, hyperkeratotic or proliferative), M4.1 (chronically recurring). The M-2 stage is a painful acute ulcer with a strawberry-like surface that is painful even on slightest touch. DD causes substantial economical losses mainly in dairy cows all over the world. The gold standard for treatment of M2-lesions is topical application of a tetracycline containing formulation, preferentially as a spray. Until now the application of a bandage following antibiotic treatment has not been evaluated systematically. To this end, the question of the first part of the present study was whether a bandage would improve the healing process following topical treatment with chlortetracycline. All hoofs were cleaned and trimmed by a professional hoof trimmer or a veterinarian. The M2 lesions of cows in the first arm of the study (N=85) were treated with topical application of CTC by a spray (CTC-Blauspray, WdT, Garbsen, Germany). Cows were then randomly assigned either to a group receiving no bandage (N=41, 48%, Group 1) or a group receiving a bandage following treatment (N=44, 52%, Group 2). In the second part of the study a non-antibiotic gel, containing activated copper and zinc chelate (Intra Hoof-fit Gel IHF, Intracare, Veghel, the Netherlands) was applied to the M2 lesions of cows (N=78). Cows were then randomly assigned either to a non-bandaged (N=40, 51%, Group 3) or bandaged group (N=38, 49%, Group 4). In all four groups lesions were evaluated on a weekly basis by the outer appearance (M-stage) and extension. 38 (=86%) cows in group 2 that had received a bandage following topical application of CTC versus 18 (=44%) on group 1 left the study as “healed”. A survival analysis demonstrated a significantly higher (p<0.001) cure rate in cows that had received a bandage. In addition lesions in cows from the latter group less likely showed transition into the chronic M4 stage. Similar results were observed for the cows in the group receiving a bandage when the copper-zinc-chelate complex had been used as a therapeutic. In conclusion, results suggest that bandaging accelerates the healing process in acute (M2) DD-lesions and more frequently results in the M0 stage when compared to cows receiving a topical treatment alone and prevents the transition of an M2 stage to a M4 stage.

Introduction
Digital dermatitis (DD) is an inflammation preferentially of the interdigital skin that was first reported in the seventies by Mortellaro. DD affects mainly dairy cattle all over the world and leads to substantial economic losses due to reduction in milk yield, and secondary diseases caused by disruption of skin and claw horn integrity (1). DD primarily affects the skin near the coronary band of the bulb region next to the interdigital space, but can also be found on the surface of tylomas, sole ulcers and white line abscesses. Such lesions have been termed “non-healing lesions” due to unfavorable prognosis following intensive treatment. Although DD is multifactorial by its origin, bacteria belonging to the genus Treponema are a constant finding in DD
lesions (2). Various risk factors have been reported including deficits in hygienic conditions on the farms, keeping cows in-house the whole year around, unsuitable hoof baths, high stocking density, genetics, and young age. (3). DD is characterized by a great variety in its clinical appearance. The acute disease, however, likely turns into chronicity with flare-ups of the acute stage in intervals. Döpfer et al. (2) described different stages of the diseases characterized by distinct lesions of the skin. The M-0 stage designates the absence of DD as reflected by healthy skin. The M-1 stadium is formed by a small ulceration (<2 cm) of the interdigital skin which most often becomes visible when a forceps is used to widen the interdigital cleft. The M-2 stage is the acute form of DD, which on most farms is the only disease entity that is noticed by the farmer. The M-2 stage is characterized by a painful skin lesion with a diameter of more than 2 cm in diameter. The surface of such lesions has a strawberry-like appearance from which a distinct odor evaporates (4). Following (topical) treatment with an antibiotic (tetracycline) or a non-antibiotic (salicylate, zinc-copper-chelate) containing spray or ointment the lesions proceed to the M3 stage, which is characterized by a scab on the surface of the lesion. This lesion is painless and can proceed into the M-0 stage, but most often develops into the chronic M-4 stage, which is either characterized by hyperkeratosis (M4H) or a proliferative stage (M4P). The chronic stages are not painful and – in most cases do not cause lameness, although DD interferes with the process of horn production in acute and chronic stages. Treponema in a cyst stadium are found in the deeper layers of the skin in patients displaying the chronic forms of DD. Treponema evade the recognition by the host’s immune system by encystation. Although the disease can remain for months in the M4 stage, the M2 stage can evolve from a small ulceration that is present within the hyperkeratotic skin of the M4 stage. This stage is termed M4.1. Once DD has turned into the M-4 stage complete resolution of the process (M-0) is less likely than chronicity. Due to the constant irritation of the skin next to the bulb region or at other sites enhanced horn growth has been observed. The newly-formed horn, however, is of inferior quality and can on its own cause secondary disorders of the claw horn such as heel horn erosion and bulb ulceration. Early treatment of youngstock for DD has been shown to contribute to a complete recovery. The golden standard of DD-treatment still is the topical application of an antibiotic spray like oxytetracycline (OTC), which is most effective in combatting the M-2 stage. Most lesions, however have been demonstrated to turn into the chronic stage after topical treatment with tetracyclines. In addition, hoof baths are used for combatting DD on the farms. The use of bandaging in treating DD has been discussed controversial. The present study was performed to answer the question if bandaging results in the same or even better cure rates as simply applying tetracycline spray.

Materials and Methods
Dairy cows of the breed Holstein Friesian in the first or higher lactation, kept in a stable fitted with cubicles and concrete floors, were included in the study when the ulcerative stage of DD (M2) was diagnosed. All hoofs were cleaned and trimmed by a professional hoof trimmer or a veterinarian. The M2 lesions of cows in the first arm of the study (N=85) were treated with topical application of CTC by a spray (CTC-Blauspray, WdT, Garbsen, Germany). Cows were then randomly assigned either to a group receiving no bandage (N=41, 48%, Group1) or a group receiving a bandage following treatment (N=44, 52%, Group 2). In the second part of the study a non-antibiotic gel, containing activated copper and zinc chelate (Intra Hoof-fit Gel IHF, Intracare, Veghel, Netherlands) was applied to the M2 lesions of cows (N=78). Cows were then randomly assigned either to a non-bandaged (N=40, 51%, Group 3) or bandaged group (N=38, 49%, Group 4). In all four groups lesions were evaluated on a weekly basis by the outer appearance (M-stage) and extension as determined by application of a software program (Jalomd, Lübeck, Germany). Lesions were deemed as “healed” by a healthy skin (M0).

Results
38 (=86%) cows in group 2 that had received a bandage following topical application of CTC versus 18 (=44%) on group 1 left the study as "healed". A survival analysis demonstrated a significantly higher (p<0.001) cure rate in cows that had received a bandage. In addition, lesions in cows from the latter group less
likely showed transition into the chronic M4 stage. Similar results were observed for the cows in the group receiving a bandage when the copper-zinc-chelate complex had been used as a therapeutic. In conclusion, results suggest that bandaging accelerates the healing process in acute (M2) DD-lesions and more frequently results in the M0 (healed) stage when compared to cows receiving a topical treatment alone.

**keywords:** Lameness, Digital Dermatitis, Bandage, Dairy Cow, M Stages

**References**

**Bovine Respiratory Disease - Etiopathogenesis, Diagnosis, Treatment, Prevention**

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**Summary**

The objective of the present study was to evaluate the importance of selected animal-related and management related parameters on the clinical outcome of „Bovine Respiratory Disease“ (BRD) in calves. Pre weaned dairy calves kept on a farm specialized in rearing of dairy calves were observed during one season lasting from October to March for the occurrence of BRD. Calves that demonstrated evidence of BRD in the period following arrival on the farm were included in the study and underwent a thorough clinical examination. Whenever the calves fit the inclusion criteria an extensive sampling protocol was followed which included trans-tracheal washings (TTL) for bacteriological examination and blood sampling for serological examination of paired sera. Data from in total 104 calves demonstrated first symptoms of BRD on average at 10.1 ± 5.3 (SD) days following arrival at the farm at an age of on average 30 ± 7.5 (SD). Bacterial cultivation resulted in detection of *Pasteurella multocida* in 33 (31.7%) animals, *M. haemolytica* in 19 (18.3%), *H. somni* on one calf and *M. bovis* in none of the calves. Calves with *M. haemolytica* in TTL fluid demonstrated more severe disease symptoms (P≤0.05) and had a significantly (P≤0.05) lower body mass at inclusion than calves with *P. multocida* in TTL. 54 of 96 (56.3%) calves experienced a relapse of BRD and needed a second antibiotic treatment.

**Introduction**

The term „Bovine Respiratory Disease “(BRD) comprises disorders of the respiratory tract of cattle that evolve from a complex interaction of environmental and host-related factors (1). In calves the disorder occurs in two different manifestations: while the seasonal form is observed in late autumn, winter and spring, the non-seasonal form resembles crowding disease and affects calves preferentially within the first two weeks following the exposure to various stress factors such as transport, disbudding, commingling and weaning (2). Although the risk factors contributing to the disease are well-known, economical losses due to the disease are still increasing (3). BRD in calves has a multifactorial pathogenesis including host-related and management related factors as well as the Bovine Respiratory Syncytialvirus (BRSV), Bovine Viral Diarrhea Virus (BVDV), Bovine Herpesvirus Type 1 (Bo-HV1), Parainfluenza-3 Virus (PI-3), *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Trueperella pyogenes* and *Mycoplasma bovis*. The objective of the present study was to examine factors that might contribute to the occurrence and outcome of BRD in calves (4).

**Materials and Methods**

The present study was conducted during the winter months on a commercial farm that reared calves on behalf of dairy farms of the region. Calves that arrived at the farm at an age of two weeks were observed daily for evidence of respiratory disease until the day of weaning at 12 weeks. Whenever clinical evidence for respiratory disease was observed, a clinical examination was performed. Inclusion criteria were: body temperature ≥ 40 °C, abnormal breathing (respiratory score ≥1), abnormal demeanor (Habitus Score ≥1).
Transtracheal lavages (TTL) were performed for microbiological examination of the lavage fluid on respiratory pathogens including Mycoplasma. Paired serum samples were obtained for serological examination on antibodies directed at BHV-1, BRSV, BPIV-3, BVDV and M. bovis. The calves were treated with a single injection of a macrolide antibiotic and were observed for 22 days. In case of persistent respiratory disease or a relapse a second TTL was obtained. The number of treatments for respiratory disease was registered up to a period of three months following arrival.

**Results**

104 female dairy calves of the German Holstein breed fulfilled the inclusion criteria. 10 calves had to be excluded later due to additional diseases. The calves were aged at 20 ± 5.7 (SD) days at inclusion. 50% of the animals displayed clinical symptoms of BRD within the second week (10.1 ± 5.3 days) following arrival. From 65 out of 104 (65%) TTL samples at least one bacterial species was cultivated. In 6.7% (7/104) of the TTL-samples more than one species was isolated. From six animals of the latter group P. multocida and M. haemolytica were cultivated. Cultivation was negative in 30.8% (32/104) of the animals. P. multocida was detected in 31.7% (33/104) of the samples, M. haemolytica in 18.3% (19/104). From 25% (26/104) of the samples environmental bacteria species were cultivated (Figure 1).

![Figure 1. Enzootic bovine bronchopneumonia. Results of bacteriological examination.](image)

A second TTL was performed in 22 calves displaying persistent BRD or a relapse of BRD following recovery from clinical symptoms. In 54.5% (12/22) of the latter cases bacteria were cultivated from TTL-fluid. P. multocida was detected in 31.8% (7/22) of these samples, while M. haemolytica was isolated from only one sample. One calf that died due to bronchopneumonia was necropsied and P. multocida and M. haemolytica were cultivated from lung tissues. In all but one calf that showed a higher titer versus BRSV at the second sampling, serological examination of paired serum samples did not show seroconversion to BHV-1, BPIV-3, BRSV and BVDV.

When M. haemolytica was cultured from TTL clinical symptoms of BRD were more severe (P≤0.05) compared to P. multocida in TTL (P≤0.05). Calves with P. multocida cultured from TTL as well as calves with a negative cultivation result at inclusion demonstrated rapid improvement (within six hours) of clinical symptoms following antibiotic treatment (P≤0.01) compared to calves with M. haemolytica cultivated from TTL. Calves with persistent or recurrent BRD demonstrated more severe clinical symptoms at inclusion and had a lower body weight compared to calves that recovered completely (P≤0.05). At 30 hours following treatment clinical scores of calves that recovered were significantly lower when compared with calves that recurrently suffered
from BRD. Data obtained from 96 animals were evaluated over a period of three months following arrival at the farm despite antibiotic treatments. Within three months following arrival 56.3% (54/96) animals suffered from recurrent BRD. In 14.6% (14/96) three or more treatments were required. Calves receiving a single treatment were significantly older at the time they developed first signs of BRD than those that suffered from recurrent disease. Calves with recurrent bouts of disease had a significantly lower bodyweight already at arrival at the farm.

Conclusions
The present study demonstrates the prominent role of *P. multocida* and *M. haemolytica* in the pathogenesis of calf bronchopneumonia. *M. haemolytica* causes more damage and more severe symptoms than *P. multocida*. Examination of TTL-samples revealed no bacterial pathogens in one third of the animals, which could be due to the sampling procedure or due to a viral etiology. Viral infections – however - were not demonstrated in the present study. To this end bronchopneumonia more likely relied on an imbalance of the microbiome and damage of the upper and lower respiratory tract due to stress or insufficient housing and management. Treatment of affected calves should start as early as possible and rely on TTL-sampling, bacteriological cultivation and resistance testing. Therapy should be reevaluated at the fourth day of treatment.

References
Calf Rearing – Effect of Calf Management on Health and Disease

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Summary
Calf health and animal welfare have an impact on lifetime productivity and longevity of the dairy cow. In order to study the impact of management and housing conditions on calf mortality and growth rates a case control study was performed including 50 dairy farms located in the eastern and northern part of Germany. Following an interview with the farmer, housing conditions and management practice were evaluated and the epidemiological associations between these factors with calf mortality rates and weight gain were estimated by using two logistic regression models including 14 and 23 preselected factors each. An important factor associated with increased calf mortality (>5%) was a high percentage of neonates displaying failure of passive transfer (FPT) on the farm. Metaphylactic use of halofuginone against cryptosporidiosis was associated with increased mortality rates, probably due to the farmer’s assumption that metaphylaxis would serve as a substitution for a proper cleaning and disinfection protocol. Daily weight gains from birth to weaning ranged between 414g and 1027g. The risk factors significantly associated with poor growth were frequent commingling of calves in the period from birth till weaning, offering hay before weaning, obtaining first colostrum later than two hours following parturition, changing the bedding in the calf pen at least once every two weeks, feeding less than three liters of first colostrum to newborn calves and leaving the farm more than two hours per day unattended. The study demonstrated great potential for advisory services regarding improvement of calf health that can be delivered by the practicing veterinarian.

Introduction
Calf health and animal welfare have an impact on lifetime productivity and longevity of the dairy cow. Consumers are more and more interested in the well-being of food-producing animals. To this end, measures to improve management practices and the conditions under which the animals are kept get into the focus of todays’ herd-managers. The objectives of this study were to evaluate management practices and housing conditions on 50 dairy farms in northeastern Germany that could compromise calf health and welfare (1). Risk factors should be discovered that are related to increased calf mortality and poor growth in young dairy replacement heifers in order to support farmers to improve the health status of the offspring.

Material and Methods
A case-control study was performed in 50 dairy herds, which were selected based on acceptance to participate in the study (1). Each herd was visited once between 2012 and 2014. A questionnaire on farm management practices, morbidity and calf mortality was completed by an interview at the farm visit. Colostral, blood and fecal samples were collected at the farm visit and evaluated for colostrum quality, failure of passive transfer (FPT). Heart girth measurements were conducted to estimate average daily weight gain. In addition, housing and management conditions were evaluated by applying the Animal Suitability Index (TGI) as described by Sundrum et al. (2). A total score concerning animal suitability was determined. The epidemiological
associations between these factors with calf mortality rates and weight gain were estimated by using two logistic regression models including 14 and 23 preselected factors each.

Results
Calf mortality on dairy farms included ranged between 0.0 and 17.7%. The factors significantly associated with calf mortality exceeding a level of 5.0% were a high number of calves displaying failure of passive transfer of colostral antibodies (OR: 8.1). In addition, the metaphylactic administration of halofuginone on a regular basis was related to increased calf mortality (OR: 10.0). In contrast, the access to hay in the first week of life compared to no hay before weaning was demonstrated to be a protective factor related to lower calf mortality rates (OR: 0.2).

On herd-level the median average daily weight gain in calves three month of age was 675 grams per day and ranged between 414 and 1027 g. The risk factors significantly associated with poor growth were frequent (more than twice) commingling of calves in the period from birth till weaning (-119 g), offering hay before weaning (-142 g), obtaining first colostrum later than two hours following parturition (-142 g), changing the bedding in the calf pen at least once every two weeks (-96 g), relocating calves more than twice from birth till weaning (-93 g), feeding less than three liters of first colostrum to newborn calves (-88 g) and leaving the farm more than two hours per day unattended (-84 g). These results indicate that management practices including the early administration of sufficient amounts high quality colostrum, adequate nutrition and suitable husbandry conditions have a major impact on calf health and well-being.

Conclusions
The present study demonstrates various risk factors as well as protective factors for increased c.q. decreased mortality and weight gain of calves on dairy farms. The results of the present study could support the advisory practice of veterinarians in order to reduce calf mortality rates and increase weight gain and development in dairy heifer calves. Further research on a greater number of farms, however, is required to understand the contribution of distinct risk factors to the outbreak of multifactorial diseases such as neonatal diarrhea and calf respiratory disease as well as navel ill.

Keywords: Calf, Mortality Rate, Growth Rates, Risk Factors, Management

References
Common Fractures in Thoroughbred Racehorses: 
Prevention, Diagnosis and Treatment 

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Summary

The majority of fractures that occur in Thoroughbred racehorses training and racing on the flat are manifestations of repetitive stress injury. Bones become weakened following accumulation of fatigue damage that arises as a consequence of large numbers of high magnitude, cyclical loads applied repetitively over months and years. The damage and, as importantly, the biological response to repair it, create focal defects in the bone that act as stress-risers that predispose to catastrophic fracture. Bones of the distal limb are at greatest risk, particularly those in the fetlock region. In addition, specific anatomical locations are consistently affected, resulting in fractures that show remarkable uniformity in their morphology. Common injuries that share this pathogenesis include sagittal fractures of the first phalanx (PI) and various extensions of this; parasagittal fractures of the distal condyles of the third metacarpal (McIII) and third metatarsal bones (MtIII); transverse fractures of the proximal sesamoid bones (PSBs); transverse fractures of the distal metaphyseal region of McIII; oblique, intra-cortical fractures of the dorsolateral cortex of McIII; slab and osteochondral chip fractures of the carpal bones; stress fractures of the humerus, scapula, tibia and pelvis.

Different training programmes, that reduce the risk of bone sustaining fatigue damage and accommodate the biological repair process of bone (intra-cortical remodelling), offer the ultimate solution to preventing these fractures. However, the current model of racing prohibits application of suitable practices, which inevitably require time, and so the emphasis is on early detection of fatigue damage in bone to allow intervention before it progresses to a catastrophic fracture.

While diagnosis of complete fractures is generally easy, the detection of incomplete (stress) fractures and focal damage that often precedes these is less straightforward. Clinical signs are variable, non-specific and may be mild to non-existent in nature. Lameness is the most common finding and when accompanied by localising signs, such as focal pain on deep palpation, is an indication for focal radiographic and/or ultrasonographic imaging. As the lesions are measured in millimetres the changes in radiopacity are minor and so hard to detect. Accurate use of specific radiographic projections that highlight focal areas of bones that are commonly affected are essential in order to maximize the chances of identifying early lesions. Equally, good understanding of the fine anatomy associated with these sites and ultrasonographic appearance of normal and abnormal features is a prerequisite for best practice. Nuclear scintigraphy is sensitive for detecting lesions, even in the absence of localising signs.

Horses frequently show no detectable signs before suffering a catastrophic fracture. Some form of screening is required to identify this subset of the racing population although the tools required to achieve this have not yet been developed.

Bone will heal under suitable conditions and damaged bone will be replaced with healthy tissue. This process is completed within three months except when there is a significant mass of dead bone that must be resorbed first. Surgical fixation is usually required for complete fractures and may be of benefit in the management of some incomplete cracks.
Over 80% of catastrophic injuries at Thoroughbred racetracks are the result of fractures of the appendicular skeleton. The prevalence of such incidents varies greatly between racing jurisdictions and race types but ranges between 6.6 cases per 1,000 starters in jump races to 0.6 per 1,000 starters in flat racing. The loss of these horses is a significant ongoing issue for the industry, both financially, and in terms of human and equine welfare.

Pathogenesis of racing fractures
While some fractures result from accidents, most happen during galloping, without any abnormal event. Numerous studies over the past 25 years have established that these injuries are the consequence of material fatigue of the skeleton. Repetitive, high magnitude loads, associated with high-speed training and racing, lead to focal degenerative changes in affected bones, which predispose them to catastrophic failure during routine activity. Ironically, the physiological mechanism responsible for bone healing, intracortical remodelling, is implicated in the pathogenesis of a proportion of these fractures. Sometimes early fatigue damage, and the associated bone remodelling result in small, incomplete fractures (“stress fractures”), which are associated with clinical signs. If thoroughly investigated such cases can be identified before the injury extends to catastrophe. The horse can then be managed appropriately, giving the bone the opportunity to heal, and the prognosis is usually excellent. Unfortunately, a significant proportion of horses that suffer bone fatigue injury show no prodromal signs, and the first indication of a problem is peracute breakdown during a race or training gallop.

Preventing fractures
The fact that the majority of catastrophic fractures are the consequence of fatigue is a source of hope. Through an understanding of the processes involved in fatigue damage of bones in the racehorse, it is possible to implement strategies to reduce the accumulation of fatigue damage and to facilitate its repair. In addition, the progressive nature of fatigue damage means that, if we can identify the injuries early enough, we can intervene while the damage is still reversible. It is potentially feasible to significantly reduce the incidence of catastrophic fractures by intervening to stop horses that are at high risk of injury from being exposed to ongoing fatigue damage. Achieving these objectives will require the following strategies:

1. Reduce the problem of bone fatigue
   - Refine training schedules to limit the extent of accumulation of fatigue damage of relevant bones. Adopt training regimes that reduce the rate and duration of accumulation of fatigue, and periodically provide opportunities for the repair of fatigue damage.

2. Identify horses at risk of fatigue injury.
   - Develop screening tools with sufficient sensitivity and specificity to be able to identify individual horses within a population that are at high risk of suffering a fatigue-related injury.

3. Detect incipient fatigue fractures at an early stage.
   Ensure:
   - That all “at risk” cases are monitored closely,
   - That clinical signs are given due significance and are investigated appropriately,
   - That clinicians who accept the responsibility of undertaking the investigations are fully aware of subtle signs of fatigue damage (from clinical examination and diagnostic imaging) and are able to interpret their significance as accurately as possible and
   - That more care is taken not to suppress clinical signs through injudicious use of analgesic medications, thereby disguising potential warning signals.

Common racing fractures
The metacarpo- and metatarso-phalangeal joints are the most common anatomical locations for racing fractures. Parasagittal fractures of the distal condyles of McIII and MIII (“condylar” fractures), sagittal fractures of the PI and various configurations of fractures of the PSBs have been shown to account for over 70%
of all fatal fractures in Thoroughbreds while racing. These injuries all occur in varying states of severity, ranging from short, incomplete fissures to complete, comminuted, open fractures.

Osteochondral chip fractures of the dorsal articular margins of the carpal bones and slab fractures of the third carpal bone, in frontal and sagittal planes, are well recognised and are all associated with underlying degenerative bone disease, associated with osteonecrosis caused by repetitive, high intensity loading of affected regions.

Stress fractures of the third metacarpal bone, humerus, scapula, tibia and ilium are also relatively common. These fractures arise in consistent locations in each bone:

- Dorsolateral cortex and palmarolateral or palmaromedial cortex of the distal diaphysis of McIII
- Caudal cortex of distal humerus and caudolateral cortex of proximal humerus
- Lateral aspect of the scapula at the distal margin of the scapula spine
- Caudal cortex of the tibia at the junction between mid and distal thirds of the bone and caudolateral aspect of the proximal diaphyseal region
- Wing of ilium

**Diagnosis**

Complete fractures can usually be recognized by clinical signs (e.g. severe lameness, instability, localised swelling, joint effusion, pain on manipulation, crepitus etc.) and confirmed with simple diagnostic imaging (e.g. radiography or ultrasonography). When incomplete, detection of these injuries can be more challenging; lameness is variable, as is swelling of surrounding soft tissues. Firm digital pressure on the bone over the site of fracture may elicit a marked pain response and this can be a helpful aid to diagnosis. Identification of incomplete fractures on radiographs can also be difficult as the fissure may be so thin as to be undetectable unless the x-ray beam is parallel to the plane of fracture. Therefore, multiple projections that each varies only a few degrees in obliquity may be required to achieve a diagnosis. In addition, specific radiographic projections that highlight anatomical regions that are susceptible to fracture, such as proximal PI, palmar aspect of the distal condyles of McIII and MtIII and the third carpal bone, are essential to maximize the chances of detecting subtle injuries in these locations.

When clinical signs are suggestive of fracture and yet no lesion can be detected radiologically, further imaging, such as nuclear scintigraphy or MRI, are indicated. Alternatively, the horse should be restricted to its stable until repeat radiographs are made seven to 10 days later, when resorption of the fracture margins makes the fracture easier to resolve. Ultrasonography is particularly helpful for the diagnosis of fractures in regions that are difficult or impossible to radiograph, for instance the wing of ilium and body of scapula.

**Treatment**

Bone affected by focal repetitive stress injury and incomplete fractures are often best managed conservatively, with stable rest followed by progressive return to exercise over a three-month period. There is some research evidence that non steroidal anti-inflammatory drugs inhibit bone healing, although this is unlikely to be of clinical significance and use of these drugs to relieve the animal of suffering is indicated until acute lameness subsides. Stabilization of the bone by internal fixation may be useful to prevent extension of an incomplete fracture and to encourage bone healing. This can be carried out relatively easily in the standing horse, under local anaesthesia for incomplete fractures of the distal condyles of McIII, MtIII and PI.

Surgery to reduce the fracture, restore normal anatomy, particularly of articular surfaces that may be involved, and achieve stability and mechanical integrity is likely to be required to manage displaced, unstable fractures. This is usually best achieved with appropriate implants applied using AO principles.

**Keywords:** Horse, Fracture, Fatigue
Guarding the Welfare of the Thoroughbred Racehorses

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Summary
Currently most societies agree that it is ethically acceptable to use animals in sport on the strict understanding that the animals’ welfare is paramount: they must be maintained in an environment that provides a “good quality of life”, fed and watered correctly, kept free of pain and distress and provided with effective health care. In addition, they should be looked after properly when they can no longer participate in the sport.

Stakeholders in sports that involve animals, such as Thoroughbred racing, must keep these principles in mind at all times. It is not only fundamentally right to ensure good horse welfare but it is also a prerequisite to maintain support of and tacit approval from society.

Horseracing faces particular challenges. Horses are large creatures that are expensive to breed and maintain. This creates financial pressures to compete them early and frequently. In addition, horses can rapidly become a significant burden once retired and no longer able to generate revenue. The intense physical nature of racing has inherent dangers to the horses that can be minimized but not eliminated. Lastly, and most critically, large sums of money are often involved in races and some people will do bad things, including abuse the horses, to win that money.

In addition to providing good basic animal husbandry, Racing must ensure that racehorses are protected from being raced when they are physiologically not ready or when they are suffering from disease or injury. Adequate safeguards must be in place to prevent horses from racing under the influence of drugs, which may be injurious to their health, allow them to compete when they are not fit to do so or may stimulate them to race beyond their natural ability. In addition, Racing must constantly monitor racecourses and individual races to ensure that they are not affected by specific factors that increase the risk of injury to horses. There must also be suitable arrangements to provide prompt and effective care to horses in the event of injury. Lastly, Racing must ensure that horses are provided for once they can no longer race and they are retired from the sport.

The debate over whether it is ethical or not for man to use animals for entertainment is one that is of increasing concern to modern societies. Currently, the general sentiment is in favour of doing so. However, this is heavily dependent on the relevant sport retaining the confidence of society that it will care for the animals involved responsibly and effectively. Animal rights activists, who oppose all use of animals in sports, are quick to seize on examples of poor horse welfare to persuade the wider population that racing is cruel and sway opinion against the sport. Therefore, maintaining the welfare of racehorses to the highest standards is not only right morally, it is also imperative in order to retain public support for the long-term survival of the sport.

Fundamental requirements of animal welfare are enshrined in the “five freedoms”:

1. Freedom from hunger or thirst - by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from discomfort - by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury or disease - by prevention or rapid diagnosis and treatment.
4. Freedom to express (most) normal behaviour - by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from fear and distress - by ensuring conditions and treatment which avoid mental suffering. Facilities and management practices must be established to satisfy these requirements as a minimum. In addition, a range of further measures are required to manage challenges to horse welfare that are more specific to racing.

**Suitability for racing**

Training and racing exert high loads of a repetitive nature on the horse. Most Thoroughbreds are able to tolerate the extreme exertions involved within reasonable limits. However, some horses may not have a sufficiently robust constitution, either because they have not developed appropriately, they have not yet grown sufficiently, they are suffering from a genetic predisposition to disease or they are in a physiological state that temporarily reduces their ability, such as pregnancy. Measures must be in place to identify these individuals and prevent them from being trained and raced. While some conditions are relatively easy to manage, for example fillies and mares cannot race when in foal after 120 days of gestation, others are less clear. Horses with conformational abnormalities are prone to injuries in the imperfect limb although the relative risk associated with different deformities and when the risk caused by them becomes too great is not defined. There is an ever-increasing body of literature documenting genetic predisposition to certain diseases and injuries in racehorses. However, focus of breeding ultimately remains on performance and little effort has been invested in weeding out bloodlines with genotypes linked to morbidity. Perceived dangers associated with racing young horses, which have not yet reached skeletal maturity, has received much attention from welfare groups. People intuitively link risk of musculoskeletal injury to this factor when horses are race as two-year olds. Research has provided some evidence to guide this debate. Horses raced as two year olds have been shown to have healthier, longer careers than those that first start at an older age. This may partly reflect earlier starters being generally healthier individuals although there is additional, independent evidence that adaptation of the musculoskeletal system to loads is most sensitive in early youth and that some tissues, such as tendon, can only adapt up to a certain age. Therefore, preparing horses for two-year-old races may actually be beneficial to their overall health and lower their risk of injury. Nevertheless, good horsemanship and sensitivity to the individual and training programmes that account for each horse’s state of development and response to training probably remain fundamental to achieving favourable outcomes.

Horses that have been in training for sustained periods may suffer from chronic degenerative conditions that arise as a consequence of repetitive stress injuries. Continued training in the face of such conditions will lead to irreversible disease and will render the horse permanently unsound. In extreme cases, these conditions predispose to fractures that may have catastrophic consequences for the horse. The racing careers of horses with degenerative conditions can be extended through effective use of medical therapies. Achieving a balance between a long and productive racing career and the chance of reasonable quality of life post racing is difficult and may not have been debated with sufficient vigor to date.

**Suitability to race**

Horses should not be raced unless they are fit and healthy. This simple and obvious rule may be contravened consciously or subconsciously. A trainer may feel under great pressure to train and race a horse when they know it is not fit because of their desire to satisfy the owner’s expectations, they think that they can’t afford not to, they are targeting a particular race or they wish to move the horse on (e.g. in a claiming race). Alternatively, they may not identify the fact that the horse is suffering from disease or an injury that renders it unfit. The threat to horse welfare posed by this risk can be minimized by inspection of each
horse by an independent veterinarian before it races. This process can be enhanced by full access to detailed clinical histories of each horse by regulatory vets, systematic review of the records and implementation of a scheme to restrict training and ability to enter a horse to race until it has recovered fully from any disease or injury. This process has the advantage of encouraging trainers to seek appropriate veterinary clinical care and is more likely to prevent horses from being submitted to training regimes when they are not healthy.

A significant proportion of fractures that affect horses while racing arise from pre-existing defects in the bone. These defects are most commonly caused by fatigue-damage to the bone that occurs due to the cyclical, repetitive nature of loads associated with training and racing. The signs associated with fatigue damage can be subtle or even undetectable by clinical examination alone. For this reason, pre-race veterinary inspection of horses is of limited value in preventing some fractures. Early detection of lameness or even subtle changes in a horse's gait by the trainer or work rider and good clinical follow-up, with use of modalities particularly sensitive to bone disease, such a nuclear scintigraphy, will provide best protection for the horse. Conversely, over-use of anti-inflammatory drugs, particularly intra-articular corticosteroids, will mask the presence of subtle clinical signs that alert to the presence of mild but critical bone disease.

Drugs may be administered cynically to intentionally mask the fact that a horse is unfit to race or in an attempt to restore a horse's ability to perform to its normal level in the face of injury. They may also be used to stimulate a horse to perform beyond its natural limit. All of these scenarios will negatively impact horse welfare as they increase the risk of exacerbating the existing condition or causing further injury. Therefore, a thorough, effective and strictly enforced medication and doping control programme is essential to protect the welfare of racehorses.

Ensuring that horses are fitted with appropriate tack and that they are suitably shod is important for the horse's comfort and safety and hence welfare. Certain types of shoes, in particular those with toe grabs, have been shown to increase risk of injury and should be banned.

Suitability of race
There is a wide range of injury rates between different types of races and different racing jurisdictions. Much variation is due to obvious risk factors associated with the character of the race. For instance, incidence of fractures is significantly higher in jumps races than those restricted to the flat. While changes can be made to lower the risk of jumps, this type of racing will always be more dangerous and it comes down to society to decide what level of risk to horses competing is acceptable.

Numerous studies have attempted to identify particular risk factors in different race types and at different racecourses. Where high quality evidence is obtained, this should be applied as practical interventions for the benefit of horse welfare.

Racing has a responsibility to record all injuries and accidents thoroughly and accurately and to collate and share these data honestly. For this process to be of value, the data must be analysed regularly and screened effectively to enable detection of trends that may reflect subtle influence of a negative risk factor at an early stage. These data are also critical for ongoing research aimed at ever-improving the safety of racing.

Care after racing
The career of a horse in racing is inevitably limited by its age. Consequently, each year large numbers of horses are retired and need to be rehomed. The prospects for horses that are fit and healthy and able to participate in a second athletic career are much better than those that are affected by chronic degenerative conditions. Even so, finding suitable homes for these animals requires ongoing, proactive effort by Racing to be successful. The management of horses that are affected by chronic conditions that limit their future usefulness is more problematic. Unless the racing owner is committed to funding care of the horse over the rest of its natural life then frequently these horses have no future. It may be reasonable to argue that, as production animals, these creatures can justifiably be euthanized at the end of their productive lives.
Whether one accepts this argument or not, it is paramount that the welfare of all horses retired from racing should be maintained at a high standard until their death. Horses that are to be euthanized must be accorded care that maintains the five freedoms as a minimum to the end. They must not be transported if that would compromise their welfare and if they have to be relocated, they must be transferred in a manner that is sympathetic to any conditions they may be suffering from. The manner of their euthanasia must be humane and respect the animal’s dignity.

**Keywords:** Horse Racing, Welfare, Injury
Veterinary Response to the Injured Horse at Race Meetings

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Summary
Accidents and injuries are an inevitable risk of high-speed horse sports. While an increasing number of interventions in training and racing have been shown to reduce this risk it will never be eliminated. Therefore, a team of knowledgeable, skilled vet clinicians must be readily available to respond immediately and effectively at any time throughout a race meeting. The objectives of a response to a horse injury are to: 1) contain the incident and avoid other animals or people becoming injured; 2) minimize pain and suffering of the affected horse(s); 3) stabilize the animal and so maximize the chances of success of potential treatment; 4) recover the injured horse from the racecourse as smoothly as possible; 5) cause minimum disruption to the race meeting; 6) act as discretely as possible to cause minimal distress to onlookers.

The relative infrequency of severe incidents makes it difficult for individual clinicians to gain experience and confidence. This can be partially managed by training, including practical drills and review of videos of past events. The latter also provides an opportunity to critically review each response to determine what was good and what might be done differently in future.

This presentation reviews practices that help to achieve the six core objectives listed above.

Severe musculoskeletal injury is a risk of competition in any high-performance athletic sport. Despite advances in preventative strategies, occasional “breakdowns” remain a feature of Thoroughbred racing. The incidence of severe injury varies greatly among different racing jurisdictions and race types. In Hong Kong approximately one starter every 1500 competing in a race on the flat will suffer a severe injury. With eight or 10 races per card and an average of 12 starters per race, this equates to one severe injury every 12 to 15 race meetings.

Clinical cover at most racecourses is usually shared on a roster basis among several clinicians. As a consequence, a veterinarian may go several months before being presented with a catastrophic injury on the field. It may be an equally long time before they have to deal with another similar emergency. Therefore, it is difficult for individuals to develop a depth of personal experience on which to base their approach to what is potentially a frightening, dangerous and high-pressure situation. Furthermore, the manner in which the professional does respond in these circumstances may have a profound impact on the horse’s welfare and in the way in which spectators react to the incident. Most notable events now days are likely to be photographed and filmed by anyone with a mobile phone and incidents are soon published on social media. This further adds to the pressures on individuals and to the magnitude of the consequences of a poor response.

The Audiovisual Department at the Hong Kong Jockey Club is responsible for broadcasting races to the public and for providing detailed coverage for regulatory analysis by the Stipendiary Stewards during and after the race. Each meeting is filmed in high definition from seven different angles. In the event of an injury, cameramen are instructed to pan back onto the incident as soon as the race has finished. This provides valuable footage on how emergency response teams deal with each case. Analysis of these videos is useful in helping to determine useful and less effective response behaviours. In addition, it helps to identify
actions that could be taken to improve the care of the horse and perceived suffering of the animal that might otherwise go unnoticed.

In this presentation I will review observations from subjective analysis of these videos and make recommendations of factors to consider when faced with various scenarios associated with a catastrophic injury on the racecourse.

I include below a simple list of some important points, many of which arise from review of past events, which may be useful to consider when preparing to cover a race meeting.

**Table 1**

<table>
<thead>
<tr>
<th><strong>Don’t</strong></th>
<th><strong>Do</strong></th>
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<tbody>
<tr>
<td>Be taken by surprise.</td>
<td>Develop an active imagination: “live through” all the scenarios you can think of in your mind before each race meeting and develop strategies for managing them. This will help you prepare mentally for the “unexpected”.</td>
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<tr>
<td>Run to an injured horse in apparent panic.</td>
<td>Walk swiftly to the scene in a purposeful, professional manner (unless there is genuine need to run).</td>
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<tr>
<td>Stand and do nothing once you arrive</td>
<td>Rapidly take stock and be proactive (take command of the horse, sedate it…).</td>
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<tr>
<td>Ignore an injured jockey.</td>
<td>Promptly determine if there is anything you need to do to manage the horse to protect the safety of the jockey and medical personnel attending him/her and then immediately do it.</td>
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<tr>
<td>Assume that someone has hold of the horse.</td>
<td>Check that the horse is well restrained by a competent person and that the bridle and reins are sufficient and are not going to slip off. Apply a head collar and lead rope if in any doubt.</td>
</tr>
<tr>
<td>Shy away from using drugs for fear “contaminating the horse with a prohibited substance”.</td>
<td>Administer sedation and analgesia (α₂ agonist and opiate) as soon as possible if indicated. (Have these ready, pre-drawn, at hand).</td>
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<tr>
<td>Move the horse if it will cause it undue suffering.</td>
<td>Call for the race to be abandoned if necessary on grounds of safety and animal welfare.</td>
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<tr>
<td>Automatically erect screens.</td>
<td>Make a quick assessment and determine if screens are necessary – don’t call for them automatically. If they are used everyone fears the worst and may think that you are hiding something. Use screens when any of the following are present:</td>
</tr>
<tr>
<td>Leave a swinging leg fracture while you wait for screens or further help</td>
<td>Most fractures can be stabilised to some extent by the use of splints (e.g. even an open fracture/luxation of the fetlock).</td>
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<tr>
<td>Move a horse unless a swinging leg fracture has first been stabilised.</td>
<td>Practice how you would stabilise the range of different injuries you are likely to encounter. (Practice on a quiet horse. Also, use limbs post mortem, on which you have artificially recreated different injuries).</td>
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<tr>
<td>Make any impulsive/hasty decisions about euthanasia.</td>
<td>Obtain a second opinion or apply first aid and transport the horse to the on-site clinic for further assessment if any doubt.</td>
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<tr>
<td>Have inexperienced people on the screen crew.</td>
<td>Train the screen crew so that they know exactly what to do:</td>
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<tr>
<td>Trust the screens to give you privacy.</td>
<td>Remain aware that people may easily see over the top of screens (from high-rise buildings) and some will find ways of looking around them with a telephoto lens.</td>
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<tr>
<td>Don't</td>
<td>Do</td>
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<tr>
<td>Euthanize a horse on the course unless absolutely necessary.</td>
<td>Remember that it is vastly preferable to recover a horse onto an equine ambulance, make further assessment if required, and then euthanize it off course (or in the ambulance if necessary) than to destroy it on course. It is safer, encourages full assessment of the injury and portrays a more caring, professional approach. The horse’s welfare must take priority and if moving it will cause it unreasonable suffering then it should be euthanized on site. However, most injuries of the distal limb (which is the most common location of serious injuries) can be rapidly and effectively stabilized with splints, allowing the horse to be loaded onto an ambulance without causing it distress.</td>
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<tr>
<td>Euthanize a horse just because you can’t get it to stand and there is great pressure to continue with the next race.</td>
<td>Have a plan in place to allow a recumbent horse to be moved, either to the side of the course or to a recovery stall. This will require a drag mat or, preferably, a recovery sledge, slip-sheets and straps. In addition, it may be necessary to induce general anaesthesia in the horse and so you should have immediate access to necessary drugs and technical expertise.</td>
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<tr>
<td>Use a gun to euthanize a horse on the course.</td>
<td>Have drugs for inducing smooth, rapid euthanasia by intravenous injection, available for emergency situations. Shooting a horse is socially unacceptable and one cannot rely on privacy on a racecourse, even behind screens. In addition, use of a free bullet is associated with obvious dangers.</td>
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<tr>
<td>Use a technique that you are not familiar with to euthanize a horse on the course.</td>
<td>Ensure that you have current experience with the drugs that will be used to induce euthanasia and that you are familiar with their use in stressed and sedated horses.</td>
</tr>
<tr>
<td>Leave the body uncovered once the horse has been euthanized.</td>
<td>Have a body sheet big enough to completely cover the horse’s carcass available in the equine ambulance. Cover the horse as soon as it is down and unconscious and check for loss of vital signs under the sheet. Ensure that the body remains fully covered while the horse is loaded into the recovery vehicle.</td>
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<tr>
<td>Drag a horse’s carcass into the recovery vehicle in an undignified manner.</td>
<td>Recover the horse’s carcass carefully. Ideally the carcass should be rolled onto a drag mat and the mat then drawn into the recovery vehicle. However, it is often easier and faster to attach the winch line to the horse’s limbs and pull it in via the limbs. This can create a distressing spectacle and so the carcass should remain completely covered throughout this process.</td>
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<tr>
<td>Leave any equipment on the course.</td>
<td>Search the course where the incident has occurred carefully after the horse has been recovered. It is all-to-easy for someone to drop a piece of equipment (e.g. the stylet from an intravenous catheter) on the racecourse while dealing with the emergency.</td>
</tr>
<tr>
<td>Leave a horse carcass that has been recovered unattended in an unsecure area.</td>
<td>Recover the horse carcass to a secure holding compound or directly to a post mortem facility.</td>
</tr>
<tr>
<td>Fail to follow-up with the horse’s connections as soon as you can after the incident.</td>
<td>Make every effort to contact the trainer (and owner) to notify them of the situation and what is happening to the horse. File a written report and arrange for security blood and urine samples to be collected under secure conditions, depending on the policy of the officiating body.</td>
</tr>
<tr>
<td>Undertake any form of post mortem examination in a position where you may be overlooked.</td>
<td>Make arrangements for a post mortem examination to be undertaken by properly trained staff in a dedicated facility.</td>
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**Keywords:** Racehorse; Injury; Accident Response;
Monitoring Racing and Training Injuries –
The Hong Kong Perspective

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Summary
Pursuit of supremacy in athletics pushes competitors to the edge of physiological boundaries and physical limits. Under these circumstances small changes in an individual's intrinsic state, or in the extrinsic environment in which it trains and performs, can precipitate failure. In addition to affecting performance this can manifest as injury, potentially with catastrophic consequences. Human athletes can express concern and make choices; the same is not true for Thoroughbred racehorses. It is the responsibility of those overseeing the care and welfare of these horses to collect and monitor data that indicates underlying health trends, which can be used to direct intervention at an early stage if necessary.

A dedicated Department of Vet Clinical Services (DVCS) provides veterinary care for all horses at the Hong Kong Jockey Club (HKJC). A separate Department of Vet Regulation provides independent oversight of horse health-related matters that impact safety, integrity and welfare. The DVCS developed and maintains a detailed database of horse health information. This is regularly analysed to screen for trends and obtain evidence of the validity of clinical practices. These data are used to monitor racing integrity. The data also facilitates relevant clinical research.

The essence of athletic competition is to push the limits of physiology and biomechanics beyond those of fellow contestants. In so doing biological safety margins, which have evolved to protect against the unusual, are progressively narrowed. Under these circumstances small changes in an individual's intrinsic state, or in the extrinsic environment in which it trains and performs, can precipitate failure. In Thoroughbred racehorses this commonly results in a range of clinical conditions including fractures, joint disease, tendinitis and exercise induced pulmonary haemorrhage.

Closely monitoring the frequency and patterns of work-related injuries among a population that trains and competes in close proximity and shares similar facilities enables those responsible for the health and welfare of that population to identify trends. When the analysis is performed on an ongoing, regular basis emerging trends can prompt investigation and intervention much earlier than would otherwise have been possible, thereby reducing the proportion of the population exposed to a new risk that may have evolved. It also facilitates measurement of outcomes following interventions, to determine their benefit. In addition, it enables comparison with other centres and assists in the development of screening tools for intrinsic risks.

Information generated about the proportion of a racing population affected by injury at any one time is also valuable to those involved with other aspects of the racing “product”. For instance, provision of this information to Handicappers may be an important professional service to help them adjust the racing calendar to accommodate situations where a high proportion of a certain class of horse may be indisposed.

Management of Racehorses in Hong Kong
All horseracing in Hong Kong (HK) is under the direct control of the Hong Kong Jockey Club (HKJC).
Racing is conducted at two racecourses, both of which are on the flat. Approximately 88% of races are on turf, while the remainder is conducted on a dirt track (sand, silt and organic fibre). The vast majority of training is on the same dirt surface. Horses are all imported, predominantly as 3-year-old males. Approximately 60% of imports are previously unraced, and those which have raced have to meet minimal performance criteria for acceptance. Horses are compulsorily retired when they reach 10 years old, although few make it to this age and the majority are retired early due to performance or veterinary-related issues. The mean retirement age is 5.7 years.

The Department of Veterinary Services (DVCS) in the HKJC provides all clinical care for racehorses and retired racehorses in riding stables in HK. Veterinarians of the DVCS are obliged to record all findings and clinical interventions diligently in a dedicated Veterinary Management Information System (VMIS). Reports detailing relevant findings are subject to daily scrutiny by a separate veterinary department (Department of Veterinary Regulation and Biosecurity Policy – DVR-BP). Horses that are recorded as having suffered significant clinical conditions and those that perform below standard in races are required to undergo independent clinical inspection by vets from the DVR-BP before racing again. This process is managed by implementation of “Official Veterinary Examination” (OVE) and “To Watch” schemes. Implementation of an OVE may be associated with an obligatory stand down period and horses are required to gallop during the inspection process before the OVE can be lifted. All horses entered to race are subject to a simple clinical inspection by the DVR-BP the day before racing and horses with findings of concern may be forcibly withdrawn.

The Rules of Racing at the HKJC stipulate that a trainer must seek prompt veterinary attention for any racehorse that suffers injury or disease. In addition, they are required to report significant clinical events, such as bleeding from the nostril after fast work. Severe penalties for infractions together with a high level of scrutiny of horses in training and in and around the stables ensure a high level of compliance to these rules.

Medications and supplements can only be supplied via the DVCS and all treatments must be administered by or under direct control of a veterinary surgeon employed by the Club.

Recording of Clinical Data

The VMIS was developed in-house in collaboration between the HKJC Information Technology department and the DVCS between 2003 and 2005. It provides a platform to record all clinical data, share information, book procedures and facilities, manage inventory and transmit relevant data for raising accounts. The VMIS interfaces with the departmental PACS and Veterinary Data Information System, which store diagnostic images and scanned copies of relevant documents for each horse respectively, providing easy, convenient access to all relevant information for each horse, stable-side. It is designed to be simple and user friendly to access and pro formas have been developed to encourage recording of relevant clinical data in a consistent manner. For instance, a simple electronic form must be completed each time a horse is subject to an endoscopic examination and grading systems are prominently displayed in relevant areas of the Equine Hospital to establish consistency of reporting.

Clinicians are obliged to produce a written report within three days every time a significant diagnostic investigation (e.g. radiography, ultrasonography, scintigraphy, MRI exam) is performed. The system demands that the veterinarian indicates whether the investigation has revealed a significant clinical finding or not before they can submit their report on line. If it has, then the vet is required to complete an injury record form. All information that is entered into the VMIS is stored in Microsoft Access-based databases that can be easily searched.

The VMIS has been designed to automatically collate all diagnostic reports for regular review by the Head of Veterinary Clinical Services (HVCS). The HVCS confirms injuries as “new” in nature or, if there is a historical record of a similar injury at the same location, as “old”. Fractures are subdivided into those caused by trauma (accident) and those typically associated with fatigue (work-related). They are further categorized as minor (e.g. incomplete fractures, chip fractures) or major (e.g. complete fracture of weight-bearing bone) in severity.
A critical feature of the data recorded by the VMIS is that it captures the incidence of disease and injury among the entire population in racing and training. The DVR-BP monitors injuries and incidents of disease that arise during racing. In common with most published records, these data typically illustrate frequency of event in relation to number of starters for a given time interval.

**Data Analysis**

Data is only of value if it is analysed effectively and reviewed in an appropriate context to generate valid conclusions, which are then used to guide relevant decision-making processes. The large volume of clinical data accumulated by the Veterinary Departments at the HKJC is subject to relatively straightforward descriptive analysis on a regular basis. In addition, it is made available to academic partners collaborating on more detailed studies.

Data on the incidence of common exercise-related injuries, including injuries to the superficial digital flexor tendon and suspensory ligament, work-related (fatigue) fractures, epistaxis due to exercise induced pulmonary haemorrhage (EIPH) and heart irregularities are collated on a monthly basis and illustrated graphically. For ease of review, data is displayed graphically and includes information for the preceding two months, to facilitate detection of emerging trends. In addition, data for the same months for the preceding 10 years, since inception of the current system, is illustrated to allow easy comparison between seasons. The VMIS also displays frequency of injury or EIPH per month for the entire season.

The DVR-BP provides monthly summaries of the frequencies of injuries sustained in races as a proportion of starters and compares these with data up to the equivalent time point in the previous four seasons. Descriptive summaries of both sets of data are reviewed monthly by staff in the DVCS, DVR-BP, Racing Operations Department and senior management. In the event of any perceived trends further analysis can be conducted and potential causes reviewed.

**Research Studies**

Access to data from the HKJC's Racing Information System and tracks maintenance programme in conjunction with clinical data from the VMIS provides opportunity to investigate numerous risk factors for injuries in racing and training (e.g. see reference list below). Ongoing studies provide insight into trends in the incidence of injuries within and between seasons.

Examples of published studies that used data generated by the VMIS are cited below.

**Keywords:** Racehorse, Injury, Data, Analysis

**References**


career duration and racing performance in racehorses that undergo left-sided prosthetic laryngoplasty and ventriculocordectomy surgery for treatment of left-sided laryngeal hemiplegia. Equine Veterinary Journal 45, 229-234.


Pain Control in Horses with Colic

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Summary
Ever since dipyrone was taken off the market in 1977 in the USA, most horse farms have flunixin meglumine readily available and it is common for trainers to administer non-steroidal anti-inflammatory drugs (NSAIDs) for lameness or colic without consulting the veterinarian. Owners and veterinarians need to be aware that a full dose of flunixin meglumine (1.1mg/kg, IV) for treatment of colic is a potent analgesic and has duration of 8-12-hours. The manufacturers recommended dose of flunixin meglumine is every 12 hours. This time frequency should be very closely adhered to. Flunixin meglumine will not completely mask signs of colic, but it makes those signs more difficult to detect, especially for owners. This presentation will provide other approaches to pain control.

Availability of analgesics
Although there are a limited number of analgesics available, they can be divided into several categories based on mechanism of action:

Table 1

<table>
<thead>
<tr>
<th>Analgesic</th>
<th>Mechanism</th>
<th>Potency</th>
<th>Length of action</th>
<th>Dose</th>
<th>Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylazine</td>
<td>ε2-agonist</td>
<td>Moderate</td>
<td>Short</td>
<td>0.3 – 0.5mg/kg</td>
<td>prn</td>
</tr>
<tr>
<td>Detomidine</td>
<td>ε2-agonist</td>
<td>High</td>
<td>Short</td>
<td>10 – 20μg/kg</td>
<td>prn</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>opiate</td>
<td>Moderate</td>
<td>Medium</td>
<td>0.01 – 0.02mg/kg</td>
<td>prn</td>
</tr>
<tr>
<td>Flunixin</td>
<td>NSAID</td>
<td>Moderate</td>
<td>Long</td>
<td>0.25 – 1.1mg/kg</td>
<td>q8-q12h</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>NSAID</td>
<td>Moderate</td>
<td>Long</td>
<td>0.6mg/kg</td>
<td>Q24h</td>
</tr>
<tr>
<td>Firocoxib</td>
<td>NSAID</td>
<td>Moderate</td>
<td>Long</td>
<td>0.3mg/kg loading; 0.1mg/kg</td>
<td>q24h</td>
</tr>
</tbody>
</table>

There are other agents in each of the above categories, but these drugs can be used as examples in order to develop a treatment plan.

Initial Approach to Control of Pain
For a horse that is actively showing signs of colic, an abbreviated physical examination (heart rate, mucous membrane color, and capillary refill time) should be performed before administering an analgesic. If the horse is extremely painful, an attempt at taking the heart rate should be made, since the ε2-agonists can dramatically alter this parameter. Furthermore, heart rate remains one of the most useful predictors of the need for surgery. The first analgesic I administer is xylazine (150-200mg for an adult horse) because it is a moderately potent but short-duration analgesic. This gives the veterinarian an opportunity to determine whether or not the colic is going to recur within the time it takes to complete the remainder of the examination. If a non-steroidal anti-inflammatory drug (NSAID) is used as the first line analgesic, it may not be possible to gauge whether or not colic will recur within the time of the initial visit because it is relatively long-acting.
However, if the horse remains comfortable on the first dose of xylazine, and the remainder of the examination is normal or the veterinarian feels comfortable with the abnormal findings (such as a mild impaction) NSAIDs can be administered to control further mild or moderate pain. If the first dose of xylazine does not keep the horse comfortable, it can be repeated at the same dose. In addition, butorphanol can be combined to provide greater pain relief (typically 5-10mg for an adult horse). If xylazine and butorphanol have little or insufficient effect, the next drug I administer is detomidine (5-10mg for an adult horse). If the first dose of detomidine has little effect, I will repeat it. If detomidine fails to control pain, the horse should be referred as rapidly as possible. Addition of flunixin to the pain treatment plan is of little benefit in these types of cases because the majority of pain results from distension and tension on the mesenteric attachments rather than from inflammation. In addition, all of the drugs mentioned in the table can be repeated as needed except flunixin, which should only be given once every 12 hours.

One new drug in the α2-agonist class of drugs that has recently been approved for horses is romifidine. Like any analgesic, it will take some time to get used to using this pharmaceutical. In general, it is a more potent analgesic than xylazine, and less potent than detomidine. However, it has a relatively long half life (much longer than xylazine), and has good sedative properties. Therefore, at this time, I would consider use of romifidine for long-term sedation, rather than an analgesic for colic.

Cyclooxygenase (COX) Inhibitors
An additional aspect of pain management is treatment of horses that have had colic surgery. These horses may have significant gastrointestinal mucosal injury, so it is advantageous to use low doses of flunixin because NSAIDs have deleterious effects on mucosa. This subject is becoming complicated as we learn about the physiology of NSAIDs. What is known is that NSAIDs inhibit the cyclooxygenase (COX) enzyme system, and that there are at least two isomers of COX: COX-1 and COX-2. Cyclooxygenase-1 is generally responsible for elaborating prostaglandins that facilitate the physiologic function of organ systems such as the gastrointestinal tract, whereas COX-2 is typically involved in heightening pain and inflammation. One option, in countries where COX-2 preferential (Meloxicam) or selective (firocoxib) inhibitors are available is to use this in place of non-selective COX inhibitors such as flunixin meglumine. Whether or not increased safety will be noticed, or a change in efficacy will be seen is unknown. One concern is that there is too much overlap between COX-1 and COX-2, so that non-selective inhibitors will continue to be required for optimum efficacy. In other words, if COX-1 does play a role in pain, no matter how small, then a reduction in efficacy may be seen with COX-2 inhibitors. Nonetheless, it is exciting to have a new class of analgesics available to equine practitioners.

Spasmolytic medications
Another medication that has become available is Hyoscine butylbromide (Buscopan®) (0.3mg/kg, slowly IV). This is an excellent anti-spasmodic agent, but the product sold in Canada does not contain an analgesic (Buscopan® sold in Europe, also contains dipyrone and is the favored choice for initial treatment for colic). However, Buscopan® can be given with NSAIDs available in the Canada, such as flunixin meglumine (0.25-1.1mg/mg, IV). Concerns about transient elevations in heart rate with Buscopan® (approximately 20 minutes) become irrelevant if the veterinarian has already checked the heart rate prior to administration of an analgesic.

Postoperative Pain Control
Continuous infusion butorphanol (15μg/kg/hr) has recently been prospectively evaluated at Washington State University and North Carolina State University. This dosage can be approximated by adding 15mg butorphanol/5L bag of fluids at a rate of 2L/hr for the average 500kg horse. The prospective study revealed significantly improved postoperative comfort level of horses based on a behavioral scoring system. In addition, there was a significant reduction in cortisol levels and a significant reduction in weight loss compared to placebo-treated horses (all horses also received flunixin meglumine during the study). One important point
that came out of the study is that horses can be painful without showing overt signs of colic. A typical painful postoperative case tends to stand in the back of the stall, with minimal response to environmental stimuli, as compared to more comfortable horses that tend to be at the front of the stall, responding to other horses and people in the environment. The one downside of butorphanol therapy was a significant delay in the initial passage of feces from a median of 5-hours in untreated horses to 14-hours in butorphanol-treated studies. However, this did not appear to alter the outcome of these cases. Horses treated with butorphanol were discharged from the hospital earlier and had a reduced bill, reflecting the positive aspects of optimal management of pain.

Lidocaine, which is typically used in an attempt to reduce postoperative ileus, also appears to provide substantial analgesia and anti-inflammatory in horses with colic. The mechanism for the reduction in inflammation is not yet clear. We have used this drug as a constant rate infusion (0.05mg/kg/minute) for horses with continuous pain in which the owner is not willing to consider surgery (typically for financial reasons). We only do this if there is some reasonable chance of medical resolution. For any horse on lidocaine, because of its analgesic effects, the onset and progression of laminitis is more difficult to detect. Therefore, close attention to the feet, particularly in those horses with signs of endotoxemia, is indicated. Currently, all horses in the hospital judged to be at risk of laminitis have their feet iced using ice boots, ensuring cooling of the feet for an extended period of time.

Keywords: Equine, Pain, Non-Steroidal Anti-Inflammatory Drug, Lidocaine

References
Critical Decision Making in Horses with Acute Colic

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Summary
As an equine profession, the decision to refer a horse for further evaluation of colic continues to be difficult and one must question if we have made as much progress as we should have over the last decade. There are typically 2 parties involved in the decision to refer – the owner (or trainer) and the veterinarian. Although numerous parties such as an insurance company may be required to make this decision, to avoid confusion the ultimate decision should be made by the horse owner or trainer with consultation by the veterinarian. This presentation is intended to provide the vital components of a referral decision, including history and examination findings. While most of the information used to make a decision to refer a horse with colic has remained the same over time, the method used to make decisions can be approached differently. The overall goal is to identify the population of horses at risk of needing intensive care (not just the clear-cut surgical cases), and to make it very clear to the owner as soon as possible what options are available.

Introduction
Veterinarians are called on a frequent basis to evaluate a horse that has developed colic. In many cases, the colic will have either resolved or require simple medical treatment. Of all conditions in veterinary medicine, management of colic often requires clear-cut decision making rather than attempting to reach a diagnosis in every case.

Initial examination
To shorten the time needed to examine a horse with colic, consider asking your receptionist to get the signalment, treatments given, and duration of colic. Owners may be reluctant to admit to administering treatments, necessitating some skill in obtaining an accurate history. Therefore, it is important for the receptionist or the veterinarian to ask questions about administration of medications in a way that makes the owner feel comfortable about providing an accurate answer. This is all the history that is needed prior to initiating the physical examination. Additional history, such as diet, de-worming schedule, and housing can be obtained after the horse has been attended to.

On physical examination, make an educated guess as to whether the horse looks bloated. Owners can be asked to confirm if the horse is bloated, although they tend to be biased on this question when their horse has colic. The veterinarian can also make a more accurate determination of the level of colic (mild, moderate, or severe). This may require having the horse in its normal environment such as a stall or paddock so the behavior is not inhibited by being handled. The next step is to assess the cardiovascular status of the horse. This is done by assessing the color of the gums, obtaining a capillary refill time, and taking the heart rate. It is preferable to take the pulse from the facial artery so that an assessment of pulse quality (‘thready’ or strong) can be made. However, the horse sometimes makes this difficult because it is in pain; at which time auscultation of the chest is appropriate. If the horse is severely painful, obtaining the heart
rate in some way is essential because it has consistently been shown to be the best prognostic indicator. The exception is the horse with large colon volvulus or acute incarceration of intestine that, despite severe pain, may have a normal heart rate. This is possibly a result from vagal input from grossly distended intestine feeding back to the brainstem and the heart (personal communication with a board-certified anesthesiologist).

**Remainder of the physical examination**

If a horse is actively showing signs of colic, the owner will be anxious, and once the cardiovascular status has been obtained, this is the time to treat for pain. The author’s choice is xylazine (150-200mg) because it is short-acting (approximately 40-minutes), highly effective as an analgesic, and sedates the horse to facilitate the remainder of the examination. Once the horse is comfortable, the level of dehydration can be determined by tenting the skin on the neck, and looking at the position of the eye in the orbit. Horses are either not dehydrated (skin tent 2-3 seconds), 6% dehydrated (3-6 second skin tent), 8% dehydrated (6-8% skin tent, some evidence of the eye sinking back into the orbit) or 10% dehydrated (prolonged skin tent, obvious sinking of the eye). The next component of the examination is auscultatation of the chest to confirm heart rate (this may be affected by an alpha 2 agonists such as xylazine). Only a brief time is needed to listen to the lung fields because of the infrequency of lung conditions causing colic. Auscultation of the abdomen at paralumbar fossa is completed for approximately 1 minute, and at a site on the lower flank for the same length of time. Listening to these upper and lower quadrants should be completed on both sides of the abdomen. Experience is needed to classify the intestinal sounds into one of 4 categories: normal, decreased, increased, or no sounds. Percussing the abdomen while listening with the stethoscope can also be used to check for excess of gas. This is most useful with the stethoscope place over the base of the cecum in the right paralumbar fossa. The time required for this part of the examination provides a good opportunity to take the rectal temperature, and this should always be done. The temperature, which should be taken before the rectal examination, can provide evidence of an infectious disease such as the early phases of colitis.

Rectal palpation is the most useful diagnostic to determine the intestinal segment causing the cause of colic, but it is not mandatory to perform on every case of colic. It is critical to perform on horses that have repeat episodes of colic. Determining the position of the spleen is important. If it feels larger than normal, and pushed away from the body wall, the most frequent reason is that the colon is between the spleen and body wall. Phenylephrine (3 µg/kg/min for 15-minutes) and walking or jogging the horse can be very helpful to shrink the spleen to help resolve the problem. The final component of the colic examination is nasogastric intubation. Technically, anything greater than 2L is abnormal. Again, your opinion is very important. Findings of gas and pH of the fluid are of very little significance in the initial evaluation of colic. When horses have severe pain, the stomach tube should be passed early during the examination to make sure the pain is not from gastric distention that is close to rupture.

**Reasons to refer**

Unrelenting pain: There is sometimes confusion as to why a horse is so painful when all other examination findings appear normal. With a horse with unrelenting pain, which does not respond to analgesia, it most likely needs surgery and should be referred to a surgical facility. If analgesics such as flunixin meglumine or detomidine have little effect, there are no other alternatives to general anesthesia or euthanasia. At referral centers, frequently these horses are immediately taken straight into surgery with a minimal physical examination.

Failure to respond to treatment: If we consider that approximately 90% of horses with colic are simple colics requiring basic or minimal treatment, then 10% of cases will require careful examination and decisions about the type of treatment; medical or surgical. This group of horses include those horses that do not respond to your initial treatment as well as those the horses that appear to do well after initial treatment, but have recurrence of pain in several hours to a day later. This does not mean that all horses with return
of pain need surgery, but this is the time to talk to the owner about their willingness for referral. Determine whether each client wants potentially expensive treatment of their horse with the option to refer for possible surgery. Once the costs are understood the decision to refer or not becomes easier.

**Signs of endotoxemia:** All horses that have congested gums, delayed capillary refill time, and elevated heart rates need intensive care (particularly fluid administration). Horses improve after administration of flunixin meglumine (0.25-1.1mg/kg), but complete treatment for shock is needed. If an investment is made to have balanced electrolyte solutions available for intravenous administration at least 20-liters of fluid (approximately half the deficit of fluids in a moderately dehydrated horse) should be available to make fluid administration a valuable treatment.

**Signs incompatible with a simple case of colic:** Some horses that appear to respond well to analgesia, and have few signs causing concern, but have signs such as intestinal distension on rectal palpation, or large volumes of gastric reflux should be considered at high risk for surgical exploration and should be referred or placed on routine and frequent monitoring.

**Some final thoughts**
Owners look to their veterinarians to treat their horse and tell them what to do. If an owner indicates that they do not know what to do, gradually take over control of communications and make suggestions to guide the owner toward a rational decision. Anecdotal stories as to what happened with the last case of colic or reciting the way another veterinarian handled a case are irrelevant, and should be largely ignored. Make sure you give clear cut choices, with percentages (typically educated guesses) of survival and potential for future use. For horses in which referral is a consideration, always offer it no matter what your impression of the owner, the value of the horse, or the quality of the facilities. Ask owners to have patience (be quiet) while you auscultate. Having said all of this, do your utmost to have a congenial, friendly, but decisive relationship with the client. Do not hesitate to call your senior partner or the referral practice (sometimes best accomplished in private). When dealing with referral centers, make sure you and your client get the absolute best service. Multiple conversations with the referral practice as to the decisions being made once the horse has arrived, when it comes out of surgery (within reason), and its progress while in the hospital are all part of developing a team consisting of the referring veterinarian, owner, and referral hospital. If you do not think you are getting this service, call the referral hospital and ask to speak to the attending clinician, service chief, or hospital director. Referral centers need to be responsive to owners and veterinarians and keep all parties informed.

**Keywords:** Equine, Colic, Heart Rate, Triage, Emergency
Differential Diagnosis and Treatment of Impaction Colic in Horses

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Summary
Impaction colic is classified as simple obstruction during which an obstruction of the intestinal lumen occurs without compromise to the blood supply during the initial stages. The progression of clinical signs is relatively slow compared to strangulating obstructions, particularly in the case of large colon impactions. Ileal impactions are most commonly associated with type of feed (coastal Bermuda hay), and the presence of tapeworms. Cecal impaction may present as a fluid or ingesta filled impaction, and should be managed carefully to avoid rupture. Cecum is often indicated to evacuate the cecum and bypass of the cecum using ileocolostomy may be considered. Large colon impactions are typically response to medical treatment (oral and intravenous fluids), but may require surgery if unresolved with fluid therapy. The prognosis is good for ileal and large colon impaction, and fair for cecal impaction. This assumes the horse has been referred within a reasonable period of time.

Stomach Impaction
Impaction of the stomach typically consists of excessive dry, fibrous ingesta, but may also be composed of ingested materials that form a mass. Other feeds that tend to swell after ingestion, including wheat, barley, sugar beet pulp, and possibly haylage may also cause impaction. Furthermore, dental disease may increase the likelihood of gastric impaction because of improper chewing of feed. Clinical signs of colic range from acute and severe to chronic and mild. For example, in one report on 4 horses with gastric impaction, colic was moderate or severe and of 8-12 hours’ duration, whereas in another report on a pony with gastric impaction, colic was chronic (7 days’ duration), associated with prolonged recumbency, anorexia, and lethargy. Additional signs may include dysphagia, dropping of feed, and bruxism. The diagnosis of stomach impaction can be made at surgery on horses that have had uncontrollable colic or poor response to medical therapy. However, endoscopy will reveal gastric impaction, and may provide information on the specific nature of the impaction. Although this would not typically be performed on an acute colic case, it would be indicated in a horse with chronic colic.

Medical treatment includes nasogastric intubation, and attempts at softening the ingesta with water, followed by refluxing the fluid contents. At surgery, the impaction can be massaged and infused, most commonly via insertion of a needle adjacent to the greater curvature, followed by infusion of a balanced polyionic fluid such as saline.

Ileal Impaction
Ileal impactions occur most commonly occur in adult horses in the southeastern United States. Although feeding of coastal Bermuda hay has been implicated in this regional distribution of the disease, it has been difficult to separate geographical location from regional hay sources as risk factors. However, a recent study indicates that feeding coastal Bermuda hay is a clear-cut risk factor (approximately 3-fold risk) for ileal
impaction. Nonetheless, it is likely that medium-to-poor quality Bermuda hay is the problem, associated with excessively fibrous and fine stems that are poorly chewed by horses as compared to coarse hays. Furthermore, sudden changes in feed from an alternate type of hay to coastal Bermuda hay likely places a horse at risk of ileal impaction. Studies in England have revealed tapeworm infection as an important risk factor for ileal impaction. Based on risk analysis, the data suggested that in excess of 80% of the ileal impaction cases studied were associated with serologic or fecal evidence of tapeworm infection. Because of the poor sensitivity of fecal analysis for tapeworms, a serologic test (ELISA) has been developed by Proudman et al. with a sensitivity of approximately 70%, and a specificity of 95%. The importance of tapeworms has been confirmed by risk analyses in the United States, which have shown an approximate 3-fold risk of developing ileal impaction for horses that are not sufficiently treated for tapeworms. The important take home message is that horses should be de-wormed for tapeworms twice yearly, preferably with one of the newer combination products that contain praziquantel.

Clinical signs of horses with ileal impaction include onset of moderate intensity colic, and palpable loops of distended small intestine per rectum as the condition progresses. Since the ileum is the distal-most aspect of the small intestinal tract, nasogastric reflux may take considerable time to develop, and is found in approximately 50% of horses requiring surgical correction of impacted ileum. The diagnosis is usually made at surgery, although an impacted ileum may on occasion be palpated per rectum. However, practitioners that see a number of these cases will begin to readily recognize them, particularly considering the fact that there are very few causes of simple intestinal obstruction in the adult horse. One particular feature during the early phases is 2-3 loops of distended small intestine on the right side of the abdomen adjacent to the cecum. During the latter phases of obstruction, multiple loops of distended small intestine make the nature of the obstruction difficult to determine. However, horses with this condition do not usually show signs of endotoxemia suggestive of strangulating small intestinal obstruction.

Ileal impactions may resolve with medical treatment, based on re-hydrating horses with intravenous fluids. Nothing should be administered to horses by stomach tube, even if there is no reflux, as fluid will not move to the impaction because of small intestinal distension and reduced motility proximal to the obstruction. Horses can be managed medically as long as the pain is manageable (periodic doses of short-acting analgesics such as xylazine [0.3mg/kg pm] and butorphanol [0.01mg/kg pm], or low dose flunixin [0.25-0.5mg/kg q8h]), and serial abdominal taps indicate a lack of evidence of small intestinal degeneration. In other words, if pain is intractable, if signs of endotoxemia begin to develop (heart rate >60bpm, congested gums, delayed capillary refill time), or if the abdominal tap is serosanguinous, surgery is indicated. Additional signs indicating the need for surgery would be multiple loops of tightly distended small intestine, and onset of nasogastric reflux. At surgery, fluids can be directly infused into the mass allowing the surgeon to breakdown the impaction. Dioctyl sodium sulfosuccinate (DSS) may be included in the infused fluid to aid in disruption of the mass. Extensive small intestinal distention and intraoperative manipulation of the ileum may lead to postoperative ileus. Extensively impacted ileum can be evacuated with an enterotomy proximal to the impaction, and placement of a hose in the lumen. This particular method has to be performed carefully to avoid contamination, but resolves the impaction quickly. Recent studies indicate that the prognosis for survival in horses with ileal impaction is good.

Cecal Impaction

This condition may be divided into two syndromes: primary cecal impactions that result from excessive accumulation of ingesta in the cecum, and secondary cecal impactions that develop while a horse is being treated for a separate problem. Although primary impactions typically consist of impacted, relatively dry fecal material and secondary cecal impactions tend to have very fluid contents, there is considerable overlap between the two syndromes, and each case must be approached carefully. In horses with primary cecal impactions, there is a gradual onset of abdominal pain over a number of days reminiscent of the development of a large colon impaction that should be differentiated from large colon impactions based on rectal palpation.
findings. Specifically, the cecum can be identified on the basis of its attachment to the dorsal body wall and its fixed position on the right side of the abdomen. Therefore, any firm impaction on the right side should be carefully palpated to determine if it is the cecum. In the case of cecal impaction, the veterinarian will note that the palpating hand cannot be slid over the top of the impaction (from the medial surface of the impaction working dorsally). If there is any question about this, then it is safer to refer the horse. This is because cecal impactions have a propensity to rupture before the development of severe abdominal pain or systemic deterioration. Secondary cecal impactions typically develop following unrelated surgical procedures that result in postoperative pain (particularly orthopedic surgeries). Secondary cecal impactions may be even more difficult to detect because postoperative depression and decreased fecal output may be attributed to the operative procedure rather than colic. By the time horses with secondary cecal impactions show noticeable signs of colic; the cecum may be close to rupture. In many cases, there will be no signs of impending rupture. Therefore, all horses that undergo surgeries where considerable postoperative pain may develop should have feed intake and manure production closely monitored.

Treatment for horses with primary cecal impactions may include initial medical therapy, including aggressive administration of intravenous fluids and judicious use of analgesics. However, if the cecum is grossly distended, or if medical therapy has no effect within a short period of time, surgical evacuation of the cecum via a typhlotomy is indicated. In addition, an ileocolostomy in order to by-pass the cecum may be indicated as postoperative cecal motility dysfunction with recurrence of the impaction is common. The prognosis is good for surgical treatment of all cecal impactions because. However, there is the potential for the cecum to rupture during prolonged during surgical manipulation.

Cecal impactions can be difficult to recognize, and cecal impaction should be considered a major differential for any horses with a right-sided impaction. If this is confirmed based on careful palpation, immediate referral is always indicated. If this cannot be confirmed by rectal palpation, strong consideration should be given to referral, particularly if the horse does not readily respond to treatment.

Pelvic flexure impactions

These are the most common of the large colon obstructions. Early feed impactions are best treated medically, with laxatives, analgesics, and intravenous fluids in refractory cases to maintain hydration. In cases that do not respond (increasing pain, deteriorating systemic condition, lack of progress on softening the impaction), treatment is by laparotomy, pelvic flexure enterotomy, and lavage.

Sand impactions

Diagnosis can be made by rectal palpation of the mass and mixing feces with water and allowing the sample to stand. Sand typically accumulates in the right dorsal colon and transverse colon. Surgical treatment is by enterotomy and evacuation, as above. Incidence of this disorder can be decreased by decreasing pasture animal density (to prevent the grass from being so short that the animals are eating dirt with it), turning animals onto pasture after they’ve been fed, and regular administration of bulk laxatives.

Enteroliths

These most commonly occur on the west coast. Diagnosis may be made by radiography, but these horses are generally taken to surgery because of signs of large colon obstruction (gas distension, bands, and impaction). Enteroliths are typically located in the right dorsal colon, transverse colon, or small colon. Initial evacuation of intestinal contents via a pelvic flexure enterotomy is followed in most cases by an incision in the right dorsal colon (because of the size of the enterolith). If an enterolith has a flat side, look for more.

Keywords: Equine, Impaction, Stomach, Ileum, Cecum, Colon
References
Management of Chronic and Non-Healing Wounds in Horses

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Summary
Non-healing wounds are a particular concern in horses because of the relative tension of the skin and lack of underlying soft tissues on the lower limbs. Thus, any wound at or below the carpus or tarsus is immediately problematic because of difficulties ensuring primary closure of wounds combined with a propensity to produce exuberant granulation tissue (‘proud flesh’). There are also other problem areas to consider, such as wounds over joints or in areas of high motion. In addition, a chronic non-healing wound should be worked up as a clinical problem, with multiple differentials such as tumor formation, and underlying foreign body or open synovial structure.

Initial diagnostic approach
The initial examination should include cleansing of the wound to avoid pushing foreign material deeper into the wound during digital palpation. Wounds can be cleaned with water delivered through a hose, followed by use of sterile saline or betadine solution heavily diluted in tap water (to the color of weak tea). Palpation may reveal deep clefts in the wound, foreign bodies, or communication with important underlying structures. Care should be taken not to overly probe wounds that may involve a joint or tendon sheath. Most chronic wounds will have what appears to be exuberant granulation tissue, and in the majority of cases it is this tissue that is in large part responsible for the lack of healing. However, exuberant granulation tissue is difficult to differentiate from tumors, particularly sarcoids. In addition, sarcoids may develop at the site of a wound. Thus, the tissue should be biopsied and sent to a veterinary pathologist for evaluation. On evaluation of this tissue, pathologists should also be alerted to the possibility of aberrant parasite migration, particularly Habronema spp. The latter has become uncommon with the advent of efficacious broad-spectrum anthelmintics such as the avermectins, but cutaneous habronemiasis is a consideration, particularly if the wound involves the external genitalia or eyelids. Consideration should also be given to culture of biopsied tissue. All wounds will culture positive for numerous organisms, so it is the magnitude of the infection that is important. Therefore, if culture is performed, a precise amount of tissue (at least 1g) should be submitted so that the total tissue bacterial burden can be calculated. Tissue with greater than 10^6 organisms/ g is considered to be infected.

Additional diagnostics for chronic wounds
Since the majority of chronic wounds are located on the lower limbs, radiography and ultrasonography are excellent modalities to determine if there is underlying bone, joint, tendon or ligament involvement. Radiographs may reveal a sequestrum, or evidence of osteomyelitis. If a foreign body is suspected, contrast radiography can facilitate localizing a filling defect that can lead to identification of radiolucent objects such as wood. If a wound is to be infused for this purpose, involvement of an underlying synovial structure needs to be ruled out. Ultrasonography is highly effective for tracing the depths of the wound toward underlying soft tissue structures. Suspected involvement of underlying synovial structures should be confirmed by centesis of the suspected joint or tendon sheath at a site distant from the wound. Once the desired structure has been reached, if it is not possible to withdraw fluid for culture and
cytology, placement of the needle can be confirmed with ultrasound and the structure can be infused with sterile saline to determine if there is indeed a communication with the wound.

Initial treatment plan
The approach to treatment will vary, depending upon the underlying cause of non-healing, such as a sequestrum, foreign body, or synovial infection. However, in most cases, the reason equine wounds have delayed healing is exuberant granulation tissue. The initial treatment plan for this type of wound is careful and thorough debridement of the wound, with the goal of removing all excessive granulation tissue to just beneath the level of the skin. In some cases, the complexity of the wound necessitates general anesthesia, but in most cases, wound debridement can be accomplished in the standing sedated horse. Granulation tissue does not have a sensory nerve supply, although some sensation adjacent to the skin or to underlying structures may be present. Thus, the most efficient way to debride a chronic granulation bed is with a scalpel blade. The level of bleeding should be explained to the owner, and will be controlled with a bandage following the procedure. There is no need to attempt to ligate specific vessels unless a distinct vascular structure is inadvertently severed. This is not only the best way to remove granulation tissue, it should be the only way that granulation tissue is removed. Many chronic wounds have excessive scar formation because of inappropriate application of caustic agents designed to remove ‘proud flesh.’ Once debridement has been completed, a bleeding fresh well contoured wound should be apparent, which will need immediate application of a bandage with sufficient layers of cotton to stop the hemorrhage. Hemorrhage may seep through the bandage over the ensuing 24-hours, but not to any great extent. One of the most difficult lower limb wounds to bandage is a dorsal hock wound. This will at the least require application of a dressing, gauze, and bandage material in a figure eight configuration, but may require a full lower limb bandage with a mid-limb ‘stack’ bandage applied to apply sufficient pressure and bandage material to the wound. The bandage should be changed the following day in all cases, at which point the wound should appear to be flat with a healthy pink color.

Continued management of the chronic wound
Once fully debrided, the wound will require bandage changes at least 3-4 times/week, and it is this phase of management that can be frustrating, and costly. At this point, skin grafting is a consideration. One frustrating aspect of wound care is that the optimal environment for wound healing is a clean, oxygenated wound bed beneath the margin of the skin. Bandaging, although frequently necessary to keep a wound clean and to prevent edema, will reduce the oxygen tension. One method to reduce rapid development of exuberant granulation tissue is to apply topical steroid ointment. Commercial preparations that also include broad-spectrum antibiotics are helpful to reduce the surface infection that usually is also enhanced with bandaging. These preparations can be used intermittently, as they may also slow down development of new epithelium. One consideration is the possibility of leaving a wound unbanded, particularly if a wound can be kept clean between veterinary visits. However, wounds should continue to be carefully monitored for progress, with the realization that as soon as granulation tissue reaches the outer edge of the skin, wound healing will cease, and additional debridement is needed.

Keywords: Equine, Wound, Skin, Repair, Granulation Tissue, Second Intention

References
Canine Body Languages

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Summary
- It is important to know canine body language. Otherwise, we are not able to diagnose canine problem behaviors.
- Dog interact with human by using their language. Knowing their language is the best way to know them.
- When you see behavior problem treatment plan, it will make more sense if you know the canine body languages.

For the small animal clinicians and veterinary nurses, knowing canine body language will be very useful information to educate the owner about dogs. It is also very important knowledge to protect yourself from being aggressed by dogs in the clinic.

Some dog training TV information or magazine information may say that the dog owners need to “dominate” their dogs. However, when you see canine body languages, the truth might be different from “being dominant”.

When dogs interact with humans, they try to communicate with us by using their languages. Today’s topic is about reading and understanding their language so we can understand what they are trying to tell us and what we should do to communicate with them.

1. See canine body languages and observe some videotapes. We will discuss what those dogs in the videotape telling?
2. One training video will be introduced. We will see what you observe from the training and think what part is good and what part should be addressed in veterinary medicine.

Keywords: Canine, Body Language, Dog, Behavior Problem
Cat's Behavior in General

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Summary
✓ Cats are social animals that communicates with each other.
✓ Cats behaviors are important to know when treating problem behavior in cats.
✓ House soiling and scratching behavior will be discussed.

Lay people may be saying that cats are asocial animals, will not interact with other animals, will not play with humans so we should leave them alone. From ethological study, we found that cats are also social animals that they interact with each other, communicate with each other and using body language. When cats are preferred associates, they will lick each other, sleep together, and allorub. When they communicate, they use tail, ears and body posture. When you see cat is coming with its tail up, that means “friendly hello!”. You will see the video images showing those behaviors.

House soiling is the most common behavior problems that practicing veterinarians may receive. When pet parents with house-soiling problems, it is common that the behavior is due to infection, inflammatory or metabolic changes that is contributing to the problem. Make sure that the veterinarians will conduct necessary physical examination, urinalysis, CBC, serum chemistry, then imaging examination if needed. Once the cat's behavior is also from the behavior or environment, litterbox condition or cat's environment should be considered. If the behavior is due to marking, neutering might be one of the first treatment you want to discuss with the pet parent.

Inappropriate scratching behavior is also common behavior problems that the owners have. Cat scratches because they need to manage their nail and scratch for marking. It is important to find a scratch post that the individual cat likes. Each cat has different preference in scratch post so the key is to find what cat likes.

Keywords: Cat, Behavior, Behavior Problem, House Soiling, Scratching
Compulsive Disorder, Science Behind

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Summary
Discussing Compulsive disorder in dogs, its science behind, diagnosis and treatment in this section.

What is Compulsive Disorder?
Compulsive disorder is a condition in which animal displays repetitive, exaggerated, or sustained behavior out of original context. It is believed to occur in animals subjected to stressful situations resulting in conflict or frustration. Compulsive disorder can develop in many species, including dogs, cats, birds, horses, rabbits, and rats. Typical clinical signs for dogs are excessive licking and chewing possibly develop into acral lick dermatitis, tail chasing, spinning, circling, licking air or objects chasing imaginary flies, fixating on certain objects, shadow or light chasing. Cats are often presented for wool sucking, nibbling and eating cloths, tail chasing, excessive self grooming. Typical behavior in birds can be feather picking and horses are often presented for cribbing and weaving.

Compulsive disorder in dogs appears similar to human obsessive compulsive disorder in its behavioral presentation, response to medication, and in genetic predisposition. Recent imaging study with MRI revealed that there is parallel between canine compulsive disorder and human obsessive compulsive disorder (OCD), exhibited brain abnormality in MRI images similar to those of human OCD. Compulsive disorder dogs exhibited what is noted as multi-dimensional OCD.

Science Behind OCD
Studies looking at an effect of serotonergic agents such as serotonin reuptake inhibitors (eg. clomipramine, fluoxetine, fluvoxamine, sertraline and paroxetine) have provided main stream of OCD treatment. Success of pharmacological treatment with serotonin re-uptake inhibitors and atypical antipsychotic drugs suggests that both the central serotonergic and dopaminergic systems are involved in the pathophysiology of the disorder. Glutamatergic augmenting agent (mementine) provided a supportive evidence for effectiveness in treating severe OCD.

Neuroimaging studies utilizing fMRI, PET, SPECT are still on going. Increased activities in orbitofrontal cortex are repeatedly reported in many neuroimaging studies. Striatum, anterior cingulate cortex, thalamus, parietal cortex are also reported to affect in OCD patients. One of the neurobiological model from imaging data widely accepted had been OCD-loop hypothesis. OCD multi-dimensional model was developed by Matrix-Cols et. al. that unitary disorder view of OCD is flawed. The group summarized that research should focus on common and distinct etiological mechanisms but need to develop specific intervention for specific problems.

Search for candidate genes associated with OCD have been the topic in many studies. Some studies hypothesized that serotonin transporter polymorphism has been associated to OCD but still not clear and results are inconsistent. Neuronal cadherin gene (CDH2) region on chromosome 7 was highly associate with canine compulsive disorder. However, CDH2 was not associated with human OCD.

Brain-derived neurotrophic factor (BDNF) plays an important role in neural development. Studies showed...
that BDNF plasma levels are lower in OCD patients compared to healthy controls regardless of treatment status. BDFN seems to be an interesting candidate for molecular analysis in OCD reported interaction between this gene and neurotransmitters associated with this disease.

Human OCD studies and some reports from canine compulsive disorder studies suggest that different phenotype of compulsive disorders might have different pathophysiology and causes. Etiology and pathophysiology behind OCD and canine compulsive disorder are still “on going” search. Still many studies need to be conducted to fully understand the disease.

**Diagnosis**

- Rule out medical conditions. Differential diagnosis includes neurological disorders (central and peripheral), dermatological problems, endocrinological diseases.
- Rule out behavioral problems such as attention seeking behavior, conflict behaviors (e.g. behaviors to release frustrations), stress from other anxiety disorders, cognitive dysfunction.
- Observe the behavior itself. Video images of behaviors that animals presenting at home are helpful.
- Getting thorough history by using open ended questions and getting the whole story about several incidents including the response (or reaction) to the behavior of animals.

**Treatment**

Cases of compulsive disorders if identified early may be treatable without medication, but most cases require psychototropic medication and behavior modification on top of environmental modification.

**Medication**

- Anti-depressants: Fluoxetine HCl (Reconcile) Dogs 1.0-2.0 mg/kg Q24H Cats 0.5-1.0 mg/kg Q24H
- Clomipramine HCl (Clomicalm, Anafranil) Dogs 1.0-3.0mg/kg Q12H Cats 0.5-1.0mg/kg Q24H
  - Do not give anti-depressant(SRIs) with MAOIs (Selegiline).
- Anxiolytics (SSRIs will not show effect for the first 1-2month, thus if in need for quick relief of animal’s fear and stress, benzodiazepines may be helpful.
- Supplements and synthetic pheromones can be used
  - Alpha-S1-tryptic casein (Zylkene): works on GABA-A receptor to reduce anxiety, pheromones

**Environmental modification**

- Keep the environment safe for the animals as well as the family members. If aggression is involved, do not let the owner touch the dog to stop the behavior.
- No punishment for the behavior. This is a behavior from stress and frustration. Punishment will just increase stress and it will not help.
- Recognize and address stress and remove it if it is possible.
- If the animal is self-mutilating, introducing basket muzzle may help.

**Behavior modification**

- When the animal is showing the behavior or almost showing the behavior, distract the behavior, give other behavior that is incompatible, one animal give its response, reward for other behavior (response substitution).
- Be consistent especially interaction between the family and the animal should be consistent and predictable.
- Give proper stimulus to the animals. If needed, take some time to take dogs out to walk or play with cats are important.
- In order to conduct response substitution for treatment, basic obedience training and some trick teaching with positive reinforcement is recommended.
Keywords: Compulsive Disorder, OCD, Pathophysiology

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Aggression in Dogs

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Summary
✓ Category of aggression in dogs will be discussed
✓ Many of the owner directed aggression in dogs are from fear, anxiety, and learned.
✓ Treating owner directed aggressive behavior should not be done by “dominating your dog”. There is a better way to approach.

Aggression in dog is one of the most commonly asked behavior problems. Today’s topic will be owner directed aggression in dogs. covering all and every aggression in dogs will be 3 days topic. Thus, this time, the topic will focus on canine aggression directing to owners.

For many years, we taught clients with dogs who bites that the problem is likely “dominant”. The dog is possessing over space, food, or other resources to establish a hierarchical status. Some dog’s behavior is to need to guard the resources but there is no evidence that interaction with humans aim to establish ranks.

Careful observation and analysis were done to know human-dog interaction and those data indicates that aggression are commonly cause by miscommunication or anthropomorphic view.

A review of cases will be discussed in the talk and general guidelines for how to treat owner directed aggressive behavior in dogs will be focused for this talk.

Keywords: Dog, Aggression, Owner Directed Aggression
Zoonotic Influenza – The Human-Animal Interface

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Summary
Since the late 1990’s the recognition of the interchange of influenza viruses from animals to humans has captured the attention of the media and late night, talk shows with the mention of “avian influenza” and “swine influenza”. It is important to appreciate that influenza virus infections are not one directional just from animals to people but that people also play an important role in viral change and interspecies transmission. This presentation will review recent zoonotic influenza events, their public health consequences associated with this disease. In addition, a discussion about risk communication strategies to clients will be discussed.

Influenza A viruses are challenging with their ability to change and their potential to cause significant morbidity and mortality. Recently we have observed an expansion of the documented host range of animal infected (i.e. dogs and cats) and the identification of new subtypes such as those in bats. Seasonal influenza also has had dramatic impact on humans with significant costs economically and the associated health effects. (1) The number of zoonotic transmission events is far less frequent than seasonal influenza but do represent an emerging disease concern. Some animals such as pigs can serve as “mixing vessels” allowing for the development of novel reassorted viruses. In addition, the cross-over to other species has been noted such as the bird-to-marine mammal transmission of low pathogenic avian influenza (LPAI) viruses from birds to harbor seals. (2)

There are a number of recent and ongoing challenges regarding zoonotic influenza infections. This includes the ongoing H5N1 globally, the H7N9 infections in China, H3N2v infections in North American among swine exhibitors, and H7N2 among shelter cats in New York. These are just some of the events. In addition, there have been wide spread regional and global influenza outbreaks among domestic poultry with severe economic and trade effects. Many of these events have human occupational exposures requiring measures to monitor exposed employees and provide guidance and possibly therapy if indicated.

Recent human cases of H3N2v among swine exhibitors highlight the need for additional research and reasoned policies to protect the public. Swine exhibition outbreaks in 2012 and 2013 were linked to exhibited pigs at County/State fairs. There were over 300 illnesses reported with 16 individuals hospitalized. (3) Cooperation among Federal, State, industry, academic and fair organizers developed a working template to educate the public and venue organizers. (4)

These outbreaks in addition to the large highly pathogenic avian influenza outbreaks have encouraged research into the role of environmental contamination and air-borne spread. (5) Studies suggest the detection of influenza viruses from environmental surfaces and air in live animal markets. Similar research has detected high concentrations of viruses within affected barns. This has implications for inter-barn and occupational spread.
In summary, influenza viruses are constantly changing and what we know about these viruses is changing. It is important to monitor these changes through good surveillance networks both human and animal. In addition, we have an important role in helping the public understand the true risks of zoonotic transmission and to take appropriate precautions including routine hand washing, basic hygiene to reduce exposure if ill, and appropriate vaccination.

**Keywords:** Zoonotic Influenza, Human-Animal Interaction, One Health

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Antimicrobial Stewardship for Companion Animals

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Summary
Companion animal practice has not been immune to the global challenge of antibiotic resistance. Within the past decade many veterinary practitioners, now observe patients with multidrug-resistant *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus* infections, including the emergent methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). As individual companion animal practitioners, we need to be aware and encourage the practice of antimicrobial stewardship principles.

From the AVMA – Task Force for Antimicrobial Stewardship in Companion Animal Practice


Companion animal practice has not been immune to the global challenge of antibiotic resistance. Within the past decade many veterinary practitioners have been confronted with multidrug resistant infections for which there are limited effective antimicrobial drugs. Practitioners now observe patients with multidrug-resistant *Escherichia coli*, *Klebsiella* sp., and *Staphylococcus* infections, including the emergent methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). Fortunately, compared to human medicine, methicillin-resistant *Staphylococcus aureus* (MRSA) is less common in pets. Most of these may be community-acquired or occur in the clinic setting. The isolation of invasive and potentially nosocomial agents in the clinic setting are often associated with poor treatment outcomes and longer hospital stays. The antimicrobials most effective for these infections are often more expensive than commonly used approved veterinary antimicrobials, and can produce more adverse reactions. Additionally, many of these organisms are found in both humans and companion animals, highlighting the possibility of zoonotic transmission.

Today, many human patients are contracting infections that cannot be treated with currently available antibiotics (1,2). It is well recognized that with antibiotic use there is “selective pressure” for the emergence of resistant bacteria in both human and veterinary medicine. It is estimated that 50% of antibiotics are unnecessarily or inappropriately prescribed in human medicine (3). Likely, that percentage is similar in companion animal settings. As such, efforts to promote judicious use or antibiotic stewardship are needed and important to combat the rise in antimicrobial resistance. The term “stewardship” has been widely used as a way to consider how to optimize the use of antibiotics. This term considers the “benefit of antibiotic use to the patient while minimizing the development of antibiotic resistance and adverse effects on patient from unnecessary therapy” (4,5). In addition, the Report to the President on Combating Antibiotic Resistance outlines a number of incentives and approaches to encouraging antimicrobial stewardship and includes antibiotic uses in animal agriculture (4).
Over the past 10 to 15 years, nearly 50% of human health care hospitals have instituted antimicrobial stewardship programs (4). These programs often contain several strategies including prescriber education, hospital formulary restriction, required antibiotic approvals before dispensing, streamlining or de-escalating therapy, and computer assisted programs that both track use and provide clinician guidance (5). Many programs appear to be successful. Initial reviews of their value demonstrate a reduction in the percentage of antibiotic resistant organisms in hospitals, a reduction in the occurrence of *Clostridium difficile* infections, improved patient outcomes, and reduced costs (6-10). It is possible that some of the strategies that have been successful in human medicine will also be effective in companion animal practice. Yet, there may be additional appropriate strategies. In production animal medicine a number of practices such as implementation of Quality Assurance programs have been instituted to educate and encourage appropriate antibiotic use. Recent guidance documents by the U.S. Food and Drug Administration also provide recommendations and guidelines on antimicrobials use in animal agriculture (11-12).

The American Veterinary Medical Association recently formed a task force to provide guidance for implementing antimicrobial stewardship programs in companion animal practice (TFASCAP). This committee demonstrates an interdisciplinary assemblage of professionals from varied backgrounds representing clinical practice, government, infectious disease, pharmacology, industry, and public health. Their goal has been to consider the emerging impact of multidrug resistant organisms in companion animal practice and design approaches to address this challenge.

Collectively the task force has developed several strategies to help our profession combat the serious threat of antimicrobial resistance. Some of these activities include devising assessments to better understand laboratory practices and practitioner prescribing behaviors, providing general “Do's and Don'ts” of antimicrobial prescribing, supporting the development of local/regional antibiograms, and creating educational programs/materials for practitioners and clients. The objective is to roll out these efforts to practitioners over the next year and encourage an active discussion promoting stewardship in our companion animal practices.

Also, other organizations are supporting these stewardship efforts. The International Society for Companion Animal Infectious Diseases sponsored and provided guidelines for common clinical conditions in veterinary medicine, such as urinary tract disease and superficial pyoderma (13-14). These efforts represent an urgently needed collaborative and consensus building activity of our profession to provide clinical guidance to practitioners.

As a profession, we need to clearly recognize the grand challenge of antimicrobial resistance and encourage initiatives that will slow the progression of resistance. Along with the recent activities of the WHO, CDC and the Presidential Task Force, the veterinary profession can reduce the selection pressure that favors spread of antimicrobial resistant bacterial pathogens. As individual companion animal practitioners, we need to be aware and encourage the practice of antimicrobial stewardship principles. Our efforts will need to be broad, multifactorial, and incorporated at the clinic level. Collectively, we need to take action now.

**Keywords:** Companion Animal, Stewardship, Antimicrobial

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**Why Should you Know About Basic Epidemiology?**
*Because You Use It Every Day*

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**Summary**

Epidemiology is typically considered applied to food animals and wildlife because of the study of disease in herds. However, small animal veterinarians also deal with animal populations, which are even more dynamic than those of large animals. The exposure of pets to different environments and animals in those environments make the possibility of disease outbreaks more likely in small animal clinics than in large animal conditions., hence the extensive use of preventive vaccination protocols in dogs and cats. Examples will be presented of other common epidemiologic methods used daily in veterinary clinics, such as a simple TPR, demonstrating the need to understand the basic concepts and techniques in epidemiology to correctly apply their insight and improve daily practice outcome. When you know that age, breed or diet are demonstrated epidemiologic risk factors for musculo-skeletal disease, you can learn how to use published research to prevent or palliate the effect on your patients.

Epidemiology has been widely considered a discipline restricted to veterinarians working with food animals and wildlife herds because of the inherent study of disease dynamics in populations. However, veterinarians working with companion animals need to consider that their patients interact with several different and evolving populations and, therefore, companion animal veterinarians should be familiar with basic epidemiologic concepts and techniques. In fact, companion animal veterinarians should be more so focused on epidemiologic information than large animal veterinarians because their patients interact in open populations that are not common to large animals. Consider that the average dog patient seen at a small animal clinic interacts with other dogs at the dog park, other pets in the household, if they are housed at a kennel, or even at the clinic, and may be exposed to travel. A vacation that takes a dog to a different country with their owners may expose all the other patients visiting the veterinary clinic after the trip to exotic diseases that they would otherwise never be exposed to. In this case, knowing the epidemiologic concepts and techniques to evaluate, stop and prevent outbreaks will be paramount for the veterinarians working at that clinic. This is one of the major reasons for repeated vaccinations in small animal populations, compared to large animal herds.

Besides the obvious situation of disease epidemics in the situation of a veterinary clinic that acts as a point of common exposure to the patients, veterinarians working with companion animals use epidemiologic concepts every day without being aware of it. A simple TPR, which should be performed in every animal being visited, uses the epidemiologic concepts of risk factors and outcome-evaluation. When determining the rectal temperature of an animal, the veterinarian is classifying the patient as being “within normal limits” or above or below the normal limits. As such, every classification then leads the veterinarian down a path of potential “causes” and consequences of abnormal values. For example, an “above average” temperature will indicate that compared to the normal values, this specific patient is presenting clinical signs consistent with the presence of an infection, heat stroke or ovulation. The reason we know the normal range, is because
someone did the studies to determine what is normal, and therefore establish a control group. The reason we know that an elevated temperature is consistent with infection or heat stroke is because someone did the studies to evaluate the effects of those conditions on each specific species. The application of previously-acquired knowledge that evaluated the association between risk factors and outcomes is the prototype use of epidemiology.

Similarly, as clinical veterinarians, we know to ask such things as age, breed, housing environment and diet as potential risk factors for diseases that may not yet be present but are more likely to develop over time when a patient is chronically exposed to them. By knowing the risk factors, and what a case of disease looks like, we can discuss with the owners the option to decrease or eliminate them from the environment of the patient. And when the risk factor is inherent to the animal (such as age or breed), we can establish preventive and palliative measures to decrease the effect of the risk factor on the animal. For example, we know old age is a risk factor for chronic musculo-skeletal disease, and that the risk of disease is higher and the consequence worse in obese patients. Therefore, when presented with a patient of advanced age that has a high body condition score, we know to advise the client to put the patient on a diet to decrease the probability and severity of disease in future years. This is the application of epidemiology in our day-to-day practices.

To effectively use epidemiology in daily practice, veterinarians need to understand the appropriate terminology, how to evaluate what can be applied and when it can be applied. This will be divided in various sections in the following lectures to make it easier to digest.

Basic information we will cover include how to correctly define a case of disease so it can be studied and compared correctly, and what are risk factors and how to evaluate them. Another major area to be covered is to understand how to read a research paper and determine what information can be drawn from it, including information on sensitivity and specificity of diagnostic tests. To understand if the research studies that we read can deliver information we are looking for, we need to know whether the study designs are adequate or not. This is also covered in one of the lectures.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Applied Epidemiology, Clinical Practice, Population

**References**

Case Definition & Study Unit Selection, the Foundation of Epidemiologic Studies

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Summary
Case definition and study unit selection are two of the most important steps required to be done correctly so that research studies can have internal and external validity, and therefore reasonable conclusions can be drawn and applied in daily veterinary practice. Clinical veterinarians use case definition to approximate a diagnosis, or at least obtain a list of differential diagnoses. When evaluating what published research to use to guide their actions, clinicians need to understand the differences in case definition and unit of analysis between multiple studies to determine whether they are comparable and whether the results can be integrated or not. Examples are presented on how to correctly apply these two basic principles to daily practice.

The basis of epidemiology is to describe how disease occurs, because it is not a random occurrence. To evaluate risk factors that are associated with a disease, we first need to clearly define the presentation of a case of disease. Typically, this is performed by a detailed description of the clinical signs of a diseased patient. This is the case definition. However, it includes not just the clinical signs, but which animals are most likely to be affected, as well as the geographic and temporal distribution of the condition. The more detailed a diseased individual is described, the less likely it is to be confused with a different condition. For example, defining a diseased dog as one with an elevated temperature can describe many conditions that may require even opposite or conflicting treatments such as heat stroke and infection. However, defining the case as an intact adult female dog with elevated temperature, polyuria and polydipsia in the weeks following estrus, not only strongly indicates the presence of an infection, but specifically pyometra. Applying the knowledge of epidemiologic risk factors to the description of a case of disease greatly enhances clinical outcomes due to early recognition and intervention.

The importance of the case definition is further exemplified when comparing and integrating results from two or more research studies. For example, consider a study that evaluates hip dysplasia in dogs using a 5 point scale with another one using a 7 point scale. How would you compare as score of 4/5? Would it be similar to 5/7 or 6/7? Another example may be contrasting the results of two studies on bovine leucosis where one uses blood smears to evaluate the leukogram, while another one uses an ELISA test to detect antibodies against the virus.

In research studies, it is imperative to establish what is called the unit of analysis, which indicates at what level is the outcome measured. For most studies, the unit of analysis is the individual animal. Some studies however require a group of animals to be the unit of analysis, especially when dealing with defined populations such as a kennel. Yet other studies may require a smaller unit of analysis, such as one of multiple similar body parts (e.g. eyes, ears, lymph nodes, kidneys, teats or legs). Which one is used in the study will determine whether the risk factors applied to the outcome are more or less similar or not. For example, consider a
study of glaucoma in dogs. If the study is evaluating the effect of diet on glaucoma, it makes sense that the unit of analysis would be the dog, because diet would equally affect both eyes. If, however, the study is evaluating the effect of a specific eye drug, then the unit of analysis would be best set at the eye level, where one eye would be exposed to the drug while the other would not. This way the non-exposed eye would act as a matched control to the exposed eye, where all other risk factors (diet, environment, breed, age, etc.) are kept equal, because both eyes belong to the same dog.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Case Definition, Unit of Analysis,

**References**
Successes and Future Challenges in Food Safety

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Summary
Great strides have been made in improving food safety over the past few decades. In certain countries, outbreaks due to intoxications such as Staph or Bacillus have been greatly reduced due to improvements in refrigeration and food handling. In addition, on-farm measures have reduced the number of individuals exposed to Taenia solium, Trichinella spiralis, and Toxoplasma gondii. Many of these successes are due to improved processing, good agricultural practices (GAP) and consumer education. Yet, ongoing outbreaks continued to be reported with new or novel agents, such as E. coli O104 and Cyclospora. In addition, many traditional foodborne pathogens are being attributed to novel vehicles (Salmonella and peanut butter, E. coli O157 and leafy green vegetables, and Listeria and apples). What challenges awaits us in the next decade? This session will discuss some of the factors that have changed the epidemiology of food-borne disease, understand why foodborne disease is an increasing and evolving problem, and lastly, address what we, the health-care community, need to know for the future to prevent these infections.

Introduction
Despite the efforts of public health, the burden of foodborne disease continues to occur. Many factors that contribute to the incidence of foodborne disease have been changing and will continue to change. These factors include diet, greater importation of foods, commercial food service, labor shortage, and new methods of food production done on a larger scale. (1) With the ongoing challenges there have been a number of successes. In the U.S. and many countries, there has been a decrease in Taenia solium, Toxoplasma gondii, and E. coli O157. This have been attributed to inspection systems to identify and remove contaminated products. This includes the advent and incorporation of the Hazards Analysis and Critical Control Point Program (HACCP)

With the increasing wealth of countries, there is a greater demand for a variety of food. For many the varieties and volume of food have never been more available or more affordable. In addition, with the rising rates of obesity and concerns about heart health there is a call to consume more fruits and vegetables. The heart healthy and cancer preventing diet that has emerged presents a challenge for the gastrointestinal tract. In the U.S. there has been a dramatic increase in per capita consumption of fresh fruits and vegetables resulting in the increasing demand for fresh produce throughout the year. For the American diet there is an increased percentage of selected produce items coming from around the world. This is largely attributed to the growing global food network.

The result of this change has been dramatic. To put this information into perspective, most outbreak-associated illnesses were from vegetables (e.g. cucumbers and tomatoes) accounting for 1121 illnesses in 2015 in the U.S. (2) Many of these outbreaks occur in restaurant settings. Some of these are due to contamination
In this emerging global food system, we continue to see major changes in the methods of food production and distribution. Recent nationwide outbreaks of salmonellosis are associated with a number of new and novel agents. These include peanut butter, ice cream, sprouts, caramel apples to name a few. In addition, there have been a number of new and novel agents including Entertoxigenic, Enterohemorrhagic, Enteroaggregative _E. coli_, Cyclospora, and the prion associated with bovine spongiform encephalopathy. These changes represent new challenges.

With these new challenges, we must employ new tools. This should include more responsive and inclusive surveillance systems, utilizing social media to rapid detect new outbreaks, new rapid diagnostic tests, and methods that improve the detection of foodborne agents. Included in these efforts is the need to train a workforce to respond, detect and prevent foodborne diseases. Another challenge is the move away from culture-based techniques. This has potential impacts on genomic characterization of pathogens and our current ability to detect outbreaks.

**Keywords:** Food Safety, Disease Surveillance, Novel Pathogens

**References**


Why Hasn't Our Efforts Affected Human Salmonella Infections?

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Summary
There have been multiple approaches applied to control foodborne diseases. The implementation of HACCP has been cited as one of the reasons for a decline in human illness due to E. coli O157 in the US. Why have those same interventions not worked for Salmonella? This session will review the major foodborne diseases and discuss possible explanations and interventions to control Salmonella.

Background
Salmonella infections causes an estimated 1 million illnesses, 19,000 hospitalizations and 350 deaths each year in the United States. (1) Listeria monocytogenes or E. coli O157 infections have declined over the past 15 years while the rates of Salmonella infection in the US have gradually increased. During 1973 – 2012, CDC received reports of nearly 30,000 foodborne disease outbreaks, many of these are attributed to Salmonella.

In fact, Salmonella foodborne outbreaks (n=63) accounted for over 50% of the multistate outbreaks in the United States over the past 5 years. (2,3) These outbreaks accounted for the majority of illnesses (82%) and hospitalizations (65%) in these multistate outbreaks. These outbreaks were attributed to varied sources including eggs, chicken, scraped tuna product, fruit, vegetables, nuts and seeds, and sprouts. Salmonella serotypes Newport (10 outbreaks), Enteritidis (6 outbreaks), and Javiana (5 outbreaks) were the most common.

Continued challenges include the survivability of different Salmonella strains, the low infectious dose, and the development of multi-drug resistant strains. In addition, as a more heart healthy diet is encouraged the public is consuming more fresh fruits and vegetables in the U.S. diet and increasingly these products are linked to foodborne illnesses. As a result, some food safety advocates have put forth a number of recommendations to reduce the impact of Salmonella. Some include developing a “zero tolerance” policy for Salmonella in raw meat and poultry or classifying specific multi-drug resistant strains of Salmonella as adulterants in ground beef and poultry products.

Future control efforts and recommendations include continued improvement in outbreak detection, response and coordination. There is a need to adopt newer, faster, less expensive molecular methods to identify clusters and outbreaks. Salmonella performance standards and policies have recently been revised and currently the USDA has draft FSIS Compliance Guideline for controlling Salmonella and Campylobacter in Raw Poultry. Clearly there is a need for additional innovative measures to reduce the number of Salmonella illnesses attributed to food products to meet the Healthy People 2020 goals.

Keywords: Salmonella, Outbreaks, Food Safety
References
We Know Very Few Causes of Illness, but We Know a Lot of Risk Factors. What Is the Difference?

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Summary
Commonly, veterinarians talk about the cause of specific diseases, when, in reality, they are referring to risk factors. The difference between them is the fulfillment of specific criteria for a risk factor to be considered a causative factor. These criteria are known as Hill’s Criteria and are (1) temporal association, (2) strength of association, (3) consistency of association, (4) specificity of association, (5) dose-response association, (6) biological plausibility and (7) analogy of association. Not all criteria have to be fulfilled at once, but the more criteria are fulfilled, the stronger the evidence for a risk factor to be considered the cause of a disease. Different denominations of risk factors are explored.

It is common to hear veterinarians talk about the “cause” of a specific disease. In most cases, this is an inaccurate description of what is truly only a “risk factor”. So, what is the difference?

A risk factor is any situation or animal characteristic that can influence the probability of an event or disease. For example, cold weather is a risk factor for respiratory disease in most animals, but it is not the cause of the disease. To be considered a cause, a risk factor needs to fulfill some specific criteria that are known as Hill’s Criteria for Causation, which are:

1. Temporal association: the causative risk factor needs to be present before the disease appears
2. Strength of association: the causative factor needs to have a strong association with the appearance of disease, otherwise it can be a haphazard association
3. Consistency of association: the causative factor needs to be consistently present in situations in which the disease is observed
4. Specificity of association: the causative factor tends to be associated with specific disease/s only, as opposed to present in many other diseases
5. Dose-response association: for example, when a higher presence of a causative risk factor is associated with a higher incidence of disease. This is also called biological gradient and can be positive or inverse (i.e. the higher the risk factor, the lower the incidence of disease)
6. Biological plausibility: the association between the causative risk factor and disease needs to make sense biologically
7. Analogy of association: the association between the causative risk factor and disease is similar to that in other animals.

Not all of these criteria need to be fulfilled at once, but the more of these that a potential risk factor fulfills, the more likely it is to be a causative factor. The most important by far is the temporal association, which is why retrospective studies can NEVER establish causation, only association. Retrospective studies evaluate historical records after the fact, and therefore it is very difficult and sometimes impossible to establish whether
a specific risk factor was present before or after the disease.

Notice the use of the word “association” to identify that presence of a certain relationship between a risk factor and a disease. This is the way commonly agreed among epidemiologist to refer to characteristics that are somehow related to a disease, but are not the sole risk factor necessary to cause disease. This latter one is known as “necessary risk factor”, without which disease does not happen. Good examples of necessary risk factors are infectious diseases that necessarily require the presence of a specific organism for a disease to appear (e.g. rabies, tuberculosis, etc.). A “sufficient risk factor” is one that does not require other risk factors to cause disease. A good example of this type of risk factors are mutations or alleles that cause disease genetic. Their presence alone will cause disease, independent of the presence of other risk factors. An “insufficient risk factor” is one that requires other risk factors to be present to cause disease. A good example of this is the necessary risk factor of *Eimeria spp* for causing diarrhea in multiple species, which is insufficient by itself, unless it is present along with other risk factors such as extreme temperatures or nutritional impediments that lead to malnourishment, and therefore immunodepression.

For risk factors to be considered causative it is imperative that the association is repeatable over time. This is why studies are repeated multiple times and under different circumstances; to determine whether the association stays consistent.

An inverse biological gradient or dose-response association is commonly found with vaccines, up to a certain limit, exposure to more doses provides smaller opportunity for disease to appear. This is a risk factor commonly referred to as a “protective factor”. Being a risk factor implies altering the incidence of disease, as a vaccine does. Being a protective factor implies being a risk factor (because it alters the incidence of disease) but only in a specific direction of lowering the incidence of disease.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Cause, Causative Factor, Association

**References**
Epidemiologic Study Designs and When They Are Appropriate

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Summary

Epidemiologic study designs imply different methodology that can facilitate cost or feasibility of a given research question. It is important to know that studies that look into the past for potential risk factors of an outcome that can be measured today (retrospective studies) do not have the ability to establish causal relationship because it is impossible to determine if the risk factor was present before the disease or not. Typical retrospective studies in the veterinary literature are surveys, case reports and case-control studies. Each one requires their own methodology to be able to allow appropriate conclusions. In contrast, prospective studies tend to be expensive in cost and time, and therefore limited to institutions with the resources. The most common prospective studies in the veterinary literature are clinical trials and cohort studies. The main difference between the two is that in clinical trials the exposure to a risk factor is controlled, while cohort studies are observational studies where the exposure is not controlled, but occurs in nature. Somewhere in between are cross-sectional studies that measure at the same time the risk factor and the outcome in the same individuals. They tend to be the least expensive, but also the ones with the most pitfalls.

When research studies are conducted, different methods are to be used based on what the study question is and what resources are available. Both are important to determine what study design is best suited to answer a specific question. Study designs can be classified according to several characteristics. One characteristic is whether the animals are subject to some type of intervention, in which case they are clinical trials, or not, in which case they are observational studies. Another characteristic is whether the study is performed looking into outcomes in the future (prospective studies) or risk factors in the past (retrospective studies). Looking into the timeline of a study, there also are cross-sectional studies that look at risk factors and outcomes at the same time. By definition, clinical trials are prospective studies, because they begin with animals that are not exposed to the risk factors under investigation and are followed into the future to observe and measure the outcome. Another characteristic of studies related to veterinary medicine is the need for statistical analyses to evaluate the association between risk factors and outcomes (analytical studies), or simple description of findings in a population (descriptive studies). An overall view of the different study designs and how they relate to each other is presented (Fig. 1), and the flows of information direction (Fig. 2) are presented below.

Figure 1 Comparison of flow of information in prospective and retrospective studies
We will cover only the most common study designs seen in the veterinary literature. For more study designs, the reader can refer to epidemiology textbooks.

Retrospective Studies

These are probably the most commonly observed studies, especially for new conditions, where the researchers start with animals with a known status of disease and they evaluate their past to identify potential risk factors for that disease. For these types of studies to be effective, there need to be good records of each animal that detail exposures and changes over time. There are three main types of retrospective studies: descriptive studies, case-control studies and surveys.

Descriptive studies

The epitome of descriptive studies are case reports. In these types of studies, one or more case of animals with a similar presentation and clinical signs are described. Typically, the number of animals in these reports is low, and the information is often incomplete and scattered, because the condition that is being observed is unknown at the start of the evaluation of the animals, and not all cases may have the same information available. These studies are interesting starting points, but, typically, nothing more. It is impossible to determine whether a specific diagnostic technique or treatment is the best course of action.

![Figure 2 Types of study design according to different characteristics](image)

Surveys

Surveys are commonly favored by veterinarians, but they have to be conducted correctly to allow drawing appropriate conclusions. There are entire books written about how to correctly conduct surveys, so we will not delve into it. The three main rules I like to observe when designing survey questions are (a) not to use open-ended questions, (b) not allow the provided multiple-choice answers to be open to interpretation, and (c) ensure the multiple-choice answers provided cover all spectrum of possible answers. For example, simply asking what the diet of a dog is will lead some owners to a very detailed answer, while others may simply say ‘dog food’. When asking dog owners whether their pets are fed raw foods, the question may draw a ‘yes’ answer both from owners that give their pets fresh fruit and those that allow them raw meat. If the survey is intended to evaluate the availability of raw meat, asking for ‘raw food’ is a question open to interpretation, while asking for ‘raw meat’ is not. Typical questions open to interpretation are those that allow answers of ‘often’, ‘seldom’ and ‘sometimes’. Similarly asking in a survey if a dog has a white coat and allowing only the answers ‘yes’ and ‘no’ will leave some owners debating the answer if their dog has a multicolor coat with white markings.
Surveys can be used as descriptive studies or analytical studies (if control animals are enrolled). They can also be very efficiently used to identify potential risk factors, when done correctly. However, as any retrospective study, it will be impossible to establish the cause of a condition using surveys, because we don’t know if the potential risk factor was present before or after the disease or condition appeared. In other words, causality can never be tested.

The main advantage of surveys is that they can collect a lot of information in a short period. The main drawback is that the information may not be accurate, as it relies on memory and willingness to participate in the survey. These two problems with surveys are typically referred to as recall bias and selection bias, respectively.

**Case-Control Studies**

A ‘case’ is an animal that presents the condition that is being studied. A ‘control’ is one that doesn’t. Therefore, a case-control study compares information about animals that are known to have the condition of interest (cases) and animals that are known **not** to have the condition (controls). Their records are evaluated to find what exposures (risk factors) are found more commonly in cases compared to controls. Therefore, case-control studies are analytical in nature. The main advantage of case-control studies is that fewer animals are usually needed because we start the study with known affected animals, which leads to typically less expensive studies. The main drawback is their retrospective nature, which doesn’t allow these studies to be used to establish a causal relationship, only association between risk factors and outcomes. Another major problem is usually the lack of detailed or accurate records in the past to look for good information on risk factors.

**Cross-Sectional Studies**

Studies that measure at the same time the risk factors and the outcomes in the same animal are called cross-sectional studies. They are also very common in veterinary medicine, mostly to establish prevalence of a disease or condition, or to try to identify possible risk factors. Again, because they are not prospective studies, they cannot be used to determine a causal relationship.

**Prospective Studies**

These are the most expensive research studies, and, because of that, not as common in the veterinary literature as the previous ones, although they are the preferred type of study designs to consider a study as evidence, and they are the only ones capable of proving causality. They do require comparison between two or more groups that had different risk factors. Because of this, they are populating research journals more frequently in the recent past. They include clinical trials and cohort studies.

**Clinical Trials**

A clinical trial is a study in which the study group is subjected to a specific intervention, where one or more risk factors are controlled. At the same time, it will be ensured that the control group is not exposed to that risk factor. The main advantage is the possibility of controlling risk factors that can alter the result and therefore isolate the study as much as possible to one specific risk factor. The main drawback is the extent of control that is required, which increases the cost of the research. Another major drawback is that in low-incidence diseases or those with long latent periods, it takes a long time to conduct these studies, with the added control of exposures and costs. Lastly, this type of studies may cross the line to unethical treatment of animals by either withholding known treatment (control groups) or exposing animals to harmful situations (study group).
Cohort Studies
The quintessential observational studies. They entail comparison of outcomes in two or more groups that have different exposure to risk factors, but this exposure is not controlled, it is natural. The main advantage is that they allow observation of the natural interactions of animals, risk factors and disease, while the main drawback is the expense of investing in a study in populations that are not guaranteed to develop the condition.

This is a short summary of the main epidemiologic study designs used in the veterinary literature. There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

Keywords: Case-Control, Survey, Clinical Trial

References
Evaluating Published Research: How to Understand What They Did

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Summary
Not everything that is published is scientific evidence. To become scientific evidence, the results need to be consistent and, for that, the studies themselves need to be repeatable to the minimal detail. Therefore, the Materials and Methods section of papers need to be detailed, walking the reader through the study design step by step. Items to be included are: (1) which animals or populations of animals were included and excluded (preferably saying why); (2) definition of a control group for comparison; (3) how data was collected, being specific as to who, what, when, where and how, including instrument names and brands; (4) definition of the risk factors and outcomes that were studied, as well as how they were measured; and (5) detail the type of statistical analysis performed, including sample size calculation to determine whether the results are statistically significant or not, based on the biologically significance assigned by the authors. Only when the study can be mapped out completely can it be understood and the conclusions can be evaluated as to whether they are warranted or not. Common pitfalls in current literature are presented, such as the lack of an appropriate control group. Consider for example a study that evaluates the impact of flea medicine on atopy on dogs, where the paper does not specify which flea medicines were used, or doesn’t indicate the breeds of dogs included in the study or the environment in which they live. Always question if the study design allows for known potential risk factors to be influencing the results and, therefore, skew the conclusions. The Materials and Methods section of the paper is the essence of what was done in that study and, besides the results, the most important part of a paper.

Published research is considered the foundation of “Evidence-Based Medicine” (EBM) making it almost sound like anything that is published becomes truth, assuming that now there is “scientific evidence” for a specific point of view. There is a lot of pressure on peer-reviewed publication of research as the only source of scientific evidence, which has resulted in multiple new journals to accommodate all aspiring authors. Unfortunately, not everyone who writes or reviews a paper has adequate training on what constitutes appropriate epidemiologic studies, which has led to publication of multiple studies that do not have the means to prove the conclusions that the authors ended up with.

Most research used for EBM purposes is based on finding why a specific disease happens, its cause or causes and how to treat it. These three questions imply causality, and as discussed in a previous lecture, causality requires specific characteristics, one of them being repeatability of results. This means that multiple research groups undergoing the same type of experiment, should be able to obtain similar results. For this to happen, each study needs to be detailed in such a way that it will be able to be reproduced by others with the same conditions. If the conditions are identical but the results are not the same, the association of risk factors and outcomes is not consistent, indicating that the relationship between the studied risk factor/s and the outcome is not causal and the researchers need to consider other risk factors to explain the results.
If the conditions of two separate studies differ and the results differ, it will not be possible to identify if the different results originate from the different conditions of the two studies, such as the presence of another risk factor, or because the relationship between the risk factors and the outcomes are not consistent.

The details of how a study was conducted are spelled out in the Materials and Methods section of a paper. Items to be detailed in this section include anything that can be considered a risk factor or was controlled for during the study, step by step. Some simple examples are presented that may seem silly because they are obvious, but will hopefully drive the different points home, to allow later evaluation of other not-so-obvious risk factors. Always question if the study design allows for known potential risk factors to be mudding the waters.

1. Inclusion and exclusion criteria
Because it has been described at some point that age, breed, gender, body condition score, location, environment, diet and other inherent individual factors such as coat color can be risk factors for a specific disease, when a study is designed, all previously known risk factors should be considered to select the most uniform group of individuals possible. This will eliminate variability in the results due to reasons other than the studied drug or management option. Consider for example a study of the potential effects of puppy diets on hip dysplasia in dogs where it is not specified which breeds are included and excluded in the study. Imagine that by random allocation, one of the groups had more German Shepherds and Labrador Retrievers than the other group. The results of the study could be doubted because we know that one of the groups has more animals belonging to high risk individuals for hip dysplasia than the other group.

It would be best if the paper detailed why individuals are included, but especially why any individuals were excluded from a study. For example, a study of a new treatment for ketosis in cattle, should exclude any other concomitant diseases such as retained placenta, metritis, mastitis and lameness, as it is well-known that these diseases can lead to negative energy balance on their own and would mask the results of the study looking into treatment of simple ketosis.

2. Definition of the control group
Clinical studies that evaluate the effect of a specific risk factor/s (drug, treatment protocol, surgery, etc.) onto an outcome need to have a group of animals that have the same conditions of inclusion and exclusion as the study group, but that are not to be subjected to the experimental protocol. These animals make up the control group. The best possible study design is one in which the only difference between the study and control groups is the risk factor that is being investigated. However, this is often impractical, and therefore all differences with the study group need to be detailed. For example, consider a study of the effect of raw meat in the diet and allergies in cats. If the control group were to be indoor cats compared to barn cats (assuming that barn cats hunt and eat mice), the comparison is obviously including other risk factors such as environment that could affect the outcome of allergies that is being studied. The lack of an appropriate control group is one of the most common mistakes observed in published literature.

3. Data collection
This part of the paper is the most tedious in well-detailed papers, as it should include everything about the instruments, drugs, protocols and techniques used: who did what, when, where and how. Doses need to be specified, order and time interval between procedures, environmental conditions such as temperature and ventilation, drug and instrument brand names, calibration processes, data recording, etc. Imagine for example a study of anemia in foals, where some of the blood was tested via hematocrit tubes and others via smears.

In recent years, many papers include a diagram that clearly indicates inclusion and exclusion criteria for
animals into the study, as well as sample collection and drug administration. These diagrams serve as a map of how the study was conducted. If this map is not presented in the paper, the detail in the Materials and Methods section should be enough for the reader to build this diagram based on the verbal description. If it is difficult for the reader to build the study design from the information presented, it will be difficult to evaluate if the conclusions are warranted or not.

4. Define studied risk factors and outcomes, and how they are measured
This tends to be the part with the laxest definitions in the current scientific literature, probably because researchers may think it is obvious by the objective of the study. However, many studies have been published that fail to detail how some of them were measured or, suddenly, report on a risk factor in the Results or even Discussion sections of the paper that were not even mentioned in the Materials and Methods section. Consider for example a study aimed at evaluating the effect of external flea medicines on internal parasite load in cats. The obvious risk factor is the external flea medicine and the obvious outcome is internal parasite load. However, how were both measured? How many doses of flea medicine were applied? How long ago was the flea medicine applied? Which type of flea medicine? Can there be a difference between different flea medicines? How is the “internal parasite load” defined and measured? Does it include only fecal egg count, or does it include a blood smear to identify filaria and other hematologic parasites? Experts in this theme can probably think of other questions that may interfere with the study.

5. Detailed statistical analysis, including sample size calculation
This part tends to be the one that most readers ignore because they don’t favor statistical analyses. However, it is the one that determines whether the results can be considered real or not. A well-designed study allows for simpler statistical comparisons than a study that has too much variability in the risk factors or exposures. You don’t have to be a statistician to understand most of the veterinary literature. Variables in veterinary medicine can be either continuous (degrees Fahrenheit, ml/dL, mm, etc.) or discrete (BCS, lesion scores, categories, etc.). The most important rule to obey is to use parametric analysis methods on continuous data, and non-parametric analysis on discrete data. Try to get familiar (not an expert) on the most common statistical analysis used to compare the mean measurement of 2 or more groups (parametric: t-Test, ANOVA) and which ones are used to compare proportions of animals that have one result or another (non-parametric: Odds Ratio, Relative Risk). Also, understand how to interpret the results and the difference between statistical significance and biological significance. Statistical significance indicates the probability that the result obtained is due to chance alone (i.e. a freak of nature). Typically, it is set at 5%, but it can be set at a different level by the researchers, depending on the conditions of the study. It merely says how confident we are that the results may not be random. Biological significance, on the other hand, indicates the importance we give to the magnitude of the difference between the groups, and this is subjective to every reader. For example, a study showing a 10% improvement in survivability of a specific cancer may be considered a great result by one reader, while not enough for another considering all the side effects of the studied drug/procedure.

When designing a study, the second thing, after determining the risk factors and outcomes, is to determine what difference between the control and study group will be considered “enough”. That is, what difference will determine the biological significance between the groups. With that, and knowing the baseline expected in the control group, a sample size is determined that will allow to detect that difference between the groups if it exists. Studies that don’t do sample size calculations can only be considered preliminary studies that are fishing for some differences to guide a later study. For example, if we determine that we will consider adding a specific acupuncture protocol to oral carprofen to be effective against hip pain if dogs treated with that protocol show at least a 15% improvement compared to a control group treated only with oral carprofen, and we consider that carprofen alone works well in 60% of dogs, that means that our study group needs to improve by 75%. The sample size calculation for this difference from 60% to 75% can
be performed online at calculators such as this one. The result indicates that we would need 152 dogs in each group to be able to prove that the studied acupuncture method in addition to oral carprofen is indeed better than oral carprofen alone. If the study only enrolls 50 dogs per group, and the difference is indeed 75% vs 60%, respectively, it will not show statistical significance. To show statistical significance with a sample size of 50 dogs per groups, the acupuncture group would have to show at least 85% improvement, or 25 above the control group.

As it can be observed, there is a lot of information that a scientific research paper should provide in order for the readers to be able to evaluate exactly what was done, and whether the results and conclusion are warranted. In summary, the key to identify a well-reported study design is that the Materials and Methods section of a paper should detail the research in such a way that anyone reading it can duplicate the study.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Evidence-Based Medicine, Materials and Methods, Study Design

**References**

Evaluating Published Research: 
What Information Can You Take Home from It?

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Summary
As we saw in a previous lecture, not everything that is published is scientific evidence. Sometimes, the study design does not allow for unbiased conclusions. However, often, the conclusions drawn by the authors are defined by their biased views or by inadequate statistical analyses that lead to erroneous conclusions. Readers need to be able to evaluate the results on their own and make their own conclusions based on the study design and results. The conclusions drawn from a research paper may be different for various readers and even the authors, if they have different underlying beliefs or points of view of a specific disease or condition. The conclusions of a research paper in the end are the biological significance of the study. Consider for example a study showing that extensive weekly long-term chemotherapy can effectively (statistically significant) increase life-span in a certain percentage of specific cancer patients for a few extra months above the control group. Some readers may conclude that this is a satisfactory result of the study, while others may conclude with the same data that this is not a satisfactory result, as it entails long weekly veterinary visits with invasive techniques and the patients have severe side effects. The results are the same, but the biological significance assigned by the different readers is different based on their own biases and beliefs. Authors should discuss all possible angles and implications of the results based on multiple points of view. Reading the Discussion and Conclusions sections of a paper should allow readers to consider other possibilities that they may have not considered before, but there is no requirement to agree with the conclusions written on the paper. Readers should concentrate on the Results section of a paper to be able to draw their own conclusions, and whether they can use the information of the study when making decisions for their own patients, as they may not have the same conditions as in the study (external validity). Readers are warily cautioned to never use an abstract, not even the conclusions section of an abstract as take-home information, simply as an indication whether an article may contain the information they are looking for or not.

As presented in a previous lecture, “Evidence-Based Medicine” (EBM) focuses on the use of peer-reviewed scientific literature to establish the evidence. It is not uncommon for readers to ignore the Materials and Methods section in a scientific publication (which sometimes is even published in a smaller font as to be of lesser importance), and even the Results section, to go straight to the Discussion and Conclusion sections to read what the authors’ opinion is on what the results of their study mean. In the end, this is all it is, an opinion. Hopefully, it is a well-rounded and thought-of opinion, considering all the bases and possible views. Unfortunately, there are plenty of examples of published literature where the authors seemed to have been set on a path to prove a specific point, even if the results of the study didn’t warrant those conclusions. More over, many readers take their conclusions from the quick soundbite published in the abstract. This is a major source of confusion, as it is often difficult to summarize any detailed information learned from the study in a section that is often limited to 250 words, including study objectives, methods and results. It is rare for an abstract to present enough information to draw educated conclusions. Therefore, readers
need to consult the Results section of a paper, as well as the Materials and Methods section to then be able to draw their own conclusions and contrast theirs with those of the authors.

Consider for example the article most commonly used as proof that antimicrobial use in food animals is a risk factor for antimicrobial resistance in humans: Chloramphenicol-resistant Salmonella Newport traced through hamburger to dairy farms. Spika et al, 1987. New England J Med.316(10);565-570. The last sentence in the abstract reads “We conclude that food animals are a major source of antimicrobial-resistance salmonella infections in humans and that these infections are associated with antimicrobial use on farms.” This sentence will lead readers to believe that the authors tested different “sources of antimicrobial-resistant Salmonella”, of which food animals (any species) were the ones with the most biologically significant effect, statistically proven. This sentence also implies that the authors measured antimicrobial use on farms to be able to state that the infections are associated with it. However, when reading the entire article, the authors did none of these. Not only that, nowhere in the Results or Discussion sections of the paper did they mention this conclusion, only in the abstract. The conclusion in the paper was that ground beef originating from slaughtered dairy cows “was the vehicle of transmission” (not the source) for a newly recognized resistant strain of Salmonella Newport. This is one example of how only reading the abstract and taking on the conclusions of the authors can lead readers astray.

In the Conclusion section of a paper, the authors are to debate what the results of the study may mean based on their previous experience and their circumstances and whether they could answer the question they posed at the beginning of the study, given the circumstances of the study. This is called the internal validity of a study, or the ability to conclude something based on the study design and results obtained. For example, a study of a specific diet on puppy growth that weighs the puppies once at the beginning and once at the end of the study at 12 months of age will not have the same internal validity to conclude that puppies grew at a specific rate of 1 Kg/week compared to a study that did weigh the puppies once per week. In the latter, it becomes obvious that the weight gain is not linear.

In contrast to internal validity, which tells us whether the authors can draw the conclusions they make based on the way they conducted the study, we have external validity, which is the ability to apply the results and conclusions of this study to other populations with different circumstances and characteristics (i.e. breed, age, environment, etc.). This is the most important part for the reader: can they apply the conclusions of the study to their patients? To answer this question, the reader needs to understand the way the study was conducted (Materials and Methods) and what the results mean under the circumstances in which they were obtained. Knowing what other risk factors may influence the results, and whether those risk factors were controlled in the current study or accounted for in some way, will allow the reader to understand how the results may apply or not to the patients under their care.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Evidence-Based Medicine, External Validity, Study Conclusions

**References**

How Do I Use the Information on Sensitivity and Specificity of a Diagnostic Test to Decide Which Test to Use?

Aurora VILLARROEL

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Summary
Diagnostic tests are used to differentiate between diseased and non-diseased animals. This means there is a need to know what normal looks like. Then new diagnostic tests are compared to results from previous tests to determine how well they perform compared to that older test. Because no test is perfect, the resulting sensitivity and specificity values can appear marginal if a newer test performs actually better than the old test. Always ask for the gold standard for each specific disease. As diagnosticians, however, our goal should be to determine the likelihood of an animal to be truly diseased when the diagnostic test is positive. This is determined using the positive predictive value (PPV) not the sensitivity of the test. If we are interested in a negative result, we should consider the negative predictive value (NPV), not the specificity of the test. Both the PPV and NPV depend on the prevalence of the disease in the population.

A diagnostic test is anything that allows us to differentiate between a normal animal and a diseased one. Most doctors regard a diagnostic test only as something that uses a tissue or bodily fluid sample to be assayed with a specific technique or reagent. However, anything we do that allows us to differentiate a diseased animal from a normal animal is a diagnostic test. This includes looking into an animal’s eyes to determine their mental state (alert/depressed), using a thermometer to test whether there is a fever, checking heart, lung and gut sounds with a stethoscope, or palpating anatomical structures. The common denominator in all diagnostic tests is that the findings in that animal or herd are compared to the normal status. And based on the knowledge of the difference between normal and diseased animals, a diagnosis is made. For example, an animal with fever is diagnosed with an infection. However, we know that not all infections cause fever. Therefore, measuring temperature as a diagnostic test for infections is imperfect. In fact, all tests are imperfect.

The first thing to consider when evaluating a test is how we know what normal looks like. For example, how do we know what is the normal range of leukocytes in healthy animals? The answer is by testing multiple animals that “appear” normal. The interesting thing is that we then turn around and test an animal that may look normal and based on a high leukocyte count we determine it has an infection. It can be an eternal circle. By testing many animals that appear normal, the outliers are expected to be diluted in the average. Because of this, all diagnostic tests are imperfect.

Sensitivity (Se) and specificity (Sp) are characteristics of a diagnostic test that measure its ability to determine the status of animals that have been deemed to be healthy or diseased based on some other diagnostic method. Let’s assume that we have a perfect test that tells us exactly whether an animal has a specific disease or not. A sensitivity of 95% would indicate us that out of every 100 animals that are indeed diseases, 95 would test positive to the test, while there would be 5 false negatives. Conversely, 90% specificity would tell us that of every 100 non-diseased animals, 90 would test negative, leaving 10 false positives.
The best way to visualize this is in a 2 x 2 table that shows the true nature of disease status in the columns, and the results of the diagnostic test in the rows (Table 1). Sensitivity looks only at data in the first column (grey), while specificity looks only into the second column (white).

Table 1 Setup of a 2 x 2 table to calculate sensitivity and specificity of a diagnostic test

<table>
<thead>
<tr>
<th>True nature</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease</td>
<td>Non-disease</td>
</tr>
<tr>
<td>Diagnostic test result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>True positive</td>
<td>False positive</td>
</tr>
<tr>
<td>-</td>
<td>False negative</td>
<td>True negative</td>
</tr>
<tr>
<td></td>
<td>All diseased</td>
<td>All non diseased</td>
</tr>
</tbody>
</table>

The problem we face is that, because no diagnostic test is perfect, the test that was used to determine the true status of disease may be wrong. As technology advances and better diagnostic tests are developed, when they are compared to an inferior test, the resulting sensitivity and specificity will be lacking, and may appear poor, simply because the new diagnostic test is correctly categorizing animals as diseased when the reference test is saying (incorrectly) they are not diseased. The pressure here is on the companies devising these new diagnostic tests, to use the best possible reference test, which is name the “gold standard”. Always ask what the gold standard test is, and how the one you are about to use compares to that one.

In the end, sensitivity and specificity only tell us how a diagnostic test compares to another.

Positive and Negative Predictive Values

As diagnosticians, we need to move beyond the sensitivity and specificity of a diagnostic test. To be able to correctly interpret the results of the test we just performed on an animal, we need to look at positive predictive value (PPV) and negative predictive value (NPV).

PPV represents the likelihood of an animal being truly diseased when our diagnostic test is positive. A PPV=95% tells us that out of 100 animals that have tested positive with our diagnostic test, 95 are truly diseased, meaning 5 are false positives. Notice the difference with Se, where the 5% remaining of the 95% Se are false negatives. Conversely, NPV represents the likelihood of an animal being truly non-diseased when our diagnostic test is negative. An NPV=90% tells us that out of 100 animals that have tested negative with our diagnostic test, 90 are truly non-diseased, while 10 are in fact diseased (false negatives). The difference with Se and Sp is obvious when we visualizing the 2 x 2 table (Table 2). To calculate Se and Sp we looked into the columns of the 2 x 2 table. To calculate PPV and NPV we look into the rows. PPV considers only animals that tested positive (top row in gray), while NPV considers only animals that tested negative (bottom row in white).

Table 2 Setup of a 2 x 2 table to calculate positive and negative predictive values of a diagnostic test

<table>
<thead>
<tr>
<th>True nature</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease</td>
<td>Non-disease</td>
</tr>
<tr>
<td>Diagnostic test result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>True positive</td>
<td>False positive</td>
</tr>
<tr>
<td>-</td>
<td>False negative</td>
<td>True negative</td>
</tr>
<tr>
<td></td>
<td>All diseased</td>
<td>All non diseased</td>
</tr>
</tbody>
</table>

The biggest caveat of PPV and NPV is that they depend on the prevalence of disease in a population. When prevalence is low, PPV will be low, while NPV will be high. When prevalence is high, PPV will be
high, but NPV will be low. See a comparison of PPV and NPV values for the same diagnostic test with 95% Se and 90% Sp when prevalence is 5% vs. a situation with a prevalence of 60% in Table 3.

Table 3  Comparison of PPV and NPV in 2 populations of 400 animals each that are tested with the same test (95% Se, 90% Sp) but have different prevalence of disease

<table>
<thead>
<tr>
<th>Diagnostic test result</th>
<th>Diseased</th>
<th>Non-diseased</th>
<th>Diseased</th>
<th>Non-diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>19 (20% * 95%)</td>
<td>38</td>
<td>57</td>
<td>228 (240% * 95%)</td>
</tr>
<tr>
<td>-</td>
<td>1 (380% * 90%)</td>
<td>342</td>
<td>343</td>
<td>12 (160% * 90%)</td>
</tr>
<tr>
<td></td>
<td>20 (400% * 5%)</td>
<td>380</td>
<td>400</td>
<td>240</td>
</tr>
</tbody>
</table>

PPV = 19 / 57 = 33%  PPV = 228 / 240 = 93%
NPV = 342 / 343 = 100%  NPV = 144 / 160 = 92%

When using a diagnostic test on an animal, as diagnosticians, we want to know what is the likelihood of that animal to be truly diseased if the test says it is positive, and how likely the animal is to be non-diseased when the test is negative. Therefore, we need to evaluated the PPV and NPV of each diagnostic test. Because PPV and NPV depend on disease prevalence, diagnosticians need to consider if they are more willing to allow more false positive results or false negative results. This depends on what disease they are working with. For example, using a rabies test in a country with very low prevalence, with the implication that a positive animal will be sacrificed would require a test with very high confidence in a positive test (high PPV). Conversely, a test to determine whether breeding stock is allowed to be imported in a country that is free of a contagious disease would require a test with very high confidence in a negative test (high NPV).

Because there is no prefect test, for important diseases, we tend to rely on combined results from more than one test, typically run in series. This means that an initial test is run trying to catch as many potentially positive or negative animals (again, depending on the disease), and then a second test is run with the opposite characteristic trying to get the best overall result possible. For example, the initial test for bovine tuberculosis would have high NPV, meaning that if a result is negative, the likelihood of that animal being negative is very high. Then, only positive animals are tested with another test that has high PPV, trying to identify those animals that are truly positive from a pool of “maybe” positives identified with the first test.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Sensitivity, Specificity, Positive Predictive Value

**References**

Outbreak Investigations: Simple Steps to Follow Every Time

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Summary

Disease outbreaks are situations in which a new disease is identified for the first time, or the incidence of a known disease is abnormally high. There are 5 steps that need to be followed to improve the chances of determining the risk factors that allowed the outbreak to happen: (1) determining the specific clinical signs of an affected animal (case definition), (2) determining if the number of animals affected is abnormally high (magnitude of the problem), (3) determining when and where affected animals appear (temporal and spatial patterns), (4) hypothesizing and testing possible risk factors (analysis), and (5) follow-up. In the end, the target of an outbreak investigation should be foremost to determine the risk factors involved so the progression of the disease can be stopped, and eventually prevented from happening again. Being able to assign a causative agent to the outbreak would be ideal, but not necessary to stop the spread or prevent it from happening again.

Outbreaks are situations in which a new disease is identified where it wasn’t before or the incidence of a known disease is abnormally high. When you are presented with an outbreak in your daily practice, there are specific steps that must be followed to consistently arrive at a point where the outbreak can be stopped. As much as we all would want to pinpoint a causative agent to an outbreak, the ultimate outcome of an outbreak investigation should not be to name the culprit, but to determine how to stop the outbreak from propagating and, if possible, from occurring again in the future. Therefore, the ultimate goal of an outbreak is to find the risk factors to the determine how to control it. For known agents, it will be commonly possible to determine the causative agent. However, when dealing with emerging diseases, the causative agent may not be known even once it is known how it is transmitted and how the outbreak can be prevented from spreading further.

There are 5 steps in an outbreak investigation:

1. Case definition / diagnosis verification
2. Determine the magnitude of the problem
3. Describe the spatial and temporal patterns of disease
4. Analyze potential risk factors
5. Follow-up

1. Case definition / diagnosis verification

It is imperative to establish the exact definition of what an affected animal looks like. For example, simply stating animals with blisters is not the same as livestock with blisters on the coronary bands and teats. The first case definition could include any species and any location of the blisters, making the list of differential diagnoses long including viral diseases as well as sun exposure. The second case definition points more specifically to viral disease in livestock.
It seems obvious in this example, but, when dealing with an unknown causative agent, a detailed case definition will greatly help in narrowing possible risk factors, and therefore improve timeliness of the investigation, as well as the outcome.

When dealing with a known causative agent, it is important to verify the diagnosis before continuing the investigation, as a false case definition will lead to faulty results.

2. Determine the magnitude of the problem

The magnitude of the problem is determined by comparing the incidence of disease currently observed with the normal incidence for that population of animals. A baseline of zero incidence means the disease was not present in the population before. However, not every outbreak that occurs has a baseline of zero incidence. Knowing the true incidence of disease in a population before the outbreak will allow establishing the magnitude of the problem. Remember that incidence requires calculating the number of affected animals in comparison to the animals that are at risk of being affected during a finite period of time.

3. Describe the spatial and temporal patterns of disease

The next step is to specify when clinical signs appeared in different animals, and where the affected animals were at the time when clinical signs started. Notice that they may have been moved at some point during the course of the outbreak. Make sure to find out if and when that may have happened. If many animals are suddenly affected at the same time, the outbreak is likely due to a “point source” that exposed all animals at the same time, such as feed or water. If only a few animals show clinical signs at the beginning of the outbreak and then more and more, it is typical of a “propagated” outbreak commonly observed with contagious organisms.

4. Analyze potential risk factors

This step will require some calculations. First, based on the clinical signs (case definition), as well as the spatial and temporal patterns, a hypothesis will be established on some possible risk factors. This hypothesis will need to be tested to establish a conclusion. If the conclusion is that none of those risk factors fulfill the requirements of significant difference in exposure between affected and non-affected animals, then a new hypothesis will need to be established and tested, until a reasonable risk factor is identified.

The calculations required for this hypothesis testing include attack proportion (AP), attributable risk (AR) and relative risk (RR), as seen in Table 1 for an example with disease in dogs.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Exposed</th>
<th>Non-exposed</th>
<th>Attributable risk</th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Total</td>
<td>Cases</td>
<td>Total</td>
</tr>
<tr>
<td>Raw meat</td>
<td>18</td>
<td>19</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Cooked meat</td>
<td>5</td>
<td>18</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Chew bones</td>
<td>9</td>
<td>15</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Kibble</td>
<td>7</td>
<td>22</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

Once the calculations have been performed, the following risk factors need to be identified:
- The risk factor with the largest attributable proportion in exposed animals
- The risk factor with the smallest attributable proportion in non-exposed animals
- The risk factor with the largest absolute number of cases
- The risk factor with the largest attributable risk
- The risk factor with the largest relative risk

The risk factor that fulfills the most number of the above checklist is the most likely risk factor involved in the outbreak. Setting up this checklist visually as in Table 2 tends to be the easiest way to identify these risk factors.

<table>
<thead>
<tr>
<th>Exposure factors</th>
<th>Largest AP\text{exposed}</th>
<th>Smallest AP\text{non-exposed}</th>
<th>Largest absolute number of cases</th>
<th>Largest attributable risk</th>
<th>Largest relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw meat</td>
<td>■</td>
<td>■</td>
<td>□</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>Cooked meat</td>
<td>■</td>
<td>□</td>
<td>□</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>Chew bones</td>
<td>■</td>
<td>■</td>
<td>□</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>Kibble</td>
<td>■</td>
<td>■</td>
<td>□</td>
<td>■</td>
<td>■</td>
</tr>
</tbody>
</table>

5. Follow-up

If new cases appear, they should be included in the investigation going through the previous steps to confirm the current hypothesis or point towards other potential risk factors that need to be investigated. Also, it is important to divulge the findings of these investigations. This is especially important when dealing with emerging diseases, so that knowledge can be reinforced with new encounters of clinical cases.

There are several books available on veterinary epidemiology that can be used for future reference. The information presented in these lectures is presented by the author of the reference book listed below, which was designed as a light-reading book with multiple real life examples for clinical veterinarians.

**Keywords:** Outbreak, Attributable Risk, Risk Factor

**References**

Introduction to Zoological Medicine

Paolo MARTELLI
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Summary
This lecture addresses the particular nature of zoological medicine. There are approximately 15,000 species currently cared for in zoos. Although modern and rich zoos sometimes perform impressive veterinary acts, this lecture focuses on the conditions most veterinarians operate in in the range countries of the spectacular biodiversity of the Asian and South East Asian regions. In our countries many zoo animals and staff have limited access to funds, equipment, expertise and other resources. This however does not exclude the practice of good veterinary medicine, as will be illustrated and advocated.
The emphasis is placed on the importance of sound clinical methods of investigation that should always precede special examinations that require more resources. We review the duties of zoo veterinarians including our responsibility in training our peers, in protecting biodiversity and in advancing the understanding and appreciation of wildlife in a World where Wilderness has shrunk to the extreme and where what is left is up for grabs.
The importance of good husbandry and veterinary programs and the resulting benefits to animal welfare are explained through real life examples.

Zoo medicine is a marginal discipline. Over the last 30 years trade in wildlife for companion animals has steadily grown and led to the emergence of exotic medicine or new companion animal medicine as it is sometimes called. There are now many vet students and working professional with an interest in non-domestic species and who read and contribute to dedicated journals, books and internet forums etc.
A lot of the focus is on mainstream animals such as small mammals, turtles, snakes and lizards and bird.
This lecture is about veterinary medicine practiced in zoos and not about seeing exotic animals in private practice.
A number of points will be made.
Firstly that Zoo Veterinarians have a responsibility to promote good and meaningful zoos as well as wildlife conservation. Zoo vets must be aware of the role that different cultures and behaviors have on wildlife and promote the messages that will help change the predatory exploitative mindset currently prevailing in our relationship with nature. This is especially important when working in the range countries of most of the remaining wildlife and in the countries that consume much of it. For example the demand for biofuels from China or the EU or the direct consumption of wild animals such as we see in Africa and South East and Far East countries. A high demand in wild species breeds corruption and associated elements such as organized crime, social injustice, banking on extinction. It is no longer enough to show animals for its sake, modern Zoos must also promote wildlife conservation and sustainable living.
Secondly that Zoological Medicine offers fewer opportunities for employment, lower salaries, less information about the species we work with and less access to veterinary resources i.e. knowledge, expertise, money, tools. It is essential to not practice ‘recipe-based’ medicine. Real cases are used to illustrate that evidence, experience and deductive reasoning must lead to intelligent questions that will narrow down the investigations
towards case resolution.
Thirdly we explain the importance of preventative medicine including knowledge of the animals natural history, sound biosecurity practices, sound veterinary programs, staff training etc. The central role that pursuit of welfare plays in developing sound zoological operations will be formally presented. The lecture ends with a number of cases where audience interaction is solicited

**Keywords:** Zoo Medicine,
Cetaceans Medicine with Emphasis on the Importance of Operant Conditioning

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Summary
Preventative medicine is the cornerstone of sound veterinary management. Collecting samples can be challenging or not worth the risks in individuals of species that are stress sensitive, or dangerous, or difficult to restrain or anesthetize. Cetaceans are all of the above. Collecting samples routinely in apparently healthy animals is necessary to establish normal values and therefore diagnose when a morbid process is present. Early and accurate detection of disease is the predominant contributor to a successful treatment. The art and science of training dolphins to participate in their medical management is well developed and an inspiration for other species. A brief introduction to operant conditioning will allow us to introduce the audience to cetacean preventative medicine.

The importance of training cetaceans to voluntarily participate in medical husbandry and procedures is illustrated using a variety of conditions commonly affecting captive dolphins. Dolphins are trained to willingly allow detailed examination of all their bodily surface, eyes, and orifices as well as to contribute samples of blood, gastric juices, urine, feces and respiratory blows.

Breeding can be managed by voluntary collection of semen and, for the females, by ultrasound examination of the ovaries to follow the follicular or fetal development.

Inevitably some individual develop grave diseases. In one case study we will see how training allowed diagnosing and treating a gravely debilitating multi-resistant bacterial ulcerative hemorrhagic cystitis. In another case we will see how operant conditioning, networking and creativity allowed the resolution of an insidious chronic pneumonia that resisted conventional aggressive treatments by training the animal to participate in daily nebulization.

Operant conditioning is based on very simple and true principles: a behavior followed by a reward is likely to be repeated, a behavior not followed by a reward is likely to not be repeated. A signal can be associated with the reward and signal the completion of the behavior. By linking a behavior with the signal we can capture a behavior. A queue can be worked in to elicit the behavior. Simple behaviors can be tied together to form complex behaviors.

A basic form of communication can therefore be established between animals and their caretakers. The choice of behaviors to accept or to design is up to us.

In the context of veterinary management we can train dolphins to voluntarily cooperate in their preventative and therapeutic medicine.

Taking a cetacean out of the water, lowering the water level or catching the animals is always stressful and can be very dangerous. Interpreting the behavior of a dolphin is very difficult because they spend of the time under water and their basic morphologies, social behaviors and expressions are completely different form other animals that we are used to and certainly are beyond intuition. Cetacean managers have used training to create a catalogue of medical behaviors that allows monitoring the health status very closely.

The behaviors that can be performed routinely or as-needed include rectal temperature, blood sampling,
blowhole sampling, fecal sampling, urine sampling, whole body examination and palpation, oral and dental examination and cleaning.

Routine treatments that can also be performed with full cooperation of the animal (voluntary) include stomach tubing for gastric sample collection or for rehydration or feeding, gastroscopy, insemination, ultrasounds, radiographs, injections.

Animals can also be trained to enter shallow pools or stretchers or to slide out of the water to allow physical restraint without chasing or fear.

Reproduction is an important aspect of captive medicine and we will discuss reproductive management from estrus detection to birth.

Common diseases of cetacean and common treatments will be covered.

**Keywords:** Cetacean,
Introduction to Crocodilian Medicine

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Summary
Crocodiles are one of the best known and most recognizable taxon. Many Zoos exhibit crocodiles where they are a big favorite of the public and seeing a crocodile in the wild is always a memorable event. Zoo Veterinarians will truthfully tell you that crocodilians very rarely fall sick but that, when they do, they are not easily diagnosed or treated.

Crocodiles are also one of the very few wild animals that have a high commercial value and yet are doing better now than they were 40 years ago. Commercial farming or ranching operations on 6 continents breed or raise hundreds of thousands of crocodilians every year worldwide for their hides that provision the luxury leather markets. Their superior hides protect their scarred, pockmarked wild counterparts from poaching and even from habitat destruction in places where ranching is well managed.

Crocodile hatchlings are quite delicate and succumb to specific diseases. Ensuring their health and survival is fundamental to the success of farming.

Zoological medicine requires an understanding of the natural history of the species at hand. After a review of the aspects of crocodile biology that are clinically relevant we will present an overview of the medical management of farmed and zoo crocodilians with descriptions of commonly encountered diseases and their treatments including clinical techniques.

Crocodilians are classified as reptiles, but “Reptiles” is an imprecise taxon with no defining attributes. Crocodilians are actually closer to birds and dinosaurs than to snakes, lizards or turtles. The order crocodylia has 3 families and 9 genera and all will be referred to as ‘crocodiles’ from now on for simplicity sake.

Crocodiles are good patients in that they seldom fall ill, they take a long time to deteriorate when they do and have amazing powers of recovery.

The aspects of crocodile biology that are relevant to the medicine and husbandry are presented. Husbandry is central to all captive wildlife health management and welfare. Good practices and principles are discussed.
Clinical examination and clinical techniques particular to crocodile medicine are explained and discussed using real cases that cover a number of common metabolic and infectious diseases and treatments.

Keywords: Crocodile Medicine, Reptile

References
Practical Endoscopy for Zoo and Exotic Animals

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Summary
Minimally invasive surgical techniques are underappreciated and underutilized in zoological collections and in (exotic) veterinary practice. We argue that endoscopy offers the shortest learning curve, fastest progress and possibly best return on investment for veterinarians who embark in it. We will share our enthusiasm for endoscopy and 22 years of practicing endoscopy in unconventional situations in unconventional species.

We will briefly introduce the basic equipment, rigid and flexible, needed for Zoological and exotic practice. A number of examples will follow, illustrated by videos and pictures. Special weight will be given to the portability of the equipment and the suitability of endoscopic techniques for working in Zoo settings or even in the Wild, in animals big and small and across the range of vertebrate species. Common procedures of exotic or zoo practice will be presented as well as some curious or original cases.

Surgeries in zoo animals can be challenging. Some patients are very large others are very small. Some patients are arboreal, other terrestrial or fossorial or aquatic. Large incisions that require substantial postoperative care are difficult or even impossible to manage. Often, the caretaker cannot have intimate contact with the patients because that would be too stressful or too dangerous.

Endoscopy is very well suited for zoo work. Keyhole incisions allow performing routine exploratory procedures or even very invasive procedures without elaborate postoperative care, if any. With a little experience, most procedures are shorter when performed endoscopically and in some species a shorter anesthesia is an important consideration in the decision to operate. Recovery is usually shorter and that is also very important in certain species that should not be kept separated from their social group. Endoscopic surgery requires less analgesia. This last point is very important. Adequate analgesia improves the outcome yet in many zoo species we have insufficient knowledge of analgesia.

Endoscopy can be used in a very wide range of sizes spanning from ounces to tons. Some zoo or wild patients must be operated on in their living space. Endoscopy equipment is portable and keyhole surgery can accommodate less strict asepsis techniques, making it ideal for operating in the field. The endoscopic equipment needed is described and discussed. Maintenance of the equipment, available resources and the training of the staff are also discussed.

Rigid endoscopy requires a camera, a light source, insufflation and various tools that include at a minimum a palpation probe and a number of grasping, cutting and biopsy forceps. Other specialized grasping or manipulating tools and harmonic scalpels or ‘intelligent’ vessels sealing cutting forceps allow just about any procedure to be carried out endoscopically.

Flexible endoscopy also has its place in zoo medicine. A versatile 4 way video-scope is a good investment to diagnose and treat foreign body ingestion, respiratory infections, urinary diseases etc.

Sometimes the image or the technique is not to the expectation of the practitioner and we will review some common causes of disappointment showing how to overcome those
We will illustrate the points above with real life examples. We present examples of field set-ups, one used in large carnivores in Africa and another in macaques in Asia. A lot of examples and possibilities are presented as part of the lecture with many videos to illustrate the talk.

**Keywords:** Endoscopy, Zoo Medicine,

**References**
(2) Veterinary Clinics of North America, Exotic animal practica, endoscopy and endosurgery.2010 13(2)
Sustainability and the Role of Animal Welfare

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Consumers can demand from retail food companies that they use ethical production systems. Most of the public now think of livestock on farms and companion animals as sentient beings and have concerns about their local environment, global warming and other climate change. The term 'sentient' is now used in legislation about animals and means that the individual has the capacity to have feelings. The European Union Treaty of Lisbon, says in the course of a statement about animal protection and welfare (Article 6b), “since animals are sentient beings...”. This wording had the intention to protect the animals commonly used by man, for example on farm, in the laboratory, or as companions.

Scientists and legislators now use animal welfare as a term that is a scientific concept describing a potentially measurable quality of a living animal at a particular time. Such usage has rapidly become widespread during the last thirty years (Broom 2011). However, the use of the term animal welfare was not always as a scientific concept, and indeed there are still many people who are not aware of the modern approach to the subject. The author’s (Broom 1986) definition of the welfare of an individual as its state as regards its attempts to cope with its environment refers to all coping systems and so includes feelings and health. It is now used by most welfare scientists and is also, in modified form, by the O.I.E. (World Organization for Animal Health).

Welfare can be assessed using a wide variety of behavioural, physiological, clinical, brain function and other measures. Measures of animal disease are often important because health is a key part of welfare. The concepts of “one health” and “one welfare” emphasise that most of the systems involved and measures to be used in evaluation are the same in humans and non-humans (Broom 2017). Other measures, for example of behaviour, physiology, immune system function, body damage, etc. are described by Broom (2014) and Broom and Fraser (2015). Welfare is always poor when animals are diseased but pain and other aspects of poor welfare vary with severity of pathological effects and can be measured (Corke et al 2014). Pain systems are clearly demonstrated to exist in all vertebrate animals and a few invertebrates (Sneddon et al 2014). Facial expression scoring systems for pain assessment have been recently developed for use in rodents, rabbits, sheep, goats and horses (Dalla Costa et al., 2014, Broom 2015, McLennan et al 2016). The welfare of animals kept extensively can be poor because of heat-stress, parasitic and other infectious disease and low nutrient availability with associated competition. The welfare of animals in feed-lots is often worse than that of extensively-kept animals.

It is important to assess how good the welfare is as well as to evaluate poor welfare. The major changes in animal welfare science during the last 30 years have been the refinement in direct measures of animal welfare and the development of welfare outcome indicators that can be used by veterinary and other inspectors, as well as by those who use animals. Welfare outcome indicators have been developed by many scientists, including those involved in the E.U. Welfare Quality and Animal Welfare Indicators (AWIN) projects. Information on the subject is available at the Animal Welfare Science Hub www.animalwelfarehub.com.
A system or procedure is sustainable if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning and morality of action (modified after Broom 2001, 2010). What might make an animal usage system unsustainable? The system might involve so much depletion of resource that it will become unavailable. Alternatively, a product of the system might accumulate to a degree that prevents the functioning of the system. However, any effect which the general public find unacceptable makes a system unsustainable. Members of the public in all parts of the world, particularly in developed countries, are now insisting on transparency in commercial and governmental activities and on changes in methods of producing of various products. A production system might be unsustainable because of: inefficient usage of world food resources; adverse effects on human health; poor welfare of animals; harmful environmental effects such as low biodiversity or insufficient conservation; unacceptable genetic modification; not being “fair trade” in that producers in poor countries are not properly rewarded; or damage to rural communities. Any of these inadequacies could result in the quality of the product being judged as poor. In future, consumers are likely to demand that sustainable systems are used. If they are not, retail companies, production companies and countries that do not produce good quality, sustainable products are likely to be boycotted and hence forced to change (Bennett et al 2002, Broom 2014).

Many of the greatest animal welfare problems in the world at present are a consequence of conventional breeding with insufficient concern about the adaptability of the animals. Chickens, and some other animals reared for meat production, often grow in body size too fast for their legs and have severe leg and other problems. Cows selected for high milk yield often have major leg disorders, mastitis and reproductive disorders as a direct consequence of the high yield (Broom 2014, Broom and Fraser 2015). These problems may be exacerbated by genetic modification, but need not be. Cloning procedures cause such poor welfare in farm animals that the European Union does not allow their use.

In some countries, genetically modified plants are not accepted because of ethical concerns, the issue being whether or not living things should be modified in the laboratory as opposed to genetic changes that occur naturally. There is also concern because protein changes can cause allergies. Genetic modifications in animals can: benefit the animals (e.g. confer disease resistance), or help to treat human disease (e.g. a blood clotting factor in the milk of a sheep), or develop new products for other purposes, or increase efficiency of animal production. Some people accept all of these but others accept some or none as sufficient justification for genetic modification. A major reason for this is that, in some cases, animal welfare may be poorer as a result of the modification. The conclusion of many people is that any production of genetically modified animals should occur only if it has been demonstrated by scientific studies of animal welfare that the welfare of the animals is not poorer than that of unmodified animals as a consequence (Broom 2014). Three-level plant production, including pasture, shrubs with edible leaves and trees that may also have edible leaves are an example of a silvopastoral system. A cattle production system whose characteristics and aims include: using three-level or other multi-level production of edible plants, managing the soil taking account of worms and water retention, encouraging predators of harmful animals, minimising greenhouse gas emissions improving job-satisfaction for stock-people, reducing injury and stress in animals and maximising good welfare, considering how to encourage biodiversity using native shrubs and trees, and utilising the potential for obtaining wood from trees is explained by Murguetio et al (2008, 2011), Broom et al (2013).

The production of leaves and other material that can be eaten by the animals is much greater in silvopastoral systems than can be achieved by pasture-only systems. Results presented from tropical and sub-tropical studies show that cattle production can be better, biodiversity much increased, animal disease reduced, and animal welfare also improved by better availability of shade and other conditions selected by the animals. There are also possibilities for feeding tree and shrub leaves to pigs, poultry or farmed fish. Worker satisfaction is generally high in such systems. The biodiversity may be greater than that in natural forest but some wild species can only be conserved by the use of nature reserves.
The welfare of animals in silvopastoral systems has been demonstrated to be better in various ways than that on pasture-only systems. The beneficial effects of shade are substantial in hot weather with cattle skin temperatures up to 4°C lower than in pasture-only systems. High temperature reduces foraging times in paddocks fully exposed to the sun. Anxiety and fear, including fear of humans, can be reduced when partial concealment is possible. The increases in predators lowers the populations of ticks and injurious insects, such as horn flies, and hence reduces the incidence of diseases such as anaplasmosis, which has been shown to drop from 25% to <5%. The presence of nitrogen-fixing shrubs such as *Leucaena* improves animal nutrition and this, together with the better water-retention by the soil, reduces the likelihood of thirst and starvation. Feeding behaviour is improved at high temperature and humidity if the animals are in a silvopastoral system. It may be that the improvement in dietary choice contributes to this beneficial effect. Social behaviour is more normal on silvopastoral systems than on pasture-only monoculture systems (Améndola et al 2013).

Systems, such as these silvopastoral systems, should be considered by all farmers as consumers are likely to insist on sustainable systems more in the future.

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Public Health and Companion Animals: Rabies and Stray Dog Population Control

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Summary
Companion animals can bring a lot of benefit to people and society. However, with an uncontrolled population they can bring harm through the spread of rabies or through problems brought on by free-roaming dogs such as dog bites or spread of diseases. These problems can be solved in a humane way that benefits both humans and animals. With today’s knowledge and technology, it is possible to eliminate dog-mediated rabies in humans through mass vaccination of at least 70% of the dog population. The dog population can be managed through intersectoral collaboration that first assesses the problem by understanding the issues caused by free-roaming dogs, the root causes of those issues, the sources of these dogs, and the attitudes of the public towards dogs. Using these information, a comprehensive, integrative, long-term, and multi-disciplinary approach can be implemented. Both rabies control and dog population management fit well under the One Health umbrella, which requires good communication and collaboration between animal, human and environmental sectors.

The Problems
Companion animals such as cats and dogs are usually seen as beneficial to people emotionally, physically, and socially. They can provide companionship, entertainment, protection, and assistance, amongst others. However, in a poorly managed population, these animals can cause problems.

In an inadequately immune dog population, dogs can contract and then spread rabies viruses to other animals or humans. Rabies kills around 59,000 people each year, with the risk greatest in poorest regions of the world. More than 99% of these human deaths were spread through the bite of a dog. Rabies is a global problem, with the risk of a rabies outbreak present even in rabies-free countries.

In a dog population with inadequate guardianship, stray dogs or free-roaming dogs can create many different concerns such as the spread of diseases, dog bites, injury or damage to other animals or property, road traffic accidents, nuisance, pollution, and poor animal welfare. The problem of free-roaming dogs tends to intensify in less developed countries, where control methods are usually less humane.

The problem of an unmanaged dog population has multiple causes and will differ from one area to another. Some of the causes can include irresponsible pet owners, poor access to veterinary services, unregulated pet trade and breeding, availability to resources to dogs, and poor dog control strategy.

Rabies Control
Rabies is a fatal but 100% preventable disease. With the knowledge and technology we have today, it is possible to eliminate dog-mediated rabies. To achieve that, we would need commitment from both the
animal and human health sectors, as well as the right strategy.

To eliminate dog-mediated rabies in humans, the one strategy that works is by eliminating rabies in dogs through mass dog vaccinations. By vaccinating at least 70% of the at-risk dog population, herd immunity will be sufficient to control and eventually eliminate rabies. This vaccination level must be maintained to counter any turnover in the dog population through deaths, births, migration, or abandonment\(^2\). A good dog population management program can complement a rabies control program by reducing the turnover of the dog population.

The indiscriminate removal or culling of dogs to do not work as a method of rabies control as rabies spread is not dependent on dog densities. In fact, culling of dogs can have a counter-effect on rabies control\(^2\). The outbreak of rabies on Bali since 2008 is a good example of how culling does not work to control rabies, but once mass vaccination was implemented the number of rabies cases in humans and dogs significantly decreased\(^4,5\).

There is also the human element to consider in any rabies control program, especially where removal or culling is carried out. Dog owners can develop strong bonds with their dogs. When a threat to their dogs is perceived, these owners may try to save their dogs despite the risks involved, such as moving their dogs out of the endemic area. For others, their culled dogs are quickly replaced by new dogs\(^4,5\). These acts not only hinder rabies control but may also facilitate the spread of rabies.

**Dog Population Management**

The problem with an uncontrolled dog population can be managed by targeting the identified issues' root causes. As mentioned earlier, there can be many different concerns regarding stray or free-roaming dogs. The roaming of dogs itself may not be an issue in a community that accepts them. Instead, the issues can be, for example, dog bites, poor dog health, or uncontrolled breeding. Not all root causes are the same for each issue, and likewise, it may also be different for each area even if they face similar issues. Therefore, to adequately address the problem, an initial assessment is crucial to identify these issues and their causes\(^6\).

One key area to assess is the where the free-roaming dogs are coming from. Some of their sources can include the breeding of stray animals, abandonment and lost dogs. These free-roaming dogs can also be owned. In many places, all, if not most, of these free-roaming dogs are actually owned\(^7\). Ownership of dogs can take on many forms, and not just fall under two categories of ‘owned’ and ‘stray’ (or unowned). Several levels of guardianship can exist between these two categories, such as feeder, community owned, and owned but roaming. Identifying these sources of dogs and their relationship with humans are important in designing the right intervention.

It is also important to understand the attitudes of the public towards dogs, such as the acceptance of free-roaming dogs, the use of euthanasia as a method of control, perception of the problems the dogs cause, the acceptance of neutering, the value of dogs, and their expectations of owning a dog. These attitudes can be influenced by religion, socio-cultural practices and beliefs, individual personalities and experiences, or the strength of the human-animal bond. Understanding these attitudes can guide the choice of an intervention.

Some of the components of a dog population management program can include education, dog healthcare, reproduction control, identification and registration, legislation, dog catching and handling, holding and rehoming facilities, euthanasia, and controlling access to resources.
Commonly, the responsibilities to manage the dog population falls under the local government. However, many other stakeholders have a vested interest in the problem and can play an important role in the solutions. These stakeholders are, but not limited to, human health authorities, animal health authorities, local governments, veterinarians, pet owners, NGOs, academics and the media. A multi-stakeholder approach and involvement in all stages of the intervention is crucial to solving the issues.

Ultimately, there are no one-size-fits-all type of solution to the problem with stray or free-roaming dogs. A comprehensive, integrative, long-term, multi-disciplinary approach that caters to the root causes of the problem and the needs of the community is needed to ensure a successful outcome.

Animal Welfare and Public Health
Both rabies control and dog population management have an impact on both human and animal health and welfare. Improvement in animal health and welfare through elimination of canine rabies or controlling the problems caused by free-roaming dogs have a direct impact on human health and welfare. These approaches fit well under the One Health umbrella, which requires good communication and collaboration between animal, human and environmental sectors.

Keywords: Animal Welfare, Rabies Control, Dog Population Management, One Health

References
Dog Meat Production in Asia

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Summary
Nowadays, dogs are defined as companion animal and living with humans in same residence in many countries. However, in some nations, mostly in Asia, dogs are killed for human food consumption. There are no qualified inspections, no law or regulations about transportation, slaughter and husbandry system for dog meat industry. Therefore dogs for dog meat productions are exposed to diverse stress situations, pains and sufferings. Scientific Animal Welfare Assessments must be done in dog husbandry systems, transport and during slaughter. Not only live dogs should be examined and inspected, but also dog meats must inspected and controlled for the OneHealth.

In the Korean "Livestock Ordinance" paragraph 2 (definition), animals such as cattle, horse, sheep, pig, deer, chicken, duck, goose, turkey, donkey, bee, rabbit and dog are recorded. Therefore thousands of dogs can be kept in a farm when the farms meet the requirements of the livestock regulation. But in the Korean "Animal Food Hygiene Regulation" paragraph 2 (definition) dogs are not included. Therefore, there are no slaughterhouses approved for dogs and no meat controls. The dogs of the farms are transported alive to certain markets and slaughtered and sold without control. Unfortunately, there are no reliable data on the dog meat industry and dog meat consumption. For the first time, the Korean politician, Jung Mi Lee, presented data on dog farms in Korea at the "International Conference to End Dog Meat Industry of Korea" (Table 1). Only in the province of Gyeongsang-Bookdo (abbreviation: Gyeongbook) was an exact number raised, namely 719 dog farms and 107,217 kept dogs. According to the "Korean Dog Meat Association" 2 million dogs are slaughtered annually. The dogs in the farms are kept on mesh floors (fig. 1) and fed with leftover-food of humans (fig.2). Since no scientific investigations have been carried out, it is necessary to examine health, stress of the dogs and hygiene conditions in dog farms.

Table 1. Numbers of dog farms in Korea in order of regions

<table>
<thead>
<tr>
<th>Number of dogs</th>
<th>Number of farms</th>
<th>Seoul</th>
<th>Busan</th>
<th>Incheon</th>
<th>Daegu</th>
<th>Daejeon</th>
<th>Gwangju</th>
<th>Ulsan</th>
<th>Sejong</th>
<th>Gyeong gido</th>
</tr>
</thead>
<tbody>
<tr>
<td>under 500</td>
<td>1,096</td>
<td>2</td>
<td>5</td>
<td>190</td>
<td>12</td>
<td>16</td>
<td>13</td>
<td>34</td>
<td>16</td>
<td>808</td>
</tr>
<tr>
<td>500-1000</td>
<td>163</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>162</td>
</tr>
<tr>
<td>over 1000</td>
<td>22</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>total</td>
<td>1,281</td>
<td>2</td>
<td>9</td>
<td>190</td>
<td>12</td>
<td>16</td>
<td>13</td>
<td>34</td>
<td>17</td>
<td>988</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of dogs</th>
<th>Number of farms</th>
<th>Gangwon</th>
<th>Chung Book</th>
<th>Chung Nam</th>
<th>Jeon book</th>
<th>Jeon nam</th>
<th>Gyeong book</th>
<th>Gyeong Nam</th>
<th>Jeju</th>
</tr>
</thead>
<tbody>
<tr>
<td>under 500</td>
<td>15,462</td>
<td>121</td>
<td>10,546</td>
<td>386</td>
<td>292</td>
<td>3,228</td>
<td>679</td>
<td>142</td>
<td>68</td>
</tr>
<tr>
<td>500-1000</td>
<td>269</td>
<td>15</td>
<td>35</td>
<td>90</td>
<td>36</td>
<td>31</td>
<td>44</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>over 1000</td>
<td>47</td>
<td>2</td>
<td>12</td>
<td>8</td>
<td>11</td>
<td>2</td>
<td>4</td>
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<td>6</td>
</tr>
<tr>
<td>total</td>
<td>15,778</td>
<td>138</td>
<td>10,593</td>
<td>484</td>
<td>339</td>
<td>3,261</td>
<td>727</td>
<td>148</td>
<td>88</td>
</tr>
</tbody>
</table>

Lee, Jung Mi (2016)
As there are no official inspections on dog farms and no control for transport&slaughter, the condition of dogs is unknown. It was told for a long time that dog farms use antibiotic medicine, steroids and self-vaccination by farmers without prescription from veterinarians. Therefore it was necessary to examine residue of antibiotics in the dog meats. After all, antibiotic resistance after dog meat consumption in human body should be taken into consideration. 77 dog meat samples from different dog meat stores were gathered and examined. Not only antibiotic residues were examined but also microorganisms were tested.

**Keywords:** Dog Meat, Husbandry System, Food Hygiene

**References**

Animal Welfare Status of Working Animals in Africa

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Summary
Animal welfare is faced by numerous challenges in Africa. Anecdotal and documented evidence insinuate that the welfare of working animals, especially working equids is particularly poor. Working animals in Africa nonetheless are increasingly being used and their population steadily increasing. These valued animals, which include donkeys, mules, horses, cattle, camels, dogs and buffaloes, increase the resilience of resource poor communities. Foreseeable channels to be pursued in order to improve the situation are inclusive of research, training, advocacy and awareness, policy and legislative changes, partnership and networking and addressing negative socio-cultural values.

Introduction
Sustained population growth, urbanization and development in Africa has put considerable pressure on natural resources and gross domestic product of member states. This in turn has had the resultant effect of over population, increased demand for affordable services and other socio-economic challenges. Animals are increasingly becoming pivotal in attaining both food security and economic goals. Up to 250 million poor people depend on livestock for their income and livelihoods (1). Further urbanization means close interaction between man and animals, with implications on public health, animal welfare and safety.

Animal welfare has a variety of definitions and as per OIE definition it simply means how the animal is coping with its environment, both at its physical and mental state. A working animal can be described as a domestic animal trained and kept by people to perform tasks. They include canids such as guide dogs or they may be animals trained to provide tractive force, such as draught horses, mules, donkeys, camels or logging elephants. Man’s relationship with working animals predates agriculture, with dogs being used by our hunter-gatherer ancestors. In various parts of the world cattle, buffaloes, yaks, horses, donkeys, mules, camels, llamas, elephants, reindeer, goats and dogs are used for transport, crop cultivation, water-raising, milling, logging and land excavation or levelling. Cattle are the most significant work animals globally. After cattle, the main work animals are horses, donkeys and mules. This report reviews some of the welfare issues surrounding the major working animals in Africa.

Working equids
This group includes horses, donkeys and mules, their population is estimated at 26 million in Africa (2). Where they are found they commonly fall into two arbitrary categories, urban/periurban equids and rural equids. Equids found in Africa’s urban centres are mostly used for pulling carts. Due to the slow developing infrastructure, their work entails transporting water for the construction of buildings and household consumption, transportation of construction material like cement, sand, ballast, timber, etc. In Egypt, peri-urban donkeys are associated with the brick kilns, here donkeys and mules are used to pull carts loaded with bricks. Ahmed et al. (3) have estimated that in these brick kilns, 2000 donkeys and 400 mules are responsible for moving
approximately 200 million bricks per month. Refuse collection from Egypt’s large towns is also powered by these equines. Rural equids on the other hand facilitate the execution of repetitive household chores. These donkeys are commonly used for transport and as pack donkeys. Their use may include transportation of firewood, food, water, people, and household materials.

The most overt problem observable in equids working in peri-urban centres is poor harnessing. A wide range of deleterious material is used in harnessing, and additionally poorly fitted, consequently they get exposed to injuries. Carts commonly used in peri-urban equines are poorly designed. Common flaws include: short shafts causing trauma to the thighs, poorly inflated tires, poorly lubricated cart wheel bearings, and heavy and imbalanced carts leading to joint problems and lameness. In addition to direct harm, poor cart designs lead to inefficiency. Mistreatment is common. This is observed in the form of overloading, which is usually accompanied by overriding, overworking and whipping. Consequently, these animals are often suffering from chronic fatigue, are riddled with whipping wounds, dehydrated, lame, and in a general state of poor health. Other conditions observed in Africa's urban/periurban working equids include oral lesions due to poorly designed bits, parasitism and malnutrition. The prevalence of conditions such as colic is reasonably high as these animals scavenge for feed in garbage dumps. Partial or complete blindness is also a feature in these donkeys as a result of involuntary trauma by whips as they are ridden. Donkeys situated in the rural areas have been observed to suffer from lack of veterinary care as practitioners frequently opt to practice in lucrative urban centres. Additionally poor cultural practices such as ear cutting, branding and firing are commonly practiced. An emerging trade in donkey skin and its demand to fuel the ejiao market has resulted in wide spread domestic poaching, where donkeys are stolen, slaughtered illegally and inhumanely and the skin is removed for sale in the illegal markets while the carcass left behind (4) (ejiao is gelatine derived from donkey skin commonly used as an ingredient in traditional Chinese medicine).

Camels
In Africa, camels are multifunctional animals. Their population is estimated at 23.5 million (2). Communities that own this uniquely adapted ungulate use it as a financial reserve and investment; camels are also a source of milk, meat, skin and draft power. Additionally they play a role in social security. Milk from camels fetch higher market prices and are more nutritious than that from cattle, citing higher fat and protein concentrations and lower cholesterol values than cow milk. The animal boasts of a longer lactation period than cattle and goats thus availing this resource during harsh climatic conditions. Camels are also used as compensation during social conflicts, heritage, bride wealth, blood compensation and restocking. In camel owning communities, the number of camels owned bestows a family and especially the man who heads it significant pride and social standing. Welfare challenges affecting this species have not been exhaustedly explored. Recorded welfare challenges are in the form of disease (5). Adera et al., (5) describes mastitis as a prime example of an ailment that affects the camel welfare via physiological derangements including pain and discomfort and also the health and socioeconomic state of camel keeping pastoralist communities as the milk is rendered undrinkable. Chronic mastitis is also known to lead to starvation and death of calves.

Cattle and buffaloes
The recorded history of animal power in Africa starts about 6-5000 BC in Egypt with the first drawings of oxen and ploughs (6). These animals have been historically used to plough/till farms. Draft cattle increase in weight during their working lives and this represents significant meat production which in some places discourages the slaughter of male calves. Other than disease, reported welfare issues include poor body condition especially at the end of dry seasons and poor yoke and harness designs. From his observation Conroy (7) reports that farmers tend to ignore yoke design and fit and instead focus on other equipment. Introduced about a millennia ago in Egypt, water buffaloes have thrived such that their population equals that of cattle in Egypt. They are used for ploughing and water raising, but cattle (with their superior tolerance of heat) remain the main ploughing animals in Egypt. The use of water buffaloes has not spread significantly
from Egypt to other parts of Africa (8).

**Summary and Recommendations: Thematic areas affecting working animal welfare**

**Research**
This is an area which has been largely neglected in research, thus most information is secondary data, by-products and or incidental findings of different research work. Lack of research means lack of data on the socio-economic value of these working animals and technological development towards addressing potential welfare needs of working animals. The backbone for good animal welfare policies, guidelines and legislations, appropriate training and education programs and advocacy and awareness campaign messages is good research work and data. To that end, programs and funding need to be put in place to carry out elaborate and substantive research work to give credence to the need for institutionalized development of welfare improvement programs, appropriate technology, equipment and working practices guidelines with scientific basis for these group of animals.

**Socio-cultural and economic values**
Animal welfare in Africa is still to a large extent seen as a factor and conditions for international trade and western ideologies rather than a factor of good husbandry practices especially for the non-food animals. To this end animal welfare doesn't have much socio-cultural support nor economic prioritizations. To that end there is need to develop appropriate and proactive programs geared towards defining the socio-economic value of working animals with emphasis on poverty eradication, affordable transport and youth/women empowerment. This should also be backed by strong arguments about myths that work against improved working animal welfare initiatives.

**Policy and legislative frameworks**
The major impediment to policy and legislation even in countries where good laws exist emanates from a low awareness and interest by most enforcing agencies and the public in general on animal welfare practices and legislations. This is coupled by lack of animal welfare standards and guidelines. There is need to develop programs that will address the policy, legislation and enforcement gaps which will have direct impact on working animal welfare.

**Advocacy and awareness**
Most of the gaps identified leading to poor welfare and most of the recommendations given in this paper rely greatly on appropriate campaigns and awareness creation to gain wider acceptance and enforcement. The negative attitudes and beliefs need to be addressed through advocacy and awareness using various methodologies which include media campaigns and awareness creation during animal related world days like World Veterinary among others.

**Training and education**
Knowledge and skills shape attitudes and practices to a large extent. Thus development and implementation of training and education programs is key in ensuring that the above gap is filled. Most of the universities in Africa do little pertaining to training of animal health professionals on working animal health and welfare

**Partnership and networking**
The success and progress of any program or strategy is dependent heavily on availability of resources. In improving the welfare of working animals in Africa, there is need of adequate resources which include finances, trained personnel and stakeholders, infrastructure and equipment. Thus a concerted effort through development of both formal and informal partnerships and networks involving the private sector, governments both local and national, regional bodies and international organizations and bodies is key. The overarching aim here being to compliment and have synergistic outcomes.
Conclusion
This review has illustrated that research, development, resource allocation and education on working animals is limited especially for working dogs was significant. It is therefore prudent that concerned organizations and governments work together to address the gaps identified.

Keywords: Welfare, Africa, Working

References
Animal Welfare and Food Security

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Summary
Livestock farming in Germany is in general regulated by the German Animal Welfare Act (German designation: Tierschutzgesetz) and the German Order on the Protection of Animals and the Keeping of Production Animals (German designation: Tierschutz-Nutztierzuchtverordnung). Not all species have been recognised within the latter. Therefore turkey birds, waterfowl and adult cattle have not been taken into consideration. But as of February 2014 it is mandatory to all farm owners to assess suitable animal-based indicators as on-farm self-monitoring of animal welfare for all the housed farm animals.

In order to evaluate self-reliantly the well-being of the animals, farmers need reliable methods. These should be easily applicable and practical. The assessment of behavioural characteristics in combination with data on mortality, performance and health represents a feasible method. Reliable scoring methods have been established and are currently still being tested and improved. Animal welfare problems in poultry farming such as foot pad dermatitis (FPD) and poor gait occur on a regular basis in broilers and turkeys. Waterfowl such as Pekin ducks for the meat production are held without access to open water and laying hens were kept in „battery cages” without any access to perches, nests or litter areas, mainly for practicable and hygienic reasons. Such restriction in the realisation of species-specific behaviour leads to impairment of the animals’ well-being. Additional welfare concerns in the industrial poultry farming are high stocking densities and genetic traits. Problems occurring during the slaughter process of livestock are amongst others an insufficient stunning effectiveness.

Publicizing known problems in (intensive) livestock farming has led to a better consumer awareness and the demand for animal-friendly produced food. Therefore a rethink in intensive livestock farming has already taken place and leads to the need for alternatives linking the increasing demand for affordable meat with the production under improved animal welfare standards.

On the basis of own studies some of the occurring problems in livestock farming and implemented alternatives will be introduced, using mainly poultry farming as example.

1. Open water source for Pekin ducks
Currently no legally recognized standards exist for the rearing of ducks, neither in the EU nor in Germany. Therefore Pekin ducks for the meat production in Germany are held without access to open water, mainly out of practicable and hygienic reasons. The medium water is of high importance for waterfowl and is connected with many species-specific behaviours. The commonly utilized drinking systems for these water birds are nipple drinkers where the fulfillment of behavioural patterns seems nearly impossible. The aim was now to analyze the feasibility of modified bell drinkers as a species-appropriate water supply for Pekin ducks under farm condition, regarding animal health, behavior and hygienic parameters (1,2).

2. Colony cage systems as alternative to ‘battery’ cages for laying hens
Since January 1, 2010 the conventional cage housing systems for laying hens, without any access to perches,
nests and litter areas are banned in Germany and since January 1, 2012 throughout the EU. Enriched colony housing systems provided with the missing resources e.g. mats for dust bathing, nests and perches then were introduced and accredited by the German regulation as alternative housing systems. And although the conditions for the laying hens have been improved, it is discussed whether or not it is possible for the hens to use the offered resources in a species-specific manner and if there is the necessity for even further improvement. Therefore this study was carried out as a part of the joint research project “Advancement of the Colony Housing for Laying Hens”, promoted by the German Federal Ministry of Food, Agriculture, and Consumer Protection (BMELV) through the Federal Office for Agriculture and Food (BLE) where altogether five institutes throughout Germany participated. Over a time period of altogether three laying periods, each lasting for twelve months, the systems of different producers were tested, concerning to what extent the hens were able to use the offered resources to fulfill species-specific behaviour (3,4).

3. Early rearing phase and foot pad condition of BUT 6 turkeys
At present there exists no legally recognized standard, specific for the rearing of turkeys in Germany. It is commonly known, that the climate of the environment of productive livestock influences its’ well-being and productivity. Therefore the German Order on the Protection of Animals and the Keeping of Production Animals claims in the General requirements for Animal Husbandry Systems (§ 3 (3) 2.), that “barns must be sufficiently thermally insulated and equipped in such a way, that circulation, dust level, temperature, humidity and gaseous concentrations in the air must be kept in a range that is unharmed for animals” (translated from German). Aim of the present field study was to analyse the foot pad condition during the early rearing phase up to five weeks of life and to scan possible connections with climate parameters as gaseous ammonia content, dust level, air temperature and humidity (5,6).

4. Alternative housing under label conditions for broilers
In the modern meat-production broiler chickens are usually barn-raised in deep litter where a special structured environment is typically not intended. Natural behaviours of the domestic fowl, such as to run freely, peck, scratch, flap the wings, groom the plumage and rest and sleep undisturbed can be restricted by lacking opportunities. The market offers organic produced products at higher costs while the possibility for an intermediate product that meets as well animal welfare as consumer requirements as economic aspects has barely been utilized. Aim of the study was to investigate the outcome of alternatively reared broilers with provided resources and a slower growing broiler strain concerning especially animal-based measures for animal welfare (7,8).

5. Controlled atmosphere stunning in poultry
In Germany 630 billion broilers have been slaughtered and approximately 972,000 tons of poultry meat have been produced in the year 2015 (Destatis, 2016). The electrical water bath stunning and systems based on the usage of gas are the two authorized methods during the slaughter of poultry, based on the regulation on the protection of animals in connection with slaughter or killing and for the implementation of the council regulation (EG) Nr. 1099/2009 (German designation: Tierschutz-Schlachtverordnung - TierSchlV). Diverging and additional provisions about the permitted stunning methods are regulated within the annex I of the TierSchlV according to annex I of the regulation (EG) Nr. 1099/2009. Deviating from annex I chapter I table 3 numbers 1 to 4 of the regulation (EG) Nr. 1099/2009 exclusively pigs and turkeys are allowed to be stunned with carbon dioxide for slaughter. If other poultry than the mentioned should be stunned with this method the competent authority can allow other stunning or killing methods for the purpose of testing according to § 13 section 1 number 1 of the TierSchlV. Concerning methods involving the usage of gas, „carbon dioxide in two phases“ is mentioned amongst others. This is defined within the TierSchlV as „progressive exposure of conscious animals to a gas mixture containing up to 40 % of carbon dioxide, followed by a higher concentration of carbon dioxide when animals have lost consciousness.” The multiphase CAS-system (Controlled Atmosphere Stunning) has already been in use since 1996 throughout Europe and
underlies continuous improvements (9). One of the major benefits of CAS towards the water bath stunning is that birds are not being handled or shackled while conscious.

6. Captive bolt stunning in cattle
The most common practiced method of stunning cattle in the EU is the captive bolt gun stunning. The penetrating type causes at best, due to its impact on the cranium and the extensive damage to the brain, a concussion of the brain with consecutively loss of cognition and consciousness. Up to the year 2001 phiting rods were used directly after stunning of cattle to ensure that every animal was killed fast, reliable and with avoidance of pain and suffering and were forbidden throughout the EU by the means of the European Commission decision 2000/418/EC from July 2011, due to biosecurity reasons. Insufficient stunned individuals during the exsanguination process could then be seen in common practice, even when stunned at the recommended point of encounter. Aim of the study was to investigate possible influencing metric variables that may lead to a deviation of the point of encounter and therefore to a possible insufficient stunning when the brain stem and the caudal parts of the hemispheres haven’t been suitable damaged (10).

Major developments and future prospects
Results and specific recommendations out of the described studies have been recognised and partly implemented for the keeping and slaughter of livestock in Germany. Further efforts concerning the improvement of animal welfare in livestock farming are in progress throughout the EU and Germany by different research institutions and organisations. It has in general been realized that animal farming is subject to change and that consumers expect food products made from animals to be produced under animal-friendly conditions during all stages.

Keywords: On-Farm Animal-Based Indicators, Livestock Farming, Environmental Complexity, Husbandry Systems, Meat Production, Alternatives

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Implementation of the OIE Animal Welfare Standards as an Aspect of Sustainable Development Goals

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Summary
Since its founding in 1924, the OIE has been responsible for setting intergovernmental animal health standards. In 2002, at the request of its Member Countries, and noting that animal health is a key component of animal welfare, the OIE broadened its mandate to include animal welfare standards and to take the lead internationally in this field.

To build upon this new mandate, the OIE has convened a cycle of Global Conferences on animal welfare, starting in 2004, to address its goal of helping all Member Countries implement those animal welfare standards that have been adopted, while taking into account the cultural and economic variations among the world’s regions and countries.

The first OIE international standards on animal welfare were published in 2005 and new standards continue to be added. The standards currently cover the welfare of terrestrial animals and farmed fish in key sectors. OIE standards are based on globally accepted guiding principles and adopted by consensus of the World Assembly of national OIE Delegates, which means that all Member Countries endorse their application at the national level, regardless of their cultural and economic situation. OIE standards note that animal welfare contributes to the wellbeing of people through potential improvement of productivity and food safety.

The issue of animal welfare can also be seen from the higher, overarching framework of the UN Sustainable Development Goals (SDGs) adopted in 2015 to end poverty, protect the planet, and ensure prosperity for all. Rearing animals under proper welfare conditions contributes to animal health and improvement of livestock production. It contributes to SDG-2, aiming to ‘end hunger, achieve food security and improved nutrition and promote sustainable agriculture.’ Participants of the 4th OIE Global Conference on Animal Welfare in 2016, thus, requested OIE Member Countries to take action to support animal welfare measures that can contribute to the achievement of the United Nations Sustainable Development Goals.

In order to encourage Members’ implementation of OIE animal welfare standards, OIE provides various supports including regularly holding regional seminars for National Focal Points for Animal Welfare, development of regional strategies on animal welfare and evaluating, on a voluntary basis, capacity of national veterinary services to comply with OIE standards. The OIE appoints Animal Welfare Collaborating Centres, and this international network is expected to contribute to the implementation of standards in the context of regional strategies, conducting research on implementation challenges and developing tools to support Members. One such example is the training materials developed to improve animal welfare during transport and slaughter developed under the OIE’s Asia Far East and Oceania Regional Animal Welfare Strategy.

A further example of the implementation support provided by OIE is the cooperation with the International
Standards Organisation (ISO) to develop a Technical Specification for *Animal Welfare Management – General Requirements and guidance for organisations in the food supply chain*[^1]. This technical specification targets Member Countries and private sector organisations, and is fully aligned with the OIE’s animal welfare standards.

The veterinary profession plays a key role in assuring animal health and welfare and the training of veterinarians is increasingly including a wider range of courses on subjects relevant to animal welfare. This being so, in various countries and regions the best results are obtained when national Veterinary Services work together with the agro-industries and NGOs to improve animal welfare.

In May 2017, the OIE adopted its first Global Animal Welfare Strategy, with the vision of a world where the welfare of animals is respected, promoted and advanced, in ways that complement the pursuit of animal health, human well-being, socio-economic development and environmental sustainability. Accordingly, the OIE will continue to put in place their existing tools, and will also look for new ones, to effectively support Member Countries to implement their animal welfare standards.

**Keywords:** Standard, Implementation, Sustainable Development Goals

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Religious Slaughter and Animal Welfare

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Summary
Since cruelty to animals occurs during production, handling, transport, and slaughter in most countries where Islam is a major religion, Muslims and Islamic religious leaders need to be sensitized to this issue with reference to the teachings of animal welfare in the Qur’an and the Hadiths. To achieve this objective a campaign is needed with the help of animal welfare organisations and the World Organisation for Animal Health (OIE). This will greatly influence the majority of Muslims in the livestock trade in treating animals more humanely.

Introduction
There is considerable debate on the role of religion in animal welfare, with implications for the study of welfare for welfare assessment and for implementation of solutions to welfare problems. In Islam, the law is a privileged means of access to the sacred. For most Muslims, Islamic normativity (fiqh or shari’a) is an essential part of being a Muslim. The demand for and production of authoritative rulings is one form of social expression of normative Islam.

There are many published papers on how Islam provides an ethic of environmental concern and nonhuman animal protection Islamic law is most prescriptive in its insistence on humane treatment. The killing of nonhuman animals for meat and hides by halāl (that is, permissible based on a set of ethical and religious standards) methods is obligatory, with meat considered forbidden (Makrooh) if the nonhuman animal has in any way been subjected to inhumane treatment.

The Relevance of Animal Welfare under Islam
Islam provides considerable support for the importance of animal welfare. There is a rich tradition of the Prophet Mohammad’s (pbuh) concern for animals to be found in the Hadith and Sunna, and Islam provides considerable support for the importance of animal welfare.

The Qur’an is explicit with regard to using animals for human purposes. A closer look at the teachings of the Qur’an and tradition reveals teachings of kindness and concern for animals.

For example:
• And cattle He has created for you (men); from them ye derive warmth and numerous benefits, and of their (meat) ye eat. Surrah An-Nahl 16:5
• And they carry your heavy loads to lands that ye could not (otherwise) reach except with souls distressed: for your Lord is indeed Most Kind, Most Merciful. Surrah An-Nahl 16:7
• And (He has created) horses, mules, and donkeys, for you to ride and as an adornment; And he has created other things of which ye have no knowledge. Surrah An-Nahl 16:8
• We have made animals subject to you, that ye may be grateful. Surrah Al
• Haj 22:36
• There is not a moving (living) creature on earth, nor a bird that flies with its two wings, but are communities like you. We have neglected nothing in the Book, then unto their Lord they (all) shall
be gathered. **Surah Al-Anam 6:38**

- Seest thou not that it is Allah Whose praise all beings in the heavens and on earth do celebrate, and the birds (of the air) with wings outspread? Each one knows its own (mode of) prayer and praise, and Allah knows well all that they do. **Surah An-Noor 24:41**
- Qur’an actually forbids human actions which may lead to harm; transgress not in the balance, and weigh with justice, and skimp not in the balance … earth, He set it down for all beings **Surah Ar-Rahman 55:8-10**

We now have a view of animals that shows them not merely as resources, but as creatures dependent on God (Allah). Animals are seen to have their own lives and purpose, valuable to themselves and to Allah above and beyond any material value they may provide to humanity. The Qur’an is not the only Islamic source for messages of kindness towards animals. There is a rich tradition of the Prophet Mohammed’s (pbuh) concern for animals to be found in the Hadith and Sunna. For example,
- the Prophet Muhammad (pbuh) condemned the beating of animals and forbade striking, branding, or marking them on the face.
- He cursed and chastised those who mistreated animals and gave praise to those who showed kindness;
- He also instituted radical changes against the practice of cutting off the tails and humps of living animals for food.

One Hadith quotes Prophet Muhammad (pbuh) as saying:
A good deed done to an animal is as meritorious as a good deed done to a human being, while an act of cruelty to an animal is as bad as an act of cruelty to a human being.” (Hadith: Bukhari and Muslim)

Prophet Muhammad (pbuh) was especially vocal in his disapproval of the cruel practices of notching and slitting of ears of animals and the practice of putting painful rings around the necks of camels. (Hadith: Bukhari)

Below are just a few well-known examples from the hadith (traditions):
- “There is a reward (ajr) for helping any living creature.” (Hadith: Bukhari and Muslim)
- “It is a great sin for man to imprison those animals which are in his power.” (Hadith: Muslim)
- “The worst of shepherds is the ungentle, who causes the beasts to crush or bruise one another.” (Hadith: Muslim)
- “You will not have secure faith until you love one another and have mercy on those who live upon the earth.” (Hadiths: Bukhari, Muslim, and Abu Dawud)
- “Fear God in these mute animals, and ride them when they are fit to be ridden, and let them go free when … they (need to) rest.” (Hadith: Abu Dawud)
- “There is no man who kills a sparrow or anything beyond that, without its deserving it, but God will ask him about it.” (Hadiths: Ahmad and al-Nasai)
- The grievous things are: shirk (polytheism); disobedience to parents; the killing of breathing beings ...
- “May god curse anyone who maims animals.” (Hadith: Bukhari)
- “Whoever is kind to the creatures of God is kind to himself.” (Hadith: Bukhari)
- “there is none amongst the Muslims, who plants a tree or sows seeds, and then a bird, or a person or an animals eats from it, but is regarded as a charitable gift for him”(Hadith: Bukhari)

These examples clearly indicate how Islam treats any animal with kindness.

**Islam and Rules Concerning the Slaughter of Animals**

The humane slaughter of animals is strongly supported in the Islamic tradition. For example, Sahih Muslim (Book 21, Chapter 11, Number 4810) records Prophet Mohammad (pbuh) saying:
“Verily Allah has enjoined goodness to everything; so when you kill, kill in a good way and when you slaughter, slaughter in a good way. So every one of you should sharpen his knife, and let the slaughtered animal die comfortably.”
Prophet Muhammad (pbuh) has also said, “When one of you slaughters, let him complete it”, meaning that one should sharpen the knife well and feed, water, and soothe the animal before killing it. He also said, “Do you intend (on) inflicting death on the animal twice—once by sharpening the knife within its sight, and once by cutting its throat?”

Islam has also laid down Other Rules for humane slaughter as indicated by a combination of Hadiths, including the following:

1. Animals should have a preslaughter rest, and be well fed and well looked after at the point of slaughter.
2. The animals must be alive or deemed to be alive at the time of slaughter.
3. Slaughter must be performed by a Muslim (who is of sound mind, mature, and fully understands the Islamic procedure and conditions for slaughtering of animals).
4. Animals that are slaughtered should be securely restrained, particularly the head and neck, before cutting the throat.
5. Operator competence is of great importance in order to carry out satisfactory halāl slaughter.
6. Slaughtering tools and other implements used must be for the slaughter of halāl animals only.
7. The knife must be razor sharp and without blemishes and damage. For animals with normal necks, the act of slaughter must begin with an incision on the animal’s neck just before the glottis, and for animals with long necks such as chicken, turkeys, ostriches, camels, etc., the incision must be before the glottis.
8. The animal’s trachea and esophagus must be severed. The spinal cord should not be cut and the head not severed completely so as to induce immediate and massive hemorrhage. In certain mazhab (school of thought), uttering the phrase “bismillah” immediately before the slaughter is compulsory. In others, such utterance is highly encouraged.
9. Slaughtering must be done once only. The slaughtering implement must not be lifted off the animal during slaughtering. Any lifting is construed as one act of slaughter. Multiple acts of slaughter on one animal are prohibited.
10. Slaughter the animal in such a way that its life departs quickly, and it is not left to suffer.
11. Bleeding must be spontaneous and complete.
12. Animals should not be shackled and hoisted before bleeding.
13. Hoisting should be done only after the animal has lost consciousness. Restraining equipment should be comfortable for the animal.
14. Further preparation and dressing of the carcass must be delayed until all signs of life and cerebral reflex have disappeared.

Shackling and hoisting conscious animals seems to violate both the humane intent of Islamic slaughter law, and Prophet Muhammad’s (pbuh) comments on the process of slaughter. Stunning has been declared as acceptable by a fatwa (unanimous verdict) of the Al-Azhar University in Cairo. Furthermore, the Muslim World League declared in 1986 that pre-slaughter stunning is lawful when the weakest electric current renders a nonhuman animal unconscious before slaughter. Requirements and methods of stunning which are acceptable by Islamic authorities in countries such as Malaysia have been published. Eating meat produced using cruel methods violates the Prophet Muhammad’s (pbuh) general precept to cause animals no pain before their slaughter, as well as more specific injunctions regarding the treatment of food animals. Indeed, if animals have been subjected to cruelty in transport and slaughter, or to general cruelty, meat from them is considered by Islam as impure and unlawful to eat (Makrooh). The flesh of animals killed by cruel methods (Al-Muthiah) is carrion (Al-Mujaththamah). Even if these animals have been slaughtered in the strictest Islamic manner, if cruelties were otherwise inflicted on them, their flesh is still forbidden (Haram) food.

All the Islamic laws on the treatment of animals, including the method of slaughter, are based on compassion, fellow-feeling, and benevolence.
What is Prevalent Today?
Many current practices are not in accordance with the above teachings and may result in great cruelty to animals. Handling of animals before and during transport is often cruel. Needless suffering is inflicted on animals that are transported three or four days together in overcrowded, ill-ventilated, trucks, especially in hot, humid weather. Harsh conditions also occur at slaughter plants. Animals may be held in primitive facilities without shade, and animals may be restrained by short tethers. At the point of slaughter, animals are often struck and beaten to make them enter the slaughter facilities.

What Needs to be Done?
Many Muslims and Islamic religious leaders are not aware of the cruelty that is routinely inflicted on animals during transport, at pre-slaughter, and at slaughter in many Islamic countries. There is an urgent need to sensitize all Muslims to the teachings on animal welfare in the Qur’an and the Hadiths. This approach is bound to be effective in influencing the majority of Muslims in the livestock trade, especially the slaughter man in treating animals more humanely. This needs to be done by intervention at the highest level by religious bodies and organizations, which could be most effective in giving rulings (fatwas) on this issue. Poor practices and animal welfare abuses occurring during halal meat production has been reviewed with ways and means suggested to improve animal welfare especially using Mosque-based sermons by Imams to increase awareness of animal welfare issues. The Dialrel project reviewed current practices during halal and Sechita slaughter in cattle, sheep, goat, and poultry in Belgium, Germany, Italy, the Netherlands, Spain, UK, Turkey, and Australia, and the report discussed various stakeholders including Muslim and Jewish representatives.

Progress might be achieved by taking the following measures.
1. A campaign is needed to apprise religious leaders of the current cruelty that occurs during transport and slaughter, for example by slides and videos. This should be done by competent and knowledgeable individuals who are also aware of the Islamic principles of animal welfare, preferably by Muslims in order to give authenticity to their claims.
2. The creation of animal welfare legislation, including animal transport and slaughter, according to the World Organisation for Animal Health (OIE) standards and Islamic principles.
3. Government officials in charge of livestock, especially at abattoirs, should be sensitized to the concepts of animal welfare and how these relate to Islamic principles.
4. Abattoirs should be equipped with the facilities required for the good application of animal welfare standards, including unloading facilities, slaughtering boxes, and well-trained personnel to implement correct halal slaughter.
5. The OIE animal welfare standards, especially those dealing with land transport and slaughter of animals for human consumption, which were adopted in 2005 by OIE Members, need to be more strictly implemented by governments. The OIE encourages Veterinary Services to enter into dialogue with religious authorities with the objective of raising awareness of the importance of animal welfare and reducing animal suffering globally.

Keywords: Islam, Animal Welfare, Halal

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Summary
Wild animals are under threat for a host of reasons, including human encroachment, habitat destruction, human-wildlife conflict and poaching for meat, products or live use of the animals. The welfare impact on wild animals, particularly through live trade and wildlife farming are of serious concern. The use of wild animals for entertainment, as exotic pets and as ingredient in traditional medicine are of particular concern from a welfare perspective, while also having significant conservation impact. Wildlife tourism attractions involve between 230,000 – 550,000 animals, of which a large portion are used for captive entertainment, such as rides, shows, close encounters and feeding. For example, a World Animal Protection study exposed that between 3,000-4,000 captive Asian elephants are used for tourism entertainment in Asia. 3 out of 4 of these animals are kept in severely inadequate conditions, facing severe restraint, cruel training and inability to express natural behaviour. Demand from tourism for elephant rides is high but some signs for change can be identified in travel company policies of many global stakeholders. These changes prefer humane alternatives, such as observational activities in sanctuaries, over exploitative practices, such as rides and shows.

Traditional medicine may at times contain wild animal products, such as bear bile, tiger bone, reptile parts and other ingredients. Recent decades led to more affluent customers, which increased demand for such products, irrespective of legality. Up to 20,000 bears are used for bear bile production in China and neighbouring countries, over 3 million Tokay geckos are captured and traded from Indonesia to supply the demand for miracle HIV cures, and many million other animals face similar destiny. The welfare concerns extend from mutilation during poaching, inadequate slaughtering methods, to severely inadequate husbandry conditions and extraction methods. For many products, herbal or synthetic alternatives exist and the need to recognize and promote these, while phasing out products based on wild animals is imperative.

Exotic pets are of similar concern and the scale of the trade is huge. Over 5 million reptiles, mammals and birds are traded annually, of which many are destined for the exotic pet trade. Often understood as an animal lovers’ activity, the keeping of exotic pets is cause of massive animal welfare and conservation concern. The mortality rates of captive bred animals along the trade route from breeder to customer, the often inadequate conditions at a less experienced owner’s facility and the mostly unmonitored or unregulated poaching of wild animals for the trade are all reason for alarm. Additionally, serious public health risks persist with the international live trade of animals for the exotic pet trade.

As veterinarians, we have a responsibility to address the above concerns and educate our customers, expose these threats through high quality research and raise our voices in the appropriate forums as animal experts. Furthermore, by supporting efforts that aim to phase out such bad practices, we can ensure that eventually wild animals can stay in the wild – where they belong.
Body

Wild animals are facing increasing threats due to human encroachment, habitat destruction, human-wildlife conflict and poaching. While usually these threats are seen from a conservation perspective, severe welfare concerns exist as well that need to be recognized and addressed wherever possible. While humans have hunted, captured or bred wild animals for thousands of years, a significant shift occurred over the last century with globalisation and increased mobility of people. Today, global demand for wild animal products, entertainment or pets is causing dramatic suffering for millions of animals. These three uses are considered particularly damaging to wild animals and are crucial to be recognized by the veterinary community in order to decrease animal suffering.

Wildlife entertainment

A study by Oxford University estimated that wildlife attractions for tourists cause a negative welfare impact for between 230,000-550,000 wild animals (1). A large fraction of this includes wildlife entertainment, where captive wild animals perform in shows, are posing for selfies or can be touched or ridden by visitors. This industry is mostly depending on charismatic species, such as tigers, elephants, sloths, primates or turtles. Often these species are endangered, yet under the claims of benefitting conservation or cultural preservation thousands of wild animals are often suffering from inadequate husbandry, cruel training and insufficient veterinary care. Approximately 3,000 – 4,000 endangered Asiatic elephants are kept in captivity for tourism entertainment, such as rides or shows (2). While an increasing number of elephants are bred in captivity, the same welfare concerns apply to them as to wild caught elephants: Mostly chained, kept in isolation or without adequate socialisation opportunities, enduring painful training and demanding or stressful daily activities these animals suffer on a daily basis. The demand is largely coming from tourists that seek unique, exciting and exotic experiences during their holiday. Between 30-40% of tourists to Thailand take an elephant ride, suggesting a demand by up to 12 million people per year in just this one country. While small family-run operations still exist, the trend is to supply this demand through large-scale tourism venues, catering to package tourists. Such venues can receive over 1,000 visitors daily, generating 20,000-40,000 USD in ticket sales per day (2). Very little of this profit is benefitting the animals or their care-takers. 4 out of 5 elephants in the tourism industry in Asia are kept in severely inadequate conditions day by day. Increasingly this is also being recognized by visitors and 11% of all tourists have seen elephants being treated cruelly during their visit to Thailand. Educational and campaign efforts globally have also started detecting a shift through a decrease in people finding it acceptable to ride elephants. It is essential to build on this momentum and enable humane alternatives for the existing elephants that also benefit their care-takers, while exploring a phase out of cruel practices.

Traditional medicine

Traditional medicine containing wild animal products is another topic involving severe animal welfare concerns. Demand for alleged miracle cures derived from products of rare, exotic or otherwise valued wild animals drive this industry. While many of these products have existed for centuries, recent decades have led to massive increase of the industry and unsustainable, cruel practices. For example, bile extracted from gall bladders of bears is being used to treat liver or gall bladder diseases in humans. The bile is harvested from live bears kept on large farms in China, Vietnam, Laos and Myanmar. Up to 20,000 bears are kept in absolutely inadequate conditions, facing regular bile extraction with questionable methods for the duration of their life (3,4). While efforts in South Korea and Vietnam to phase out these practices are starting to see some success (5), the demand persists and continues to drive a cruel industry in China and neighbouring countries. Another example is the use of Tokay geckos for traditional medicine, which experienced a massive surge after rumours of being able to treat HIV. In 2014 Indonesia has legalised the annual export of 3 million live Tokay geckos for the pet trade, yet in most cases the true reason for trade is traditional medicine. Captive breeding is not feasible of such large numbers, so the majority is being poached in the wild, causing severe animal welfare and conservation concerns (6).
Exotic pets
While most of these geckos may end up in the traditional medicine trade, they are also in demand as pets. The exotic pet trade is another major wildlife trade sector that spans the globe and involves millions of animals. A 2005 study estimates over 40,000 primates, 4 million birds, 640,000 reptiles, and hundreds of millions of fish being traded alive every year (7). Many of these are destined as exotic pets. The demand for exotic pets can be rooted in actual love for animals, but also in the desire for status symbols or as collector items. While exotic pet owners may often believe they are providing well for their animal, this is not always the case as the often high turnovers of exotic pets suggest (8). Also the mortality rates at captive breeding facilities or during poaching and transporting long distances are often not acknowledged by the end consumers. Lastly, the live trade of exotic wild animals is a severe threat to public health – not only on an individual level but also for the risk of epidemic and pandemic outbreaks. 70% of emerging infectious diseases originate from wild animals and recent outbreaks include SARS or Ebola (7,9). The presence of large varieties of species at a market or a fair, many of which may carry diseases, and the high numbers of people passing through these venues is a nightmare scenario for epidemiologists (10). The demand for exotic pets and wild animal products, coupled with insufficient regulations is perpetuating this risk.
Yet, these public health concerns should not distract from the persisting severe welfare concerns for the animals. Millions of wild animals destined as exotic pets suffer at every step of this trade, no matter how well-meant the intentions of the customers are.

The role of veterinarians
We as veterinarians, advocates for animals, have an important role to play in preventing suffering of wild animals – not only by treating wild animals as patients but by actively educating our clients and colleagues, speaking out at appropriate forums and meetings, and supporting efforts that aim to phase out such bad practices, so that eventually wild animals can stay in the wild – where they belong.

Keywords: Wildlife Trade, Exotic Pets, Wildlife Entertainment, Traditional Medicine, Animal Welfare, Wildlife Conservation

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International Trends in 3Rs Development:
Human Behaviour Change to Promote Animal Welfare

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Summary
A significant body of research on research animal refinement has been developed. Despite this, visits to research animal facilities demonstrate that gaps are still common in routine implementation of tools, practices, and techniques to enhance the environment and care of research rodents. While part of the answer for this may lie in a need for further animal welfare science and 3R’s research, some of this is also related to resistance to changing behaviours and practices in those working with research rodents. This talk will focus on the need for understanding principles of human behaviour change to improve animal welfare.

The 3Rs (replacement, reduction, and refinement) are key ethical principles that guide the care and use of animals for research and teaching internationally. There is strong and continued interest in laboratory animal science in promoting the 3Rs as they apply to research animal use. While many positive concepts have been implemented to provide for a life worth living for research animals, there is still significant work to be done to consistently refine their care and use. Despite the significant body of research that exists for best practices in caring for and managing animals used for research and teaching, there is a significant delay in widely implementing improvements that have been well characterized. Of recent interest is the study of human behaviour change for enhancing animal welfare practices. This concept draws on a seemingly obvious principle – that further advances in animal welfare can only be made through changes in human behaviour towards animals. However, changing behaviours and accepted practices are sometimes the most difficult of hurdles when attempting to refine animal welfare. Use of a social science approach to consider animal welfare issues is helpful in trying to understand the disconnect that sometimes exists between knowledge gained through animal welfare science and intended to improve animal well-being and the reality of how animals may be managed and cared for on a day to day basis (1).

One example of this, in the research animal context, is the use of PVC tubes to handle mice. Research has demonstrated very strong preferences of multiple strains of mice to handling with a tube versus being picked up by the tail (2). Despite that this research was published over 7 years ago and that many other articles and guidelines have promoted implementation of the handling method very little consistent uptake of the method has occurred. While it seems like a simple and inexpensive method to introduce into facilities there can be significant resistance to its use. Further discussion with facility and research personnel have suggested that those working regularly with mice have a range of opinions regarding use of tubes for restraining mice. For example, they may be unaware of the technique, they may not perceive a benefit to using tube restraint because it is such a rapid procedure, they may not believe that recommendations apply to them because of their extensive experience in handling mice, and some may believe that it will slow their work processes to use a tube, and possibly interfere with their relationship with the mice that they are handling. Overcoming resistance to adopting the new technique needs to address all of these perceived biases and can involve the use of video examples that demonstrate strong preference of mice to specific handling
techniques, specific training in the technique, as well as persuasive discussions that emphasize other ways that caregivers and researchers can interact with animals and demonstrate their care.

Similar issues surround the consistent use and reporting of analgesics in research rodents to refine their care and well-being. Inadequate analgesia continues to be one of the most important welfare issues in laboratory animal science and also one that has the capacity to profoundly introduce variability and issues related to reproducibility (3,4,5). Understanding and addressing the root causes underlying resistance to giving analgesics is a critical factor in promoting refinement of research rodent care.

Through similar examples, this talk will explore the need for better understanding of human behaviour and motivation for improving research animal well-being.

**Keywords:** Human Behaviour Change, Refinements, Laboratory Animal Welfare

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‘One Welfare’ and Veterinary Education

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Summary
Animal welfare often means different things to different people, and opinions are varied and debates often heated. But if we are to achieve higher standards of animal welfare worldwide, we need to be able to rely on more than emotional arguments relating to animal suffering. We need to utilize scientifically validated evidence, and empower credible professionals to help persuade those with competing agendas and from different parts of the world where animals and their needs are low priority, that animal welfare matters, not just to animals but also for human wellbeing. Providing accessible and relevant animal welfare education as an integral component of the modern veterinary curriculum, as well as improving the capability of veterinary educators so they can confidently deliver teaching and research in this area, are required if future veterinarians are to be influential in ensuring higher standards of animal welfare worldwide.

International concern for animal welfare continues to grow with increasing demand for measures to protect animals and improve animal quality of life and a call for veterinarians to recognize the important role they play in the area of applied animal welfare. It is now clear that animal welfare, in its entirety, should become a major subject in the curriculum of every veterinary school. However how animal welfare science, and animal ethics should be taught within the curriculum is a subject for much discussion. Traditional methods for imparting knowledge to veterinary and animal science students particularly in developing countries where animal welfare is problematic, have tended to be didactic with the focus being on delivering content rather than on ensuring learning and empowering learners. Whilst there has been a move within modern veterinary schools mainly in Europe, North America and Australasia to utilize a more problem- based approach to learning, promoting more integration of subjects and critical thinking, this is not yet the case in many developing countries, where a more traditional pedagogical approach is applied.

One area of concern is the way in which traditional veterinary teaching methods frequently make use of live animals as models for instruction of future veterinarians. In many cases this practice results in welfare concerns since these sentient creatures are at risk of experiencing fear, pain and stress as students learn the handling and treatment skills essential for their future success as trained professionals. From the limited research there has been, it seems clear that over the course of a veterinary degree, empathy for animals reduced significantly with both male and female students rating animals as being less sentient in their later years of study. Whilst there may be an argument for the objectifying of animals to help students develop the detachment they need for dealing with sick and dying animals, there is the risk that this translates into a lack of real concern for animal welfare. For example, it has been shown that there is an apparent decrease in likelihood of senior veterinary students making use of analgesia.

Given that access to animals in their training is essential, it must be the responsibility of veterinary schools to minimize negative experiences for animals wherever possible, and ensure that the utilitarian approach to education, does not result in mixed messages for students regarding the worth of an animal. This extends
to all aspects of teaching use of animals. Investment in teaching approaches and facilities, using innovative ways of delivering the learning students need at each stage of their journey to becoming a veterinarian, can provide a supportive and effective way of enabling students to be able to practice their clinical and surgical skills development in a safe, risk-free environment. Utilizing animal manikins and training models provides a means of reducing live animal use, eliminating any potential for stress or trauma to a live animal patient as students practise handling, investigation and treatment techniques. Skills can be developed in a ‘safe learning environment’ through stress-free demonstration and practice, ensuring that when a student is ready to be ‘hands on’ with a live animal, clinical skills are well-honed, and there is less risk to student and animal. A wider benefit is the underlying message, that animals are valuable, that they are not inanimate objects on which to practice, but living sentient beings, worthy of respect and protection, even from those with the best intentions.

Another area for educational attention is the way in which internationally relevant subjects such as Animal Welfare, are often misunderstood, considered to be lacking in credibility and as a consequence under-represented within the veterinary curriculum. This means that veterinarians educated in many countries have little understanding of the central role they play in promoting and informing higher animal welfare standards. As elegantly stated by Linda Keeling (2010), ‘Most people agree that a good education does not tell people what to think, but aims to give them the knowledge to think for themselves. Nowhere is this more important than in the emotionally charged subject of Animal Welfare. We may use our knowledge differently, and we may have different values ourselves, but we need to know the science underpinning it and the ethical views associated with it to make progress.’

Many developing countries are members of the OIE, and as such animal welfare should be a major focus of the veterinary curriculum, but lack of veterinary knowledge of animal welfare along with a traditional ‘silo-ed’ approach to veterinary education, and an unwillingness by students to question their lecturers, means that informed teaching of animal welfare science, ethical decision making and critical thinking is often lacking. This is certainly an area where there is scope for veterinary schools in parts of the world where knowledge and training in animal welfare is more advanced, to provide support to vet schools where knowledge and resource is much needed.

It has become increasingly obvious that in order to enhance the acceptability of animal welfare it must be made relevant by relating it to human health and welfare. Although human welfare, social welfare, and animal welfare have traditionally been seen as distinct discipline, a new integrating concept, ‘One Welfare’, has been proposed as a way for exploring and explaining the interconnectedness of human and animal welfare. This approach is especially useful when devising and implementing interventions for developing knowledge and practice related to improving standards of animal welfare in communities where animals and people live in close proximity and/or co-dependently, and where health and welfare for both are a problem.

In this paper I will discuss the importance of integrating animal welfare into the international veterinary curriculum, and how a ‘One Welfare’ approach can help improve acceptability and understanding.
The Future of Animal Welfare Education in the Veterinary Profession

Malcolm Kwok Wei CHONG, Lara SCHERER, Michael HUANG, Hilary ANTOSH

Introduction
Animal Welfare, as defined by the OIE during the 76th General Session in May 2008, means “how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment.”¹
As it currently stands in the world, Animal Welfare is a very vague topic, especially since there are so many different definitions and standards globally. A person from a European country (the Netherlands, for example), with more Animal Welfare education may, for example, perceive Animal Welfare very differently from a farmer in an Asian country with less exposure to Animal Welfare issues. As such, advancements and reliance on science and the objectivity of empirical evidence would be very helpful in the quest to understand Animal Welfare, while an understanding of local values and cultures will help to translate and spread that message globally.

What we would like to achieve
The International Veterinary Students’ Association (IVSA) has the mission: “To benefit the animals and humans of the world by harnessing the potential and dedication of veterinary students to promote the international application of veterinary skills, education and knowledge.”² As such, we do believe that it is possible to improve the lives, and the quality of life of animals. To that end, we would like to see an increase in global standards of Animal Welfare, as well as an improved standardization of Animal Welfare globally, as well as better transparency and legislation in the field of Animal Welfare. This should be tied in with modules and more education about the importance of Animal Welfare within the Veterinary Education system.
We are aware of the different cultural sensitivities that may affect the efficiency of Animal Welfare efforts globally, but we do believe that an open discussions, paired with good, shared knowledge, techniques, and technology, will help make global Animal Welfare a success, and is a dream we long for in the future.

What is being done?
As IVSA, in line with our mission statement, we do several projects and events throughout the year to promote that discussion and improve the Animal Welfare climate globally.
We publish materials, articles, and toolkits to train and prepare our students to face Animal Welfare issues that they may face in the future. This helps train them to look out for Animal Welfare problems that should not be there, and to react appropriately.
We create partnerships with organizations that are dedicated to protecting the welfare of animals (such as Wild Welfare, etc.) This gives opportunities for our students to work with these organizations and intern with them, allowing a deeper appreciation and understanding of Animal Welfare issues locally and internationally.
We organize events and workshops, lectures, webinars, etc. at IVSA events, promote local events on a global scale, and support the work of our students in engaging the community about issues regarding Animal Welfare.

**How can you get involved?**
Students may have the enthusiasm, ideas, mobility, and energy to go out and try to change the world, but are generally lacking in many areas that working professionals can and must fill in order for Animal Welfare to truly succeed globally.

Experience: Working professionals have the experience and have seen how the world works, and can guide the next generation on where to direct their steps.

Knowledge and understanding: Working professionals have been in the field longer and know the true underlying issues, and where the more complicated issues truly stem from. They can also help by aiding and supporting endeavours within research to improve our understanding of Animal Welfare in the world, and in years to come.

Support: Working professionals, professors, and lecturers have the ability to enable the students to go and do what is necessary to improve Animal Welfare, whether it is by people they know, monetary means, support and encouragement, or just being a good example of how Animal Welfare can be practiced well within their daily lives and in the profession.

**Conclusion**
If we truly want to see an improvement in Animal Welfare, the best way is to start with ourselves. What better way to change ourselves then, but to change the way we think? And what better way to change the way we think, than to change the way we learn?

The key to an improved world and improved Animal Welfare lies not in law, punishment, and rewards, but rather by words, textbooks, and action, which will convince the hearts and minds of tomorrow that there is more work to be done.

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New EU Policies on Pig Welfare

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About 150 million pigs live in Europe. Animals are recognised as sentient beings in EU law. The welfare of pigs is covered by Council Directive 2008/120/EC, which lays down minimum standards for their protection. Amongst other requirements, this Directive bans the use of individual stalls for pregnant sows and gilts from 4 weeks after service to 1 week before the expected time of farrowing, bans tethering of sows and gilts, requires pigs to have permanent access to fresh water and to materials for rooting and playing. It also requires that tail docking is not carried out routinely but only where there is evidence that injuries to pigs’ tails have occurred. Further recommendations are published by the European Commission on measures to reduce the need for tail-docking and best practices regarding the provision of enrichment materials to pigs.

In addition to these legislative requirements, a voluntary sector declaration was agreed in respect to pig castration. The end goal of this declaration is to phase out surgical castration by 2018. The declaration also requests that since 1 January 2012, surgical castration of pigs shall only be performed with prolonged analgesia and/or anaesthesia.

The Federation of Veterinarians of Europe (FVE) carried out end 2015 a survey on pig castration in order to see the progress made in respect to the European declaration on pig castration. Results show that the deadline of 2012 has not been met; 61% of pigs were still surgically castrated, of which the majority without analgesia or anaesthesia (Figure 1). Commonly agreed and effective protocols are lacking. Regarding banning surgical castration by 2018, we are far from the aimed goal. Also it should be recognised that from an ethical point of view, the 3 alternatives to pig castration (castration with analgesia/anaesthesia, immunocastration and raising entire boars) are not equal.

Figure 1 Castration methods male pigs
In 2017, FVE also started an investigation on the use of enrichment materials and the prevention of tail biting and reduction of tail docking. The preliminary results of this currently running study will be presented at the WVC.

Although legislation exists to cover pig welfare, a Europe-wide survey in 2015 showed that 82% of Europeans believe the welfare of farmed animals should be better protected than it is now. The majority (64%) of EU citizens also request more information about the conditions under which farmed animals are treated, a number we have seen growing over the years in Europe.

At the same time, pig farming in Europe has been of low profitability in the last years. Given this negative economic climate and the pig crisis, it is unlikely that traditional pig farmers will be able to make strong investments in animal welfare. A more sustainable method to farm pigs has to be found, both from the perspective of the animals and the farmers, in order that farmers can raise pigs in a sustainable and welfare friendly way and to get a fair price for the meat produced. Taking away the pain and stress associated with the housing and management of pigs is in this way connected to the future of the European pig sector.

References
Mouse Modeling to Inform Precision Medicine

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Summary

Precision medicine represents a new paradigm in clinical medical practice by which a patient’s own individual biological and pathophysiological characteristics, rather than commonly-shared signs and symptoms, dictate a specific molecular diagnosis, targeted therapeutic intervention, and, if applicable, an effective prevention strategy. The “precision” inherent in this approach to medicine is dependent on a person’s “-omic” profile (e.g., genome sequence, metabolomic profile, transcriptome, microbiome characteristics, etc), environmental influences, and individual behavioral patterns and lifestyle choices. This triad is aimed at delivering the correct diagnosis and/or treatment to the right patient at the right time. Efforts aimed at adopting these principles to the practice of precision medicine have largely focused on statistical analysis and interpretation of large data sets devoid of experimental testing or validation in vivo. Although in some instances, in silico analysis of data available today might be sufficient for establishing a precision medical approach to diagnosing, managing, and treating disease in a patient, in most cases there will be insufficient relevant and interpretable functional data to achieve a high degree of clinical success. At the very least, precision modeling in animals, such as mutant mice that recapitulate genomic variants of disease observed in patients, will be needed for developing and testing the effectiveness of targeted molecular therapies prior to administration clinically. Optimally, precision modeling in vivo will become integral to the development and practice of precision medicine now and into the future.

Lecture Notes

In his 2015 State of the Union address to the nation, US President Barak Obama announced the launch of the Precision Medicine Initiative, an effort involving government (e.g., the National Institutes of Health, Food and Drug Administration, etc), health providers, academic medical centers, and drug and device makers to re-envision how medicine could be delivered to individuals based on one’s personal biological characteristics, environmental exposures, and behavior and lifestyle choices. The ultimate impact would be to deliver the right therapy in the right amount to the right patient under the right conditions at the right time. At the time, the convergence of numerous technological advances in -omics profiling, such as what is encompassed by next generation whole exome and genome sequencing, measuring and recording innumerous environmental variables, and wearable and mobile health monitoring devices, was thought to make this process primarily data driven, somewhat along the lines of “digitized” evidence-based medicine.

The concept of precision medicine is not entirely new, as one no less than Hippocrates (460-370 BCE) himself observed that “it is far more important to know what person the disease has, than what disease the person has”. Some 2,500 years on, in a 2011 National Research Council report (1), Susan Desmond-Hellmann and colleagues introduced precision medicine to the modern age as “a potential framework for creating a new taxonomy of disease based on molecular biology (rather than clinical symptomatology)…to improve health outcomes”. Today, precision medicine, also referred to as personalized or individualized
medicine, is considered a natural evolution of clinical medicine in which therapy is matched precisely to a specific diagnosis based on three foundation pillars: 1) an individual’s molecular characteristics, 2) environmental exposures and influences, and 3) lifestyle choices and behaviors.

The concept of creating a new taxonomy of disease is critical to understanding the fundamental nature of precision medicine. Within that concept are two driving principles: pleiotropy and heterogeneity. With regard to pleiotropy, a singular diagnosis can be based on the molecular characteristics of a disease process, irrespective of the potential variety of organs and tissues involved, or the variability in overt symptoms observed. Take for instance the numerous syndromes associated with mutations in the LMNA gene: Emery-Dreyfus muscular dystrophy, Charcot-Marie-Tooth axonal neuropathy, lipodystrophy, and premature aging disorders (2). Each of these apparently “different” diseases have distinctly unique clinical presentations, yet they are all associated with mutations in the LMNA gene. Therefore, precision medicine guides one to define a diagnosis and treatment regimen on the molecular mechanism of disease, rather than on multiple, symptom-based treatments. The mirror image to pleiotropy is heterogeneity, the second driving principle of precision medicine. In this case, precise diagnoses and treatments are based on the molecular mechanism of disease, irrespective of a phenomenological diagnosis based on similar symptoms. For example, colorectal cancer is a histopathological, tissue-based diagnosis with multiple associated molecular mechanisms determined by the genetic variant identified in either RAS, BRAF, PI3K, cMET, EGFR, or the MMR System (3). In precision medicine, an effective treatment strategy targets the genetic disease mechanism, not the descriptive appearance, of the disease.

While understanding disease mechanism is necessary for the effective application of precision medicine, it is not sufficient. For example, knowing the mechanism of disease is necessary to achieve a precise diagnosis, but itself is not sufficient to design an effective therapy. An example of this is sickle cell anemia, the mechanism of which has been known for decades, yet an effective therapeutic intervention targeted at the very well described molecular mechanism still eludes us. On the other hand, for precision medicine to be effective, one must be able to demonstrate a predictable outcome in response to a targeted therapy. In other words, precision diagnostics plus proven effective therapeutics equals a predictive outcome, the hallmark of precision medicine.

Despite the logical arguments behind it, the scientific knowledge and indeed capability to adopt the precision medicine paradigm fully today is lacking. It should go without saying that there remains much to learn about the molecular mechanisms of not only simple, rare inherited “Mendelian” diseases, but also of complex and more common “multifactorial” diseases and disease syndromes. To date, of the ~7,000 known inherited diseases, we know the genetic basis for only about half. Further, the focus of genetic medicine has been on mutations and other anomalies that occur in less than 5% of the genome that encodes for proteins. As originally envisioned, clinical actions guided by precision medicine will need to derive from the statistical interpretation of -omic, environmental, and behavioral data accumulated over time from patients who share common disease mechanisms at the molecular level. It is likely that this approach, which relies solely on measurements and observations in patients alone, will require a very long time to gather sufficient evidence of disease causation to achieve a high enough level of confidence with which to make clinical decisions regarding diagnosis, therapy, and prevention. Instead, a more holistic approach that integrates in vivo testing and precision modeling in animals with clinical diagnostics and therapeutic trials will not only catalyze a better understanding of disease mechanism but also accelerate the practice of precision medicine (4).

The approach to future precision modeling in animals differs distinctly from traditional animal models of disease in the past. In an effort to functionally annotate the (protein-coding) mammalian genome, the International Mouse Phenotyping Consortium (IMPC) is producing gene knockout mouse models for comprehensive, broad-based phenotyping (5). This goal is required in order to understand the functional
role of each gene, and to begin to identify “disease genes”…genes for which the induced null mutation in the mouse predicts an inherited disease phenotype caused by a functional mutation in a human. In this way, an induced mutation in a mouse model is first made and studied, and then used to match up with a relevant human phenotype. On the other hand, “precision modeling” is a disruptive approach by which a genetically-defined human phenotype informs the creation and study of the relevant animal model. For example, a mutant mouse which is generated to incorporate genetic variants of unknown significance (VUS) found in a human patient can be used to differentiate disease-associated from disease-causative factors. With exponentially increasing numbers of VUS becoming identified through whole exome and whole genome sequencing, the ability to test their precise pathophysiological role in vivo will be essential to confirming molecular diagnoses and advancing targeted molecular therapies. Further, projects such as the IMPC will generate new knowledge of gene function in mice essential for prioritizing the assessment of potential disease alleles in humans.

In summary, precision medicine represents the next stage of evolution of clinical medicine. Its focus on disease mechanism at the molecular level, in context with the impact of environmental factors and behaviors, will greatly accelerate the delivery of highly effective treatment and care. Integral to the timely success of precision medicine will be the incorporation of precision modeling in animals, especially mice, in which precise genetic variants identified in people can be recapitulated and tested, including in context with relevant environmental influences and behavioral conditions.

Keywords: Precision Modeling, Genome, Mutant Mice, CRISPR/Cas9

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Extending Availability and Enhancing Accessibility of Mouse Resources Worldwide

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Summary
Unencumbered sharing and dissemination of research resources is essential for ensuring that advances in biomedical sciences continue unabated. A principal component of these necessary resources are animals that are used as human surrogates for understanding components, mechanisms, and pathways of disease. A number of programs, including the Knockout Mouse Project (KOMP), a member of the International Mouse Phenotyping Consortium (IMPC), and the Mutant Mouse Resource and Research Centers (MMRRC) Consortium, both supported by the US National Institutes of Health (NIH), have led efforts to make transgenic and gene knockout mouse models more available and accessible to the global research community. For example, since 2011, repositories and associated IMPC members have together distributed thousands of knockout mouse models to an equivalent number of scientists in dozens of countries on 4 continents. These resources are maintained and disseminated as live mice, frozen germplasm (embryos and sperm), targeted embryonic stem (ES) cells, and/or gene targeting vectors. Recently, the advent of CRISPR/Cas9 technology has greatly increased the potential for making even more varied mutant mouse models of human disease variants possible. These too, like the transgenic and traditional knockout models before them, will be archived, maintained, and distributed by KOMP and MMRRC repositories to research scientists around the world.

Lecture Notes
Future advances in science are built upon the successes and failures of past scientific experimentation. In the field of biomedical research, many recent advances are made using animal, mostly mouse, models of human disease. A number of mouse models that are created using a variety of technologies (e.g., transgenic, homologous recombination, ENU-induced mutagenesis, transposons, etc) are generated by scientists in individual laboratories and large research programs all over the world. To maximize their usefulness for catalyzing research activities and accelerating scientific advances, mutant mouse lines can be submitted to mutant mouse repositories for archiving, maintenance, and distribution purposes so as to ensure their broadest possible availability and accessibility by the research community.

Over the years the scope of mouse repositories has broadened to activities and services beyond archiving, maintenance, and distribution (1). Repositories now have added roles and responsibilities to enhance and extend the scientific value of mutant mouse models for biomedical research. For example, by providing technical and support services to users, interfacing with complementary resources and databases, and ensuring the protection, preservation, and perpetuity of mouse resources for future research, repositories encourage the use of extant, quality controlled mouse resources over the temptation for scientists to recreate individual mouse lines, maximize awareness of mouse lines available for research, and reduce barriers to accessing mice and resources. These and other value-added services and activities aim to promote scientific rigor
and reproducibility, reduce the overall time and cost burden of biomedical research, and advance scientific knowledge and research productivity.

A recent example of how newly generated mutant mouse resources have been made available and accessible to the scientific community is the Knockout Mouse Production and Phenotyping (KOMP2) project, an effort led by 3 teams at the University of California Davis, Baylor College of Medicine, and The Jackson Laboratory and funded by the US National Institutes of Health beginning in 2011. The three KOMP2 groups, in association with their 12 collaborating partners in 11 countries on 3 continents within the International Mouse Phenotyping Consortium (IMPC), aim to knockout and phenotype in the mouse every protein-coding gene in the mammalian genome (2). Mouse production and phenotyping is conducted according to basic principles of scientific experimental design, such as a statistically-validated group sizes, including both sexes in the analysis, randomization and blindedness in selection of animals to study, sufficient and appropriate controls, and other characteristics necessary to ensure rigor and reproducibility (3). The IMPC has to date produced more than 5,000 mutant mouse lines from both gene-targeted ES cells and CRISPR/Cas9 technology, generating more than 37.5 million data points, 29,000 phenotype annotations, and 271,000 images from approximately 500 different phenotyping parameters measured during embryo development, early adulthood, and, most recently, at later, older adult stage of life. Because most of the genes selected for study were those with limited functional annotation or no known biological or pathophysiological association, the new knowledge generated and contributed to our understanding of the mammalian genome has been immense (4). The phenotyping data and images produced by the KOMP2 project and other members of the IMPC have together resulted in more than 1,200 publications, notably and most recently a paper describing the pathophysiological relevance of hundreds of genes related to known and heretofore undescribed inherited disorders and diseases.

If kept solely within the confines of the laboratories where they were produced and phenotyped, this extraordinarily valuable resource of both mice and data would have limited impact on advancing science and our understanding of the genetic basis for many human diseases and syndromes. Instead, KOMP2 and other members of the IMPC have actively engaged in outreach to inform the biomedical research community of the availability of mice and utilization of the data generated for hypothesis-driven research. For example, although all deposit their lines in a regional distribution repository, 7 of the 15 IMPC members also provide direct-to-consumer (DTC) access to the mutant mouse lines they produce, as either live mice, ES cells, and/or frozen germplasm. This extra-repository activity accelerates the availability of mutant mouse lines to the public, as it enables access to mutant mouse lines faster and potentially more likely as live mice before they are shipped to a public repository and archived as frozen germplasm. In many instances, access to mutant mouse lines is not limited to just academic centers, but is granted to commercial, for-profit entities as well. To date, more than 1,500 mutant mouse lines have been distributed by IMPC production centers to an almost equal number of investigators around the world.

Because of consortium-wide adoption of standard operating procedures, harmonized testing protocols, and quality control/quality assurance practices, the reliability of DTC access from production centers is on-par with that of distribution from bonafide public mouse repositories. And, there are several additional benefits to having a production center actively engaged in distributing mouse lines. For example, most production centers also provide ancillary services to facilitate maximum utilization of the distributed resource, such as blastocyst microinjection of targeted ES cells to generate live mice, PCR-based gene-specific genotyping, and colony breeding and management services. In addition, most production centers provide customer and technical assistance to investigators using mouse resources produced by and obtained from the IMPC member. Further, the production center has the technical knowledge of the allele type and construction, which is especially useful if an investigator wants to conduct further genetic manipulation (e.g., CRE recombination) of a mutant strain. Finally, a production center that is also a phenotyping center will fully understand the
analytical strategy and outcomes of phenotyping a mutant strain, knowledge of which is often greater and more in-depth than at a distribution repository, especially one not co-located with an IMPC member.

On the other hand, public repositories have as their primary operating principle the responsibility to serve all requesting investigators equally and without reservation, a mandate not required of an IMPC member. Ten repositories and repository systems around the world have established formal relationships with IMPC members to receive, verify, and cryopreserve their mouse lines for archiving and distribution purposes. Of these 10, two repository systems (the Mutant Mouse Resource and Research Center [MMRRC] Consortium in the United States and the European Mouse Mutant Archive [EMMA] in Europe) receive mutant mice from more than one IMPC production center. The consolidation of mouse lines from multiple IMPC production centers into a smaller number of repositories facilitates the search for, identification of, and acquisition of the optimal mouse lines for research. Further, the interaction between repositories and repository systems (e.g., Federation of International Mouse Resources [FIMRe]) ensures an efficient, global responsiveness to requests from researchers world over. As a result, participating distribution repositories have archived over 5,000 mutant mouse lines produced by IMPC members, and distributed more than 2,000 of these lines to investigators in Asia, North America, and Europe. To date, repositories have fulfilled over 3,000 orders from more than 2,400 investigators for mutant lines maintained in various formats, including as molecular targeting vectors, ES cells, cryopreserved embryos and sperm, and, of course, live mice, many of which are recovered on-demand from the cryoarchive. Access to the mutant mouse resources maintained in public repositories is facilitated by web-based searchable catalogs, online ordering systems, liberal and non-exclusive material transfer agreements or electronic “conditions of use” statements, and modest service and handling fees (5).

In the case of the KOMP2 program, all mice are archived and maintained by the four repositories in the MMRRC Consortium in the United States. The MMRRC was established in 1999 as a national system of four regionally distributed mouse archive and distribution repositories and research centers. Together with and an Informatics, Coordination and Service Center (ICSC), the individual MMRRC centers function as a fully integrated repository system. The four centers import, quality control, maintain, archive, and distribute mouse lines upon request. The ICSC provides an online searchable catalogue, dynamic website, strain curation services, data resources, technical assistance, outreach, and education activities. Although all four MMRRC centers archive and distribute KOMP2 mice, two of the centers (UC Davis and JAX) also host and operate KOMP2/IMPC production and phenotyping centers. Coordination between the KOMP2 program and the MMRRC Consortium, and especially the colocation of MMRRC repositories with IMPC centers, greatly expedites and streamlines the availability and accessibility of mutant mouse lines for the benefit of the research community. The IMPC production centers ship batches of lines to a designated MMRRC center where they undergo quality control verification procedures, gene-specific genotyping, cryopreservation, and archiving. Reciprocal data integration occurs through data mirroring between the MMRRC Consortium and the IMPC, a visible presence on each others’ websites, IMPC referral of researchers to an MMRRC center for ordering mouse lines and information on husbandry, care, and genotyping, and MMRRC referral of researchers to an IMPC member for production and phenotyping data, images, and other relevant information.

In conclusion, national repository systems have coordinated their efforts and agreed to abide by the highest standards of care and best practices in order to provide certified mutant mouse resources to the global research community. Beyond traditional biobanking functions, recent efforts by repositories have been aimed at increasing the availability of scientifically valuable mutant mouse lines and to reducing barriers to access to those mouse lines by everyone and anyone conducting experimental research. For example, many repositories now have earned “approved vendor status” recognition by receiving institutions, allowing them to import mice directly from a repository without quarantine and with minimal delay. Finally, many repositories offer customizable complementary procedures and services that increase the potential for developing scientific
collaborations with investigators.

**Keywords:** Mouse, Biobank, Knockout, Repository, Distribution

**References**
Large-Scale Pathology Phenotyping

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Summary
The laboratory mouse is an invaluable model for functional annotation of mammalian genomes, for improving our understanding of the genetic basis of normal human development, biology, and disease, and for preclinical translational research. The International Mouse Phenotyping Consortium (IMCP) is developing a comprehensive catalogue of mammalian gene function. The IMPC aims to produce a mouse strain with a null mutation for every protein-coding gene in the mouse genome, to acquire primary broad-based phenotype data for each mutation, and to publically disseminate the mutant resources and phenotype data to the global scientific community. By the beginning of 2017, the IMPC had generated over 6,000 mouse mutant strains and phenotyped nearly 5,000. All phenotype data is available via the IMPC web portal (www.mousephenotype.org).

The IMPC phenotyping pipeline is standardized across all participating Centres. The pipeline uses a battery of clinical and terminal tests assembled and designed to provide high-throughput, cost-efficient, sensitive, and systematic analysis for phenotypes associated with single gene mutations and deliver the raw, quality-controlled, and statistically analyzed phenotype descriptions to the community for further analysis. The pipeline includes necropsy and collection of a standardized panel of tissue from each mouse strain, histology (processing and embedding of the tissue to glass slides) and histopathology (analysis of the tissue and annotation of findings).

Pathology plays a pivotal role in bespoke, hypothesis-driven research using mouse models, providing important insights into the morphological consequences and mechanisms of gene function. It is also known from basic and applied research using the mouse, that it is often the description of the underlying tissue dysmorphology (histopathology) that moves a mutant mouse phenotype to a bona fide phenocopy model of human disease. In large-scale hypothesis-generating phenotyping, expected and unexpected phenotypes will only be found if they are screened for in a phenotyping pipeline. A pilot histopathology screen using 2 female and 2 male mice from 50 IMPC knockout lines showed that histopathology adds either correlating morphological data that adds essential tissue-level detail to the phenotype description, or novel findings not predicted from the clinical screen in 23 of 30 (76%) lines that had phenotypes in the clinical pipeline (5). Histopathology screening also identified and described phenotypes in 4 of 20 (20%) lines that had no phenotype detected in the clinical pipeline.

Pathology has a complementary and unique contribution to high-throughput primary phenotyping. Examples will be provided of histopathology rescuing clinical phenotypes as models of human disease, identifying phenotype(s) in strains with no clinical phenotype, characterizing and extending clinical phenotypes at the tissue level, and defining cross-species genotype-phenotype relationships and mechanisms of disease.
**Keywords:** Mouse Models of Human Disease, Mutagenesis, Phenotyping, Pathology

**References**


Progress and Prospects for the NIH Knockout Mouse Phenotyping Project

Colin FLETCHER

Knock Out Mouse Phenotyping Program (KOMP2)
National Human Genome Research Institute National Institutes of Health, Maryland, USA

Summary
The NIH funded KOMP project marks its 10th year in 2016. The project was envisioned as an effort to determine the phenotypes of a comprehensive set of null mutation in the mouse.

Lecture Notes
The NIH funded KOMP project marks its 10th year in 2016. The project was envisioned as an effort to determine the phenotypes of a comprehensive set of null mutation in the mouse. The project was launched in 2006 with the support of 18 institutes and centers at NIH, and operated in conjunction with similar European efforts. The first iteration of the project, from 2006 – 2011, accomplished the task of creating a library of 9,000 knockouts in the form of embryonic stem cells. In 2011, the focus shifted to animate of live mice from this library and the broad phenotyping of the strains. The project has been renewed for an additional five years and intends to complete the phenotyping of over 5,500 knockouts by 2021. KOMP, as part of the IMPC, has delivered key scientific insights into gene pleiotropy and the importance of sex as a biological variable. In addition, KOMP leads the NIH efforts to improve rigor and reproducibility in the scientific enterprise.

Keywords: KOMP, IMPC
Gut Microbiota and Reproducibility of Rodent Models of Disease

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Summary
The gut microbiota (GM) is the community of commensal, symbiotic microorganisms that occupy the intestinal tracts of animal species. In recent years, the interest in microbiota has exploded due to advancement and availability of technologies to define and analyze these complex populations. Of importance to the biomedical research community is the growing wealth of data showing that the differences in GM are associated with differences in model phenotypes. These include models of intestinal disease, but also a surprisingly diverse range of physiological processes such as neurodevelopment and behavior. Our laboratory and others have shown that a host of common factors in rodent husbandry, such as diet, housing, bedding, shipping, water decontamination and animal source can alter the composition of the GM. A causal role for GM in phenotype change can be assessed using a variety of procedures ranging from reconstituting germfree mice with defined flora to complex microbiota targeted rederivation (CMTR). With CMTR, mice of the desired genotype are rederived using embryo transfer into surrogate dams with one or more desired GM profiles. The pups are then seeded with the GM of their respective surrogate dams, resulting in genetically identical animals with different, defined, complex GM. Using CMTR, we have shown that different, naturally occurring GM profiles significantly influence lesion score severity in a mouse model of inflammatory bowel disease and tumor burden in a rat model of hereditary colorectal cancer. Collectively, these findings underscore how differences in the composition of the GM can contribute to phenotypic differences of animal models. The influence of the GM on animal models can thus be considered as a potential hindrance to model reproducibility that can now be controlled, but also as a tool to identify which microbes might be investigated further as potential contributors to health and disease. For use in these studies, we have created colonies of mice with four standardized complex GM. The latter have been maintained for over ten generations with minimal drift in GM composition, richness and diversity. In addition to serving as sources of surrogates for CMTR, these standardized colonies may also be used as fecal donors of complex GM through co-housing, cross-fostering and fecal transplantation. As a result, these colonies may prove to be invaluable as tools for addressing how complex GM impact rodent model reproducibility.

Abbreviations: GM = gut microbiota

- Overarching objectives:
  - Determine if complex GM vary in contemporary rodent colonies and assess what factors cause (or correlate with) such variation
  - Determine whether existing variation of GM among contemporary rodent colonies can influence model phenotypes of inflammatory bowel disease, colorectal cancer and other disease models
  - Establish means to manipulate complex GM to better assess its role in model refinement,
model optimization, translatability and applicability to precision medicine

- Factors found to influence complex GM composition, richness and/or diversity in contemporary rodent colonies
  - Age of rodent (marked changes during early life)
  - Rodent producer
  - Antibiotic manipulation
  - Shipping
  - Location of sampling (e.g. jejunum vs cecum vs colon)
  - Housing type (i.e. ventilated rack vs static microisolator)
  - Bedding (interaction with housing type)
  - Diet (acute change)

- Factors found to slightly influence complex GM, but less likely to be of biologic relevance
  - Water decontamination
  - Facility within institution
  - Standard rodent chow diets

- Factors under investigation
  - Sex of rodents
  - Enrichment strategies
  - Quarantine procedures

- Model phenotypes influenced by differing complex GM that exist in contemporary rodent colonies
  - Inflammatory Bowel Disease (e.g. IL-10 knockout model)
  - Colorectal Cancer (e.g. PIRC rat)

- Methods to manipulate complex microbiota
  - Fecal (or intestinal content) transfer to axenic mice
    - **Pros** – can be well controlled; can also be used for transfer of defined flora, monoclonization or xenogenic GM (e.g. humanizing)
    - **Cons** – expensive; requires specialized facilities; requires use of second generation animals to account for early life events; incomplete transfer of xenogenic GM and lack of normal immune development in xenogenic transplants
  - Co-housing
    - **Pros** – ease of use; low cost
    - **Cons** – incomplete transfer resulting in hybridization of GM; requires use of second generation animals to account for early life events
  - Cross-fostering
    - **Pros** – ease of use; low cost; early (w/in 24 hours) transfer of GM
    - **Cons** – poorly studied; requires timed mating; may also result in some hybridization; minimal vaginal microbiota contribution to GM
  - Fecal transplants (post-antibiotic)
    - **Pros** – moderate cost; doable in most lab setting
    - **Cons** – incomplete transfer with success limited to transfer of high richness GM to recipient with low richness GM

- Standardized complex microbiota colonies as tools for study
  - Can be used for all of the above methods as well as complex microbiota targeted rederivation (CMTR)
• Colonies created represent four major producers in the US
  • Stable GM for over more than 10 generations
  • Disparate GMs maintained when rodents shipped to other institutions with minimal GM drift in composition, richness or diversity

• Impact on animal welfare
  • Troubleshoot and improve study design and reproducibility
  • Define and monitor GM
  • Environmental factors
  • Early life events in axenic mice studies
  • Optimize models and ultimately decrease animal numbers
  • Biomarkers for non-terminal endpoints?

• Future directions
  • More complex GM for studies (i.e., wild mouse GM)
  • Impact of differing complex GM on other model phenotypes
  • Trans-kingdom considerations – contribution of viruses, fungi, protozoa, etc.
  • Improved bioinformatics and statistical analysis strategies
  • Coupling of GM identity with function (metabolomics, etc)
  • Coupling of GM with host genetics in model phenotype

• Paradigm shift - Incorporating complex GM studies into GM studies which currently are highly focused on more reductionist approaches – improve translatability and/or model precision medicine

Keywords: Microbiota (complex), Metagenomics, Model Reproducibility, Microbiota Manipulation.
References


Macaque Medicine for Beginners

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(American Expatriate)

Summary
Veterinarians assigned to begin caring for macaques may feel apprehensive and inadequate, but if they have training and experience providing care to common pets and/or livestock they can apply much of what they already know to caring for macaques. However, it is critical to learn important behavioral, anatomic, and other differences in the macaques, compared to other species. These differences influence the types of health conditions a veterinarian can expect to see and the treatment methods they should employ. The laboratory animal research environment provides advantages over the clinical practice environment, because of the ability to more closely monitor the animals on a daily basis, to detect health problems early and to more closely track responses to therapies. Veterinarians must be prepared to recognize pain and distress in macaques and know which treatments are most effective. The two most common health conditions seen in macaques in an animal research facility are diarrhea and wounds. There are many types and causes of diarrhea in macaques and the veterinarian should generally resist the temptation to aggressively treat every case in the same way. Wounds are most often caused by fighting, but macaques can also find ways to injure themselves with cage parts or other items within reach. Fight wounds inflicted by female macaques may require different treatment than wounds inflicted by males. Acute gastric dilatation occurs in macaques, but it does not usually involve torsion. Macaques are susceptible to a range of infectious diseases caused by viruses, bacteria, fungus, and parasites, a few of which are unique to the species, but many of which can pass between species. It is also important to be aware of zoonotic diseases that macaques may carry. Macacine herpesvirus 1 (aka Herpes B) is probably the most feared because of its role in documented cases of human fatalities. It is important to know how to prevent exposure of humans to Herpes B and how to conduct effective post exposure follow up. Miscellaneous conditions may be encountered and will be discussed briefly in this presentation, such as nutritional diseases, alopecia, frost-bite, behavioral problems, dental problems, foreign bodies, dystocia, cancer, and diabetes. The learning curve required to progress from a beginner to a competent primate veterinarian may not be as steep as some fear, but a 45 minute lecture is not nearly enough time to cover everything. One should take comfort in and take advantage of the published information, as well as the ready network of colleagues in the laboratory animal field who are willing to share their expertise. We all want to see the animals receive the best care possible.

A selection of important features of macaques:

**Behavioral** – relatively intelligent; diurnal; intricate social structure/hierarchy, matriarchal; males protect and help keep peace; social grooming; quadrapedal, but can walk upright; arboreal and terrestrial; skilled swimmers; tend to flee vertically; very threatened by direct stare; spend large amount of time foraging for food when in wild; need enriched environment to promote psychological well-being; some self-awareness

**Anatomic** – binocular, color vision; hands with opposable thumbs; dexterous fingers; grasping toes; nails,
ischial callus; variable tail length between macaque species cheek pouches; long, sharp canine teeth in males; sexually dimorphic; two pectoral mammae;

**Other** - Vitamin C requirement; omnivorous, with preferences for fruits, vegetables and nuts;

### Rhesus Normal Values (1)

<table>
<thead>
<tr>
<th>Value</th>
<th>Value</th>
<th>Weanlings</th>
<th>Juvenile</th>
<th>Adult Male Indoor</th>
<th>Adult Male Outdoor</th>
<th>Adult Female Indoor</th>
<th>Pregnant Female</th>
<th>Geriatric Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC x106/μl</td>
<td></td>
<td>4.9-5.7</td>
<td>4.85-5.9</td>
<td>5.7-6.4</td>
<td>4.95-6.1</td>
<td>5.46-6.0</td>
<td>4.8-5.5</td>
<td>5.85-6.94</td>
</tr>
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<td>Hgb (gm/dl)</td>
<td></td>
<td>11.0-12.8</td>
<td>10.7-12.7</td>
<td>12.9-15.1</td>
<td>11.7-14.3</td>
<td>12.2-13.4</td>
<td>12.1-13.0</td>
<td>13.7-15.5</td>
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<tr>
<td>Hct (%)</td>
<td></td>
<td>34.2-39.6</td>
<td>32.2-38.0</td>
<td>42.6-49.6</td>
<td>35.9-44.3</td>
<td>38.3-42.3</td>
<td>34.8-38.2</td>
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<tr>
<td>MCV (fl)</td>
<td></td>
<td>66-74</td>
<td>61-71</td>
<td>72-76</td>
<td>67-74</td>
<td>67-73</td>
<td>68-74</td>
<td>67-73</td>
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<td>MCH (pg)</td>
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<td>21.8-24.6</td>
<td>22.3-24.7</td>
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<td>MCHC (gm/dl)</td>
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<td>31.5-33.1</td>
<td>32.6-34.0</td>
<td>29.6-31.2</td>
<td>31.3-33.5</td>
<td>31.0-32.4</td>
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<td>WBC x103/μl</td>
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<td>7.4-13.0</td>
<td>9.6-20.0</td>
<td>5.8-10.4</td>
<td>8.3-18.1</td>
<td>6.4-10.2</td>
<td>8.2-18.8</td>
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<td>Mono x103/μl</td>
<td></td>
<td>1-5</td>
<td>0-8</td>
<td>0.4</td>
<td>1-5</td>
<td>1-7</td>
<td>2-6</td>
<td>4-10</td>
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<td>Lymph x103/μl</td>
<td></td>
<td>49-72</td>
<td>17-45</td>
<td>38-60</td>
<td>16-42</td>
<td>33-51</td>
<td>9-25</td>
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<tr>
<td>Neut x103/μl</td>
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<td>25-47</td>
<td>46-76</td>
<td>34-58</td>
<td>54-78</td>
<td>43-61</td>
<td>68-84</td>
<td>39-63</td>
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<tr>
<td>Eosin x103/μl</td>
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<td>1-3</td>
<td>0-4</td>
<td>0-4</td>
<td>0-2</td>
<td>0-4</td>
<td>1-3</td>
<td>1-7</td>
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<tr>
<td>Platelets x106/μl</td>
<td></td>
<td>3.54-6.52</td>
<td>4.47-6.47</td>
<td>2.97-4.99</td>
<td>3.13-4.57</td>
<td>3.56-6.54</td>
<td>2.46-4.56</td>
<td></td>
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<tr>
<td>Plasma Protein (gm/dl)</td>
<td></td>
<td>6.2-6.9</td>
<td>6.1-6.7</td>
<td>6.8-8.0</td>
<td>6.8-7.6</td>
<td>7.2-8.0</td>
<td>6.3-7.1</td>
<td>6.9-7.9</td>
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<tr>
<td>Fibrinogen (mg/dl)</td>
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<td>100-300</td>
<td>100-300</td>
<td>100-300</td>
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### Serum Chemistry

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<tr>
<th>Value</th>
<th>Value</th>
<th>Adult Males Indoor</th>
<th>Adult Male Outdoor</th>
<th>Adult Female Indoor</th>
<th>Geriatric Females</th>
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</thead>
<tbody>
<tr>
<td>Sodium (mM/l)</td>
<td></td>
<td>144-152</td>
<td>147-155</td>
<td>146-150</td>
<td>145-151</td>
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<tr>
<td>Potassium (mM/l)</td>
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<td>3.1-4.2</td>
<td>4.7-5.7</td>
<td>4.3-5.3</td>
<td>4.6-5.8</td>
</tr>
<tr>
<td>Chloride (mM/l)</td>
<td></td>
<td>104-112</td>
<td>106-110</td>
<td>112-116</td>
<td>106-114</td>
</tr>
<tr>
<td>Total CO2 (mM/l)</td>
<td></td>
<td>18-29</td>
<td>21-27</td>
<td>22-28</td>
<td>20-26</td>
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<tr>
<td>Anion Gap (mM/l)</td>
<td></td>
<td>15-23</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Calcium (mM/l)</td>
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<td>8.7-10.8</td>
<td>9.9-10.9</td>
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<tr>
<td>Phosphorus (mg/dl)</td>
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<td>3.8-5.6</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td>0.7-1.4</td>
<td>1.0-1.4</td>
<td>0.8-1.2</td>
<td>0.9-1.3</td>
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<tr>
<td>BUN (mg/dl)</td>
<td></td>
<td>17-29</td>
<td>22-30</td>
<td>16-22</td>
<td>13-21</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td></td>
<td>38-74</td>
<td>55-89</td>
<td>43-71</td>
<td>40-72</td>
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<td>ALT (U/l)</td>
<td></td>
<td>1-40</td>
<td>24-78</td>
<td>26-52</td>
<td>41-79</td>
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<tr>
<td>Alk Phos (U/l)</td>
<td></td>
<td>0-603</td>
<td>55-237</td>
<td>91-181</td>
<td>98-216</td>
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<tr>
<td>Total Protein (g/dl)</td>
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<td>5.9-7.9</td>
<td>7.3-8.3</td>
<td>7.2-8.0</td>
<td>6.5-7.9</td>
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<tr>
<td>Albumin (g/dl)</td>
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</tr>
<tr>
<td>GGT (U/l)</td>
<td></td>
<td>21-50</td>
<td>51-85</td>
<td>48-76</td>
<td>35-59</td>
</tr>
<tr>
<td>CPK (U/l)</td>
<td></td>
<td>88-1796</td>
<td>63-237</td>
<td>0-436</td>
<td>0-3003</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td></td>
<td>23-68</td>
<td>27-45</td>
<td>24-38</td>
<td>40-88</td>
</tr>
<tr>
<td>Tot bilirubin (mg/dl)</td>
<td></td>
<td>0-0.2</td>
<td>0-0.3</td>
<td>0.1-0.3</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>LDH (U/l)</td>
<td></td>
<td>193-598</td>
<td>223-505</td>
<td>217-419</td>
<td>216-870</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td>68-190</td>
<td>132-190</td>
<td>148-200</td>
<td>127-187</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td></td>
<td>11-64</td>
<td>72-256</td>
<td>18-92</td>
<td>40-306</td>
</tr>
</tbody>
</table>

(rectal temp: 99.0-102.5 F  
HR: 120-180bpm  
RR: 30-70/min  
gestation: 160-170 days)
NHP Formulary (2)

**ANALGESICS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buprenorphine</td>
<td>.01-.03mg/kg IM BID</td>
</tr>
<tr>
<td>Butorphanol</td>
<td>.05mg/kg IM TID</td>
</tr>
<tr>
<td>Carprofen</td>
<td>2mg/kg PO BID</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>7-10mcg/kg/hr IV CRI; .05-.15mcg/kg IM PRN; Patch: 25mcg/5-10kg, 50mcg/10kg animal q48-72h</td>
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<tr>
<td>Flunixin Meglumine</td>
<td>2mg/kg IM BID</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>7mg/kg PO BID</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>2mg/kg IV/IM SID</td>
</tr>
<tr>
<td>Ketorolac</td>
<td>15-30mg/animal initially; then 10-15mg/animal q8h</td>
</tr>
<tr>
<td>Morphine Sulfate</td>
<td>1-2mg/kg IV/IM/SQ q4h</td>
</tr>
<tr>
<td>Nalbuphine</td>
<td>2.5-5mg/kg IM q3-4h</td>
</tr>
<tr>
<td>Naloxone</td>
<td>.1mg/kg IV/IM/SQ PRN</td>
</tr>
<tr>
<td>Oxymorphone</td>
<td>.15mg/kg IV/IM/SQ q4-6h;</td>
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**ANESTHETICS / SEDATIVES**

<table>
<thead>
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<tbody>
<tr>
<td>Acepromazine</td>
<td>0.1-.5mg/kg IV/IM</td>
</tr>
<tr>
<td>Atipamezole</td>
<td>.15mg/kg IV/IM</td>
</tr>
<tr>
<td>Diazepam</td>
<td>seizure: .5-1mg/kg IV/IM</td>
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<td>Droperidol</td>
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<tr>
<td>Fentanyl/Droperidol</td>
<td>0.1-0.3ml/kg IM</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>.03-.05mg/kg IM BID</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10mg/kg IM; 5mg/kg IV</td>
</tr>
<tr>
<td>Ketamine/Xylazine</td>
<td>Ket 7mg/kg IM; Xyl 0.6mg/kg</td>
</tr>
<tr>
<td>Meditomidine</td>
<td>10-35mcg/kg IM</td>
</tr>
<tr>
<td>Midazolam</td>
<td>.05-.1mg/kg IV(slow)/IM</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>adult: 25mg/kg IV slowly to effect; juvenile 15mg/kg IV slowly to effect</td>
</tr>
<tr>
<td>Phenoobarbital</td>
<td>Seizure: 2mg/kg IV; 1-6mg/kg PO</td>
</tr>
<tr>
<td>Propofol</td>
<td>2.5-5mg/kg bolus then 0.3-0.4mg/kg/min CRI</td>
</tr>
<tr>
<td>Thiethyl Sodium</td>
<td>15-25mg/kg IV to effect</td>
</tr>
<tr>
<td>Thiopental</td>
<td>25mg/kg IV to effect</td>
</tr>
<tr>
<td>Tiletamine/Zolazepam</td>
<td>5-8mg/kg IM</td>
</tr>
<tr>
<td>Xylazine</td>
<td>.5-6mg/kg IM</td>
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<tr>
<td>Yohimbine</td>
<td>.1mg/kg IV/IM</td>
</tr>
</tbody>
</table>

**Keywords:** Macaque, Veterinary Medical, Laboratory Animal

**References**

(1) NHP Normative Data from California National Primate Research Center. Posted on Association of Primate Veterinarians (APV) Member Website, https://www.primatevets.org/education

(2) Lee DR, Doane CJ. NHP Formulary, Almagordo Primate Facility. Posted on APV Member Website, https://www.primatevets.org/education
Macaque Models of Human Disease

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Summary
Nonhuman primates are valuable animal models for studying many human conditions because of their close similarities to humans across a wide spectrum of physiologic and disease research areas. The macaque species most commonly used as models of human disease are cynomolgus macaques (Macaca fascicularis) and rhesus macaques (Macaca mulatta). Other species of macaques are used to a lesser extent, depending largely on regional availability and current preferences in the scientific community. Some macaque species used as animal models in the past are no longer readily available for biomedical research. During the past 29 years the presenter has had experience supporting a wide variety of research projects using macaques as models of human disease. This presentation will provide an overview of selected studies using macaque models in the following areas: neuroscience; pharmaceutical research, development and toxicology; infectious diseases; obesity and diabetes; reproduction; ophthalmology; developmental origins of health and disease; intrauterine gene therapy; bone regeneration and repair; and organ and tissue transplants. Project support issues and lessons learned will be shared as time allows in this brief introduction to macaque models of human disease.

Notes:
Because of their close phylogenetic relationship to humans, nonhuman primates are valuable animal models for many areas of research, including, but not limited to neurobehavior, physiology, developmental biology, immunology and genetics (Vallender and Miller, 2013). Among nonhuman primates, macaques are used with greatest frequency as animal models of human disease. The macaque species most commonly used are rhesus (Macacacmulatta) and cynomolgus macaques (Macacafascicularis, aka cyno). Japanese (Macacafuscata), pigtail (Macacanemestrina), bonnet (Macacaradiata), and Barbary macaques (Macacasylvanus) are used to a lesser extent, depending largely on regional availability and investigator preference. Among researchers, there are preferences for certain species over others for selected types of research. Historically, stumptail macaques (Macacaarctoides) were used as models for baldness research, leading to the discovery of minoxidil. Some behavioral researchers prefer rhesus over cynos for cognitive testing, claiming cynos have a shorter attention span. Even within species there appear to be differences in suitability as models of human disease depending on origin or subspecies. Indian-origin rhesus macaques have been favored over Chinese-origin rhesus for HIV-related research. Cynomolgus macaques are used in greater numbers than any other macaque species in the pharmaceutical industry. Significant body size variations are seen between subspecies of cynomolgus macaques, with use of larger subspecies providing the advantage of larger blood volume for sample collection during PK studies. Body size may also be a consideration for organ transplant studies, with larger macaques, such as rhesus, Japanese, and pigtail macaques preferred over smaller cynos. This presentation will focus on a selection of macaque models with which the presenter has had some experience. These are outlined below:
1. Neuroscience
   a. Behavior and cognition
   b. Parkinson's Disease
   c. Cranial Implants
2. Pharmaceutical and CRO
   a. PK and Safety Toxicology
   b. Cardiovascular Telemetry
   c. Cisterna Magna Cannulation
3. Infectious Diseases
   a. Immunodeficiency Viruses
      i. HIV
      ii. SIV
      iii. SHIV
   b. Tropical diseases
      i. Malaria
      ii. Dengue
      iii. Zika virus
4. Obesity and Diabetes
   a. High fat diet
   b. STZ Induced diabetes
   c. Metabolic Syndrome Macaques
   d. Pancreatic Islet Cell Transplant
5. Reproductive
   a. Assisted Reproductive Technology (ART)
   b. Cloning
   c. Genetically Modified Macaques
6. Ophthalmology
   a. Ocular Hypertension
   b. Glaucoma
   c. Retinal Disease
   d. Diabetic Retinopathy
7. Developmental Origins of Health and Disease
   a. Nutritional deficiencies
   b. Maternal nutrition
   c. Epigenetics
8. Intrauterine Gene Therapy
   a. Single gene disorders
   b. Viral Vectors
9. Bone Regeneration and Repair
   a. Osteoporosis
   b. Mandibular reconstruction
10. Organ and Tissue Transplant
    a. Heart-lung transplant
    b. Heterotopic heart transplant
    c. Human tissue/stem cells
    d. Ethical Issues of Human Animal Combinations

There is often a steep learning curve involved with introduction of a macaque model into a research program. It is important to share lessons learned and best practices with veterinary colleagues in the research community.
to optimize model development and use. Examples include the use of individual macaque temperament screening to aid selection of suitable candidates for cognitive testing (Coleman et al., 2005) and adopting refinements in chronic collection of cerebral spinal fluid. (Gilberto et al., 2003).

Keeping current with the latest refinements in macaque care and use is essential to humane and high quality science in pursuit of improvements in prevention and treatment of human diseases.

**Keywords:** Macaque, Animal Model, Research

**References**


Translational Research from Mouse to Human Clinics

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Professor, Seoul National University College of Veterinary Medicine, Korea

Summary

Mouse models are crucial for the functional annotation of human genome. Gene modification techniques including gene targeting and gene trap in mouse have provided powerful tools in the form of genetically engineered mice (GEM) for understanding the molecular pathogenesis of human diseases. Several international consortium and programs are under way to deliver mutations in every gene in mouse genome. The information from studying these GEM can be shared through international collaboration. However, there are many limitations in utility because not all human genes are knocked out in mouse and they are not yet phenotypically characterized by standardized ways which is required for sharing and evaluating data from GEM.

Body

The recent improvement in mouse genetics has now moved the bottleneck in mouse functional genomics from the production of GEM to the systematic mouse phenotype analysis of GEM. Enhanced, reproducible and comprehensive mouse phenotype analysis has thus emerged as a prerequisite for effectively engaging the phenotyping bottleneck. In adipose tissue, agonists of the β3-adrenergic receptor (ADRB3) regulate lipolysis, lipid oxidation, and thermogenesis. The deficiency in the thermogenesis induced by neuroblast differentiation-associated protein AHNAK in white adipose tissue (WAT) of mice fed a high-fat diet suggests that AHNAK may stimulate energy expenditure via development of beige fat. Here, we report that AHNAK deficiency promoted browning and thermogenic gene expression in WAT but not in brown adipose tissue of mice stimulated with the ADRB3 agonist CL-316243. Consistent with the increased thermogenesis, Ahnak(-/-) mice exhibited an increase in energy expenditure, accompanied by elevated mitochondrial biogenesis in WAT depots in response to CL-316243. Additionally, AHNAK-deficient WAT contained more eosinophils and higher levels of type 2 cytokines (IL-4/IL-13) to promote browning of WAT in response to CL-316243. This was associated with enhanced sympathetic tone in the WAT via upregulation of adrb3 and tyrosine hydroxylase (TH) in response to β-adrenergic activation. CL-316243 activated PKA signalling and enhanced lipolysis, as evidenced by increased phosphorylation of hormone-sensitive lipase and release of free glycerol in Ahnak(-/-) mice compared to wild-type mice. Overall, these findings suggest an important role of AHNAK in the regulation of thermogenesis and lipolysis in WAT via β-adrenergic signalling.

Keywords: Mouse, Phenotyping, Obesity, Ahnak, Knockout Mouse

References

(1) Shin JH, Lee SH, Kim YN, Kim IY, Kim YJ, Kyeong DS, Lim HJ, Cho SY, Choi J, Wi YJ, Choi JH, Yoon YS, Bae YS, Seong JK. (2016) AHNAK deficiency promotes browning and lipolysis in mice via...


Improving the Mouse as a Model for Human Diseases

Axel Kornerup HANSEN

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Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Summary
Many mouse models used in pre-clinical drug development today show little predictive value, and enormous resources are vanished in the pharmaceutical industry to work on targets ultimately not working in humans. Due to this low predictive value of many nowadays mouse models future mouse models will be ‘humanized’, i.e. made more translational in relation to human diseases. This can be done by a combination of gene modification, cell transplantation, microbiome modification and the feeding of specialized diets. This furthermore has to be combined with ethical evaluations to assume customer acceptability this ‘humanization’ approach. Different approaches are necessary to improve models within different research fields, and therefore companies and research groups are likely to work more on their own tailor made mice rather than standard shelf mice in the future.

Introduction
The need for large group sizes and standardization forces human drug development to rely on high predictive validity of mouse models. So, the global mouse model is overall estimated to be worth USD 2.7 billion by 2021. However, the mouse shows poor translationability within a range of pre-clinical fields, such as inflammatory diseases, type 1 diabetes, metabolic syndrome, amyotrophic lateral sclerosis, cancer and several other fields. Therefore, more relevant models, such as non-human primates, have been applied. However, in addition to being costly and time consuming, the European Union has substantially limited the use of primates making the need to seek other ways of improving pre-clinical drug development urgent. Today, it is possible for especially larger universities to combine several techniques to turn nowadays mouse models into more predictive next generation models to increase the efficiency of preclinical research in the pharmaceutical industry. The Department of Veterinary and Animal Sciences at the University of Copenhagen has a close collaboration with the pharmaceutical industry to make specific improvements of their models by a combination of techniques. Also several biotechnology are now selling licenses valuing customized mouse models in the millions of USD, and several large pharmaceutical companies have humanized mouse models in development. Consequently, the sub-market for humanized mouse models is projected to reach USD 116.0 million by 2021. Humanized mice are permitting significant progress in human disease studies, and some of the first models to hit the market, such as custom-designed “humanized” antibody-producing (humAb) mice, have created significant uptake.

Gene modification
Today, even multiple replacement of specific genes in mice by human genes without extensive breeding, has become much easier by nuclease based techniques, such as CRISPR/Cas9 genome editing, which has led to more advanced “humanizations”, i.e. mice which has a few human cells, a short strand of human DNA, human tissues, a human tumor, a humanized immune system, or, parts of the human microbiome. Analyses of differential expression in tissues from humans discordant for a disease using NCBI
(ncbi.nlm.nih.gov/pubmed/) can provide a list of genes highly associated with that disease, and species homology analysis of these genes may identify the genes being least homologous and thus likely to play an important role in the poor translationability, and subsequently be replaced in mice.

**Cell transplantation techniques**

Commercial breeders and universities have put essential efforts into creating severely immune deficient platform mice, such as the Taconic NOG mouse or the Jackson Laboratory NSG mouse, which can be reconstituted by stem cell transfer. E.g. it is possible to create mice with a human immune system by engrafting CD34+ human hematopoietic stem cells into pre-irradiated NOG mice. In general, humanized leucocytes survive and proliferate in a mouse setting and recapitulate human disease pathogenesis, e.g. human T-cell specific leukemia virus (HTLV-1) infect and creates adult T cell leukemia in such huNOG mice within 4-5 months. Humanization of entire organs rather than targeting individual genes may be done by linking toxin receptors to molecules, which are expressed on specific mouse cells, which are then selectively killed by dosing animals with targeting toxin after having supplied the mouse with human stem cells to create a human tissue instead, e.g. to create mice with human livers.

**Microbiome and dietary modifications**

A mouse’s own gene pool is outnumbered by a factor 150 by the gut microbiota gene pool, which differs essentially between mouse and man, and which has been found to play pivotal roles in the development of a range of human diseases, when modeled in mice. Germ-free mice have been established with a human microbiota to express a human phenotype of specific diseases, and subsequently further diet manipulated to exhibit effects observed in humans. Such mice may be housed in a stable form in housing systems commonly used in the industry, but further work on inoculation regimes and ages, as well as the subsequent feeding, is needed for a human-like microbiota in mice to be efficient in priming the mouse immune system to make it express a close-to-human phenotype.

**Ethics**

Some have seen using animals as models for human diseases as unnecessary cruelty, while some regard using mice instead of animals higher on the so-called socio-zoological scale as positive though debatable. Humanization of mice adds new dilemmas to this, as a ‘respect for nature’ point of view may challenge the scientific world acting as the creator of living creatures. Through its various departments, our university offers to make ethical and legal considerations part of each mouse development project, and to study e.g. public perceptions, formulate policies and consult stakeholders, as we have e.g. done in a previous project on improving models for life-style diseases.

**Discussion**

Mouse models need to be improved to increase efficiency in pre-clinical research. This is indeed possible today, but the goal cannot be achieved by one single approach. It, therefore, needs to be done by a collaborative effort of various experts, e.g. as these can be found on the larger universities. Future mouse models are, therefore, most likely to be based upon several modifications achieved by different techniques. Since several important interactors and modulators of the immune system in stem cell reconstituted platform mice are still of mouse origin (cells and factors from the metabolic system, gut epithelial cells and endothelial cells), the models can be improved by genome editing, and some of the improvements achieved in the first place by stem cell technique may, if properly studied, be made permanent by gene modification. As a much larger gene pool is housed by the microbiome, the mice need to be inoculated with microbiotas specifically set up for specific research fields. This microbiota needs to be fed a diet consisting of nutrients, which will feed these bacteria, many of which will be of a human origin. This will also turn mouse production from previous shelf breeding of standard mice into contract production of tailor-made mice for each individual research group.
Keywords: Mice, Animal Models, Transgenes, Stem Cells, Microbiota, Diet

References
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Housing, Handling, Animal Welfare and the Laboratory Results

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Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Summary
Animal welfare in connection with experiments on animals is a major concern for many citizens. While the ethical guidelines of each country often details the housing for each laboratory species, the ethical permissions often focus on the experimental procedures with less emphasis on the routine management of the animals. The current paper outlines the effects of three common procedures on the welfare of the animals, as well as their scientific consequences. Cage cleaning is one of the most common procedures and seems to have a strong effect especially on mice, the effect on rat behaviour and welfare is more ambiguous. Handling is another very common procedure which may be done in different ways. Attempts at managing the effect of handling have focused on minimizing the human contact with the help of tunnels (for mice), habituating the animals to human contact (e.g. rabbits) or using positive interactions (e.g. tickling of rats). Training animals to cooperate for procedures such as blood collection is typically employed on animals that may otherwise be hard to handle, e.g. primates. While most of the training procedures used have employed positive reinforcement, a recent study indicates that a combination of negative and positive reinforcement may be used minimal negative effects.

Introduction and background
Animal welfare in general is a major concern for many citizens. Experimental research using laboratory animals is an area that is and has been very much in focus (1), and the EU directive 2010/63/EU have regulated e.g. the animal species that are to be taken into consideration, the way in which these animals should be housed and handled and how the 3Rs (reduction, replacement and refinement) are to be implemented throughout the study-periode. Ethical permissions often focus primarily on the research protocol, but much less on the routine procedures. In the present presentation we will address three different areas; the routine changes and cleaning of cages, the handling of animals in connection with moving or sampling the animals and finally the training of animals.

The main negative consequence of all of these is an increase in stress and/or fear. That this will affect the scientific result in a number of different ways is evident. The most obvious behavioural effects are perhaps the effects on tests used for fear and anxiety (e.g. open field tests, elevated plus-maze). Less evident is perhaps that it can also affect e.g. pain/nociception related tests such as the tail-flick test (2). There is also a risk that stress related physiological measures of e.g. glucocorticoids and blood pressure are also affected (3).

1. Cage changes
The bedding in the cages of mice and rodents gets soiled over time and needs to be changed. In many cases both the bedding and the cage is changed, with the consequence of the animals encountering a completely new olfactory milieu after the change. There are conflicting findings, concerning the impact of this change. For mice it seems that the change of cages is stressful and may result in e.g. disturbed sleep...
patterns (4). Attempts at decreasing the novelty of the situation, and hence the stress response, by either bringing some of the old bedding over into the new cage or alternatively just changing the bedding and keeping the old cage increases the risk for aggression (5, 6). Transferring old nesting material on the other hand seems to decrease the risk for aggression (6).

The effect of cage changes on rats is inconclusive with some studies showing a minimal effect (e.g. 7), others showing non-consistent reactions (8) and finally some showing fairly strong reactions (e.g. 9, 10, 11). The study by Meller et al. (11) also investigated the use of transference of the old lid or an enrichment object (see also the mice studies above), and found that the stress reaction they had found was ameliorated by the presence of the familiar object/odour.

One factor that may explain some of the variation in results is the timing of the cage changes. In many cases the cages are changed during the light period, i.e. the period during which the mice and rats are inactive. Cage change during this period is likely to cause more stress than if they are done during the dark period (12). Other factors are e.g. the strain of mice and possibly the gender of the care taker, as mice may be more stressed by the presence of a man compared to a woman (13).

2. Handling

A naïve rodent that is picked up probably perceives this as an attack by a predator, and it is therefore not surprising that handling may result in severe stress responses in the animals. There are three different approaches to decrease the stress response. The restraint part of the handling, and in a sense the handling itself, can be minimized, the animals can be habituated to being handled and finally they can be trained to associate the handling with something positive. The minimization of handling is most often done with mice. Gouveia and Hurst have published a number of papers that document the positive effect of either a method in which the mice are allowed to creep up into the cup of the hand of the experimenter or allowed to crawl into a tube, compared to the more common "tail-picked" method (e.g. 14, 15). The far reaching consequence a choice of handling method may have has also been shown by Ghosal et al (3) who looked at the glucose tolerance of mice handled either by the cup-method described above or the traditional tail-picked method.

The discovery of the "laughter" in tickled rats (16) has led a number of researchers to investigate the possibility of having positive interactions with rats. Cloutier and co-workers have published papers reporting positive effects of a "tickling regime" (17, 18). The tickled rats were reported to be less fearful of the handler, as well as being easier to inject. The effect was most pronounced when the tickling started with the young animals (18). However also more traditional positive handling experiences, i.e. stroking of neck and back, seem to affect the fear levels of rats (19).

3. Training for experimental procedures

While rodents are easy to handle not all animals are that tractable. Primates are often kept in groups and it may be hard to handle. There has therefore been considerable interest in training them to present themselves so that scientists may take samples from them "voluntarily". The same techniques may be used on any animal that is to be kept for a longer time period and/or frequently sampled. In most cases the training method used is based on positive reinforcement (e.g. 20). This has the advantage that it allows for an open behavioural response which can easily be modified to various situations. Training with positive reinforcement can however be quite time consuming and therefore costly. One way of decreasing the training time is to spread out the training occasions over a longer time period. Using laboratory beagles a paper have demonstrated that by training the dogs once a week instead of daily, it was possible to cut down on the number of training session with 25% (21). Another approach is to combine the positive reinforcement with negative reinforcement (22). In this study on macaques it hugely increased the speed of learning to enter a specific part of the cage, as compared to only positive reinforcement (10 out of the 12 individuals trained with negative reinforcement learned the task within the allocated time, no individual trained with positive reinforcement did). However, in this study the positive reinforcement training
was performed sub-optimally (without the use of a target) due to the risk of the study interfering with other training sessions in the same animals. The use of negative reinforcement training always introduces some aversive stimuli that the animal can escape or avoid, and hence it may seem a risk that the animals could form a negative association with humans. In the study by Wergård et al (22), no such difference between the groups was detected when a human approach test was done. It should be emphasized, though, that the use of NRT is not a simple technique, and it may result in negative consequences on animal welfare.

Conclusion
In conclusion, there are strong indications that management routines may heavily influence the results of experiments on animals. Unfortunately however, we still do not know enough to be able to predict the strength or even in some cases the direction of that influence. More knowledge of this would help us to refine our experimental set ups to yield more repeatable and valid results.

Keywords: Handling, Welfare, Stress, Laboratory Animals

References
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tickle-induced reward in rats: effects of social housing and genetic variables. Behav Brain Res 115:25–38
Role of Veterinarians in Translational Medicine

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Summary
Translational medicine has numerous definitions in various fields of medicine although the term was first started to be used in the field of cancer research. One of the most commonly used definition is, translational medicine includes two areas of translation, one is the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second area of translation is research aimed at enhancing the adoption of best practices in the community. As per another definition, translational medicine is part of a unidirectional continuum in which research findings are moved from the researcher’s bench to the patient’s bedside and community. In the continuum, the first stage of translational research (T1) transfers knowledge from basic research to clinical research, while the second stage (T2) transfers findings from clinical studies or clinical trials to practice settings and communities, where the findings improve health. So, what is the role of veterinarians in the above definitions of translational medicine?. Veterinarians play an important role in both stages of translational research. With their training and background in anatomy, systems biology, physiology and biochemistry, veterinarians have unique skill sets which enable them to be critical members of interdisciplinary teams, both in academia and industry. In the first stage, they can be veterinarian-scientists, similar to clinician scientists performing basic research at molecular level, laboratory animal veterinarians managing animal studies and developing animal model of human diseases, veterinary pathologist evaluating tissues for phenotypic changes of genetically modified animals or drug efficacy, veterinary ophthalmologists supporting development of ophthalmologic therapeutic agents, veterinary cardiologists evaluating cardiovascular safety of human medications for clinical trials, veterinary oncologists developing oncology medications for both human and animal cancer and veterinary surgeons evaluating the efficacy of medical devices for human use. In the second stage, they can function as epidemiologists or manage clinical trials. The talk will discuss in detail the role of veterinarians in both stages with specific examples of animal model development and future opportunities.

What is the best definition for translational research or translational medicine? One of the definitions used by NIH states that, translational research includes two areas of translation. One is the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second area of translation concerns research aimed at enhancing the adoption of best practices in the community (1). Another definition is, Translational research is a “bench-to-bedside” enterprise of harnessing knowledge from basic sciences to produce new drugs, devices, and treatment options for patients. The endpoint is the production of a promising new treatment that can be used clinically or commercialized as a result of the interface between basic science and clinical medicine. This enterprise is vital, and has been characterized as follows: “effective translation of the new knowledge, mechanisms, and techniques generated by advances in basic science research into new approaches for prevention, diagnosis, and treatment of disease essential for improving health (2).
In an Institute of Medicine’s Clinical Research Roundtable, the definition of translational research was elaborated and described as two “translational blocks” in the clinical research enterprise and labelled them as T1 and T2. The T1 is described as the transfer of new understandings of disease mechanisms gained in the laboratory into the development of new methods for diagnosis, therapy, and prevention and their first testing in humans and T2 is described as the translation of results from clinical studies into everyday clinical practice and health decision making (3). Though both T1 and T2 research are equally important, T1 seems to overshadow T2 in the United States, most individuals have T1 in mind when they use the term translational research and T1 attracts more funding (2). As veterinarians, our role is mostly related to T1 block, although some veterinarians play a role in in T2 block also.

Veterinarians have a unique skill set because of their training, which provides them enough knowledge in the basic subjects of human medicine along with their hands on experience in working on animals, both small and large animals which help them to be critical members of interdisciplinary research teams both in industry and academia. The professional veterinary curriculum uniquely includes education in systems biology, pathobiology, clinical and comparative medicine of a wide range of small and large animal species many instances in context to human disease, surgery, epidemiology, and public health (4).

A subset of veterinarians receive advanced research training, often leading to MS or PhD degrees. These highly trained individuals, veterinarian-scientists, similar to clinician-scientists who have an MS or PhD degrees with MD degree, will typically serve as principal investigators or members of collaborative research teams in academia, industry, or government. The T1 translational block research requires advanced knowledge of molecular biology, genetics, and other basic sciences. Many veterinary schools in US have a combined DVM-PhD programs which provides training in these areas. A veterinarian-scientist has the required knowledge in this area to function as a principal investigator in academia to conduct basic research on disease mechanisms and molecular basis of diseases. These veterinarian-scientists are also involved in drug discovery activities in pharmaceutical and biotech industry where knowledge on disease mechanisms and molecular basis of diseases is very important in identifying therapeutic targets. This is one area of translational medicine where veterinarians have been successful and there is also high demand for these veterinarian-scientists in academia and pharmaceutical industry.

Many veterinarians pursue advanced clinical training in one of the many specialties (analogous to human medicine) in veterinary medicine. Currently, there are 41 distinct specialties recognized by AVMA in USA. These veterinary specialties have a post graduate training program (residency) and some of these specialist veterinarians also pursue an MS or PhD degree program. The specialist veterinarians who are trained in laboratory animal medicine, pathology, ophthalmology, surgery, cardiology and oncology have active involvement in translational medicine.

The two specialties which are most actively involved in translational medicine are laboratory animal medicine and pathology. In USA, laboratory animal veterinarians who undergo a three year residency program, do have the opportunity to interact with several translation medicine researchers during their training programs. Many of these training programs for this specialty is conducted in medical schools which increases the opportunity for the trainees to directly involve in different aspects of translational research. The other specialty which actively play a role in translational medicine is veterinary pathology. Similar to laboratory animal medicine residency programs, many of the veterinary pathology residents get opportunity to interact directly with translational medicine researchers. Also, there are few comparative pathology fellowship programs for veterinarians who have completed a veterinary pathology residency programs and these fellowship programs are exclusively conducted in medical schools or cancer centers with primary focus on translational research. Many of the students in both these specialties pursue a graduate program and select a track to work as principal scientists in academia or pharmaceutical industry.
Over the past two decades, research grants in biomedicine show an increase in the number of studies using animals. This growth has also increased the demand for veterinarians to function as veterinarian-scientists, laboratory animal veterinarians, investigative or diagnostic pathologists, and members of research oversight committees. As mentioned earlier, both veterinary pathologist and laboratory animal veterinarians who have close interaction with translational medicine researchers during their training programs gain enough knowledge and experience to provide critical input for the above mentioned functions. One area in which laboratory animal veterinarians and veterinary pathologists play a key role in translational medicine is the development and validation of animal models of human diseases. These animal models play a key role in evaluating the efficacy and safety of drugs and medical devices and also validation of efficacy and safety biomarkers. This talk will also discuss in detail with specific examples related to the challenges in the development of animal models (5) and the role of veterinarians in these activities, both in academia and pharmaceutical industry.

Both veterinary ophthalmologists and cardiologist play an important role in preclinical studies for new drug and medical device development. They are involved in evaluating the safety of drugs and medical devices using animal studies. Similarly, veterinarians trained in surgery play an important role in medical device development as most of the medical devices are tested for efficacy and safety in animal models. Veterinary oncologists who study animal cancers play an important role in translational medicine because of the similarities in human and animal cancer biology. The comparative oncology program launched by National Cancer Institute studies naturally occurring cancers in animals especially spontaneous cancers in dogs and cats which share many features with human cancer including a predictable progression similar to human cancers.

Public health professionals have become increasingly aware of the role of veterinary medicine in the prevention of human illness especially zoonotic diseases and this is an area where veterinarians have an important role in the T2 block. Currently many veterinary colleges offer advanced education in veterinary public health and epidemiology in partnership with schools and colleges of public health. These veterinarians thus play an important role in translational medicine.

Do veterinarians have a formal role in human clinical medicine? Dr. Speare, a physician and a veterinarian in Australia believe that veterinarians need to be working with physicians for diagnosing zoonotic diseases (6). The One Health Initiative, a movement to unite human and veterinary medicine is very relevant in this context, which will increase the demand and role of veterinarians in both T1 and T2 blocks of translational medicine.

Keywords: Translational Medicine, Veterinarian, Comparative Medicine

References
The Evaluation of hES Cell-Derived Dopaminergic Precursor Cells in MPTP Induced Parkinson’s Disease Model with Common Marmoset

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Parkinson’s disease (PD) is the neurodegenerative disease associated with the loss of dopaminergic cells. Although L-DOPA has been known as the most effective drug against motor symptoms up to date, it is not the fundamental therapy, thus stem cell transplantation as the alternative therapy is currently under investigation. Therefore, the aim of this study is to evaluate the efficacy of hES cell-derived dopaminergic precursor cells with suitable differentiation status in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) induced Parkinson’s disease model with marmosets. For this, marmosets were subcutaneously administrated with 1.0-2.0 mg/kg MPTP for consequent 5 days. And, they were injected hES cell-derived dopaminergic precursor cells into caudate nucleus, putamen, and substantia nigra at 4 weeks after the last MPTP administration without any immunosuppressant. In MPTP-treated group, abnormal behavioral symptom was occurred at Day 2 and maintained for 32 weeks, whereas this symptom was attenuated from 2 weeks after hES cell-derived dopaminergic precursor cell transplantation and almost recovered at 10 weeks after transplantation. And decreased expression of radioisotope tracer 18F-FP-CIT in PET image following MPTP injection was significantly recovered at 14 weeks after transplantation. In conclusion, our findings suggest that hES cell-derived dopaminergic precursor cells have the therapeutic effect against MPTP-induced PD symptoms in marmoset, and can also be potential and novel therapy for PD patients.

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**Keywords:** Dopaminergic Precursor Cell, Parkinson’s Disease Model, Common Marmoset
Zooeyia – The Health Benefits of Companion Animals and an Essential Contributor to One Health in the Community

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Summary
One Health, dedicated to improving the health of all species through the integration of human and veterinary medicine, is often focused on controlling zoonoses. However, zooeyia, the health benefits of companion animals, is an essential component of One Health. The four categories of zooeyia are discussed. Pets build social capital. Pets motivate healthy behaviour change. Pets catalyze harm reduction. Pets are a resource for healthcare providers in developing some treatment plans.

Companion animals can profoundly influence the health of their families. While concerns about zoonotic risk can dominate, the health benefits of pets are manifold. These advantages are collectively known as zooeyia.(1) The word derives from the Greek zōion (animal), and Hygeia, the ancient Greek goddess of health, daughter of the god of medicine and goddess of soothing pain. Zooeyia can be sorted into four general categories.

First, pets build social capital – a fundamental contributor to health.(2) Importantly, they provide companionship in their own right, reducing loneliness and its negative impact on health.(3,4) This is particularly significant for those who live alone.(5) Children, who are more likely to live with a pet than with a sibling or their biological father(6), consider pets among their most important relationships.(7) Moreover, there is a ripple effect. Pets facilitate meeting other people, and these encounters can develop into lasting friendships. Pet ownership is associated with increased social contact and interaction, and positive perceptions of neighbourhood friendliness.(8) Pets build communities. Pet owners are more likely to know their neighbours than non-pet owners(9), and are more civically engaged.(10)

Second, pets motivate healthy behaviour change. Dogs, in particular, are enthusiastic exercise partners. Dog owners are more likely to exercise and to maintain weight loss than non-dog owners.(11) Children with dogs are more active.(12) Significantly, veterinarians can encourage this healthy behaviour(13) by focusing on the bond owners have with their pets.(14) Riding horses is excellent aerobic exercise(15), which can improve insulin sensitivity and elevating resting metabolic rate.(16) Pets encourage the activities of daily living that maintain health – an important concern for the elderly(17), and those suffering from anxiety.(18) The social aspect of pet ownership – their caring presence – can increase appetite among seniors.(19)

Third, pets can catalyze harm reduction. Learning the hazards of second-hand smoke to pets can motivate smokers to alter their behaviour – cutting down, smoking outside the home, attempting to quit or stopping altogether.(20) Homeless youth with pets will minimize drug use and avoid criminal behaviour out of concern for their companion animals.(21) A pilot project of the Korea Internet Addiction Centre, affiliated with the Ministry of Public Administration and Security, successfully addressed internet addiction by placing a pet in the patient’s household.(22)
Finally, pets are an existing resource for healthcare providers to complement medical and psychiatric therapies. Pets are a therapeutic component of addressing the risks of cardio-vascular disease.(23) They reduce stress – a pet’s company elevates oxytocin and lowers cortisol levels.(24) Even petting an unknown animal can have this effect.(25) Pets are an important source of social support for those with long-term mental illness.(26) The benefits of pets as adjuncts to medical therapy are particularly remarkable when managing chronic conditions.(27) Incorporating pets and activities with companion animals is easily done: they are part of the person’s life, and they incur no additional cost.

All relationships have positive and negative aspects. Zooeyia has only recently been defined as a strong and beneficial influence on the health of families with pets. Pets are a health resource which can be activated by families and their caregivers.

**Keywords:** Zooeyia, Pets, One Health

**References**

Interprofessional Collaboration to Mitigate the Four Categories of Zoonotic Risk in Families and the Community

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Summary

While the health benefits of pets (zooeyia) can be profound, companion animals also present risks to their families' health. There are four categories of zoonotic risk. First, pets can be a source of bacterial, viral or fungal infectious disease. Second, interacting with pets can lead to injury, such as bites, kicks or falls. Third, the impact of pets on the shared environment can present health risks to people. Lastly, pets can challenge family resources – financial, emotional and social. Interprofessional collaboration between human and veterinary medical practitioners is an effective One Health approach to mitigate these risks to pets' families and the community.

As in all relationships, having a pet in the family has its risks. By definition, pets live in close contact with their people. Zoonotic risk can be categorized in four ways.

- **Infectious disease**: Zoonoses account for 61% of infectious agents affecting people, and 75% of new and emerging infectious agents.(1) The risk of particular zoonoses is species-specific. Kittens are a common source of ringworm, for example, and many reptile species are asymptomatic carriers of *Salmonella*. (2) The young, old, pregnant, immunocompromised(3), and those living with mental challenges(4) are at higher risk of contracting disease, including those transmitted between pets and people.

- **Zoonotic injury**: Injuries from pets include bite wounds and scratches (with and without consequent infection), tripping and falling over a companion animal, and spills from a horse. Most pet-related injuries can be avoided through appropriately training the pet, and educating the pet owner.

- **Environmental impact**: Some environmental impact of pets, such as infested animals shedding the eggs of intestinal parasites, can be easily managed through proper preventive veterinary care. Many human healthcare providers have a poor understanding of the environmental risks of pets. Their number one concern is asthma – yet early exposure to pets can protect children from allergic disease by stimulating the immune system.(5) Toxoplasmosis is a common concern of those with pregnant patients who own cats – yet the most frequent route of infection is unwashed and improperly cooked food.(6) When healthcare providers better understand the environmental impact of pets, they recalibrate their assessment of the risks and are better able to care for their patients.

- **Challenge to family resources**: The cost of caring for pets can strain owners' finances, and lead to an unsustainable balance of priorities. Emotional resources may be threatened by the grief of pet loss.(7) Some people avoid medical care for fear that hospitalization will separate them from their pet.(8) Improperly trained animals can constrain an owner's social life.

Pet owners do not fully understand the risks their animal companions can pose.(9) Medical education places little emphasis on zoonotic risk(10), such that physicians' understanding is also inadequate.(11) Of the healthcare professions, veterinarians are the best educated about zoonoses. Physicians see managing zoonotic risk as the responsibility of public health services and veterinarians.(12) In the long-term, education...
of the human healthcare professions about zoonotic risk will empower them to better care for their pet-owning patients. But that does little to help today.

The solution lies in the One Health approach of inter-professional collaboration between the human and veterinary healthcare professions. Primary healthcare providers must be aware of pets in their patients' families – they can simply ask.(13) Veterinarians are unlikely to know of the immune status of clients and their families.(14) They must learn of all who live with the pet. Veterinarians generally welcome the opportunity to collaborate with physicians in the care of clients' families.(15) They can encourage a collaborative approach by offering clients a business card to share with their physician. Our recent research indicates that when physicians are aware of their patients' zoonotic risk, they will approach the family's veterinarian for assistance. The one caveat is to ensure that full and informed consent must be obtained from the patient/pet owner before information is shared.

**Keywords:** Zoonoses, Interprofessional, One Health

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Pets in the Family
- The Multiple and Diverse Roles of Pets in the Families

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Summary
Pets are important members of families. Considering pets in the Family Life Cycle provides a framework for veterinarians to understand their diverse roles in the family as it moves through developmental stages over time. These stages include the Independent Adult, the Marital Dyad, the Infant/Toddler Family, the School Age Family, the Adolescent/Launching Family, the Empty Nest/Retired Family, and the Elderly Family. Pets can complement or interfere with the family dynamics in each stage. Understanding and predicting pets’ roles throughout the family life cycle enables better support of clients and better care of their animal companions.

Pets are important members of many families(1), regardless of ethnicity or culture.(2) When veterinarians apply their own experience and insight, the Family Life Cycle(3) provides a framework to understand pets’ roles in the family. The family life cycle can incorporate pets.(4) Pets move through the cycle with their families and can complement the family structure. Alternatively, pets can replace human family members, either augmenting or interfering with the human dynamics.

The Independent Adult
The Independent Adult is negotiating adult relationships with parents, and is developing an independent social network. Independent adults often acquire their first pet, with which they bond quite closely. The pet’s species can indicate its role for the Independent Adult: dogs tend to act as buddies or younger siblings, cats as children, and horses and parrots as partners.(4) Pets can act as a social lubricant, catalyzing the formation of new relationships. Conversely, involvement with the pet can be so intense as to exclude other social activities.

The Marital Dyad
Some Independent Adults settle into stable partnerships. Negotiating care for the pet may be a predictor of how responsibilities for any future children will be shared.(5) Pets can be a source of conflict within the Dyad. When people with pets form a partnership, difficulties can arise from keeping the animals in one home.(6) A companion animal may replace a human in the dyad, creating a person-pet partnership. This can be a healthy development, with the person continuing to cultivate a social network and fully participating in the world outside the home. Less often, the relationship with the pet can limit that person’s social life and personal development.

The Infant/Toddler Family
If a Marital Dyad acquires a pet as a couple, it moves them into the next stage of the family life cycle. With the arrival of a new baby, parents learn to nurture, manage increased financial demands, share household responsibilities, and develop mature relationships with grandparents. Pets in a growing family can augment the human dynamics, but they are also a risk that requires appropriate management.
The School Age Family
Families balance work responsibilities, developmental and emotional needs of children, and expanding social networks and resources. Sometimes, pets take on the role of a child, particularly in families without children. Here, this stage is protracted, since owners have responsibility for all the animal’s needs throughout its life. Pets fulfill a need to nurture, but bring their own complications: the “baby” will develop geriatric illnesses and the family must deal with the grief of pet loss.

The Adolescent/Launching Family
Parents negotiate greater freedoms and responsibilities for teenagers, and maintain couple time while being available when needed. The family provides the emotional and financial resources to launch young adults into college, a job or marriage. Pets can be emotionally significant companions and social lubricants for teenagers. They can also be a flashpoint for family arguments, particularly as their care needs increase with age. Pets obtained when the children were young are now elderly and frequently ailing. Adolescents may be strongly affected by the death of a pet, which may trigger serious depression.

The Empty Nest /Retired Family
The couple is advancing at work, and then transitioning into retirement. Pets are often acquired in an effort to manage empty nest syndrome.(3) The couple may cultivate new hobbies or travel. Pets, unsocialized to children, are often sent to the adult children for care during extended travel vacations, posing a potential risk to any grandchildren.

The Elderly Family
Health problems, medical costs, the death of friends or a spouse, and challenges to an independent life are all important. Pets age at the same time as their owners’ health concerns increase. However, pets are often an important source of support to the elderly, particularly as a social lubricant. For those who are less able to socialize in the community, pets can assume the role of partner. They alleviate loneliness.(7) The death of a pet can be devastating. The owners’ grief can disrupt their activities of daily living, and further distance them from their social support system.(8)

Family Life Cycle is a theoretical framework, not intended to realistically describe the life of all families. Yet its stages and transitions are readily recognizable and adaptable to the wide variety of family structures which include pets. In veterinary practice, understanding the family life cycle can focus care and better address the needs of clients and their pets.

Keywords: Family Life Cycle, Pets, One Health

References
Opportunities and Activities for One Health in Veterinary Care in the Community

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Summary
Veterinarians highly value the human animal bond, yet few rigorously provide community programs which address the emotional component of pet ownership in practice. Zooeyia, the health benefits of pets, is the evidence base of the human-animal bond. Veterinarians can strengthen the bond clients have with their pets, and consequently the health of the entire family. Several One Health activities, adaptable to clinical veterinary practice, are presented, including the Family Mosaic with Pets, Fun Activities with Pets, Healthy Habits with Pets, Celebrating Connections with Pets, and Healthier and Safer with Pets. Each addresses a significant area of human health, and builds the bond between clients and their animal companions.

While most One Health endeavours are at a governmental level, the majority of healthcare practitioners (including veterinarians) work in community practice. Pets profoundly affect the health of their owners – and communities.(1) Zooeyia, the health benefits of pets, is the evidence base of the positive impact of the human-animal bond.(2) Veterinarians value the human-animal bond very highly, yet few systematically address the emotional component of pet ownership.(3) Veterinarians can strengthen the bond clients have with their pets, and their health, by incorporating One Health activities in practice.

The Family Mosaic with Pets
A lack of awareness of all members of the family impairs both human medical and veterinary care.(1,4) When clients illustrate their immediate family, including pets, and share it with both their veterinarian and their primary healthcare provider, understanding of the potential benefits and risks of pets to family members is immediately enhanced.

Fun Activities with Pets
Identifying activities for clients to enjoy with their pets will strengthen their bond and enhance zooeyia. Pets are sources of joy. Committing to scheduled active time in fun activities with the pet can distract owners from their worries and reduce anxiety. Even simply petting an animal elevates oxytocin and depresses cortisol levels(5) – and the effect is reciprocal.(6) Pet owners may be unaware of what their pets will enjoy. Veterinarians can offer suggestions, and encourage clients to commit to – and schedule – activities with their pets.

Calming Exercises with Pets
Animals live fully in the present moment, a goal of the multiple mindfulness activities which buffer the negative effects of stress, and increase well-being.(7) Incorporating pets into mindfulness exercises enriches rewarding time with pets, and the animals’ presence provides an immediate model. Veterinarians can suggest activities which soothe pets, and simultaneously benefit their owners.
Healthy Habits with Pets
Pets can encourage healthy habits – like regular exercise. Obesity of both animals and people is a common health concern. Exercise, even at a moderate level, helps with weight loss, and brings cardio-vascular benefits. The effect is stronger and more sustained when people exercise with their pet.(8) Advice on exercising from veterinarians encourages both pets and people to exercise.(9) Walking a dog, or riding a horse are obvious examples; exercise with other pets requires some creativity. Veterinarians can suggest activities involving all kinds of pets. With regularly scheduled exercise, the health benefits to both client and pet are multiple.

Celebrating Connections with Pets
Social capital – the extent and strength of one’s social connections – is a significant determinant of health.(10) The company of a pet itself reduces loneliness and its devastating effects.(11) And there is a ripple effect. Pets can be a catalyst for meeting new people, and developing stronger relationships with neighbours.(12) Some of these acquaintances can develop into lasting friendships. Pets build communities. Pet owners are more civically engaged than their non-pet-owning counterparts.(13) Veterinarians can offer activities for clients and their pets, and suggest other means of community involvement which include animal companions.

Healthier and Safer with Pets
Pet owners are seldom adequately aware of the health risks of their animal companions.(14) Veterinarians can start a conversation with clients about who shares the home with their pets. Knowing of elderly, young, pregnant, immunocompromised or mentally challenged members of the household will inform the pet’s healthcare. Likewise, human healthcare providers are better able to care for their patients if they know of pets in the family. Pet owners can build bridges between their veterinarian and healthcare provider to improve their own health care. Offer your clients business cards to give to their primary healthcare provider, and let them know you will be available if inter-professional collaboration will improve the care of the family. Showing an interest in clients’ families is one way to build the all-important relationship.

Keywords: Zooeyia, Human-Animal Bond, One Health

References
Introduction to the Essential Basic Concepts on AMR for Veterinary Professionals

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Summary
Antimicrobial resistance (AMR) has become a global health issue in the last few years. In 2016, it was estimated that approximately 700,000 people die of resistant infections every year. If actions are not taken at this point, it is further estimated that by the year 2050, about 10 million lives a year and a cumulative 100 trillion USD of economic output will be at risk due to the rise of drug-resistant infections. Veterinarians have a significant professional responsibility and a critical role in addressing this global issue as well-informed gatekeepers of antimicrobial usage in animals. While the concept of AMR is not new to veterinary professionals, it is important to start with some basic concepts to provide the necessary background on the principles behind AMR emergence, spread, and mitigation.

Bacteria primarily resist antimicrobials through any or the combinations of the following strategies: (1) preventing or reducing the ability of the antimicrobial to penetrate into the cell to reach its target; (2) expulsion of the antimicrobial agents from the cell via general or specific efflux pumps; (3) inactivation of antimicrobial agents via modification or degradation; or (4) modification of the antimicrobial target within the bacteria. These abilities of bacterial organisms to utilize the various strategies to resist antimicrobial compounds are all genetically encoded and may be intrinsic or acquired. Intrinsic resistance is naturally coded and expressed by all (or almost all) strains of that particular bacterial species. Acquired resistance is the phenomenon of current concern, where bacteria obtain the ability to resist antimicrobials to which it was previously susceptible to. Only present in certain strains of a species or genus, this acquired ability is attributed to changes in the bacterial genome through mutation or horizontal gene acquisition. This adaptive process propels the emergence of AMR and is often a consequence of selection pressure placed on susceptible microorganisms. The abuse, misuse, and overuse of these important medical substances in animal production is not unknown and are reinforced by recognized drivers of AMR emergence in livestock and aquaculture. AMR emergence and spread in animals can negatively impact human health with increased human morbidity and mortality, reduced efficacy of related antibiotics, increased healthcare costs, increased carriage and dissemination, and facilitated emergence of resistance in human pathogens. Imprudent use of veterinary antibiotics, along with inappropriate farm waste management can contaminate the soil and the aquatic environment, complicating and expanding the issue at the animal-human-environment interface.

Introduction

An antimicrobial agent is a naturally occurring, semi-synthetic or synthetic substance that exhibits antimicrobial activity, by killing or inhibiting the growth of micro-organisms, at concentrations attainable in vivo.

Their
introduction transformed human and animal health by revolutionizing how infections are addressed, subsequently improving survivability and chances against disease-causing pathogens. Shortly after its introduction, however, it was later realized that microorganisms could in fact adapt and modify to resist their effects, propagate with these new traits, and even share such resistance traits with others in their immediate environment. This ability of a microorganism to survive and multiply in the presence of an antimicrobial agent that normally inhibits or kills this particular kind of organism is what is now known as antimicrobial resistance.

Antimicrobial resistance is one of the many adaptive traits that resilient bacterial subpopulations may possess or acquire, enabling them to out-compete and out-survive their microbial neighbors and overcome host strategies aimed against them. This phenomenon is nearly as old as the discovery of antimicrobials themselves, having been described by pioneers like Ehrlich for trypanosomes and Fleming for staphylococci². What is most alarming today is the rate at which antimicrobial resistance often develops and how quickly it spreads across the globe and among different species of bacteria. Furthermore, as a result of sequential, cumulative acquisition of resistance traits against different antibiotics, more bacterial pathogens with multiple-drug resistance are now being reported worldwide.

**Bacterial Resistance Strategies**

To survive in the presence of an antibiotic, bacterial organisms must be able to disrupt one or more of the essential steps required for the effective action of the antimicrobial agent. The intended modes of action of antibiotics may be counter-acted by bacterial organisms via several different means:

1. **Preventing or reducing the ability of the antimicrobial to penetrate into the cell to reach its target:** To interfere with the normal function of the bacterial organism, antimicrobial compounds require access into the bacterial cell to reach their target site. Some bacteria are able to protect themselves by prohibiting these compounds from entering past their cell walls. For example, a variety of Gram-negative bacteria reduce the uptake of certain antibiotics, such as aminoglycosides and beta lactams, by modifying the cell membrane porin channel frequency, size, and selectivity.

2. **Expulsion of the antimicrobial agents from the cell via general or specific efflux pumps:** To be effective, antimicrobial agents must also be present at a sufficiently high concentration within the bacterial cell. Some bacteria resist this by using membrane proteins that act as an export or efflux pump, extruding the antibiotic out of the cell as fast as it can enter. This results in low intracellular concentrations that are insufficient to elicit an effect. Some efflux pumps selectively extrude specific antibiotics such as macrolides, lincosamides, streptogramins and tetracyclines, whereas others (referred to as multiple drug resistance pumps) expel a variety of structurally diverse anti-infectives with different modes of action.

3. **Inactivation of antimicrobial agents via modification or degradation of its active components:** To roll out its desired mechanism of action and cause an effect, an antimicrobial must make full use of its active component. Some bacteria however, are able to destroy this to resist its effect. A classic example is the hydrolytic deactivation of the beta-lactam ring in penicillins and cephalosporins by the bacterial enzyme called beta lactamase. The inactivated penicilloic acid will then be ineffective in binding to PBPs (penicillin binding proteins), thereby protecting the process of cell wall synthesis.

4. **Modification of the antimicrobial target within the bacteria:** To be able to carry out its effect, the antimicrobial agent must be able to reach its target within the bacterial cell. Some bacteria however are able to evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, in spite of the presence of an intact and active antimicrobial compound, no subsequent binding or inhibition will take place. This strategy has been observed in: Staphylococci against methicillin and other beta-lactams
through changes or acquisition of different PBPs that do not sufficiently bind beta-lactams to inhibit cell wall synthesis.

No single mechanism of resistance is considered responsible for the observed resistance in a bacterial organism; in fact, several different mechanisms may work together to confer resistance to a single antimicrobial agent or cause multiple resistance to various antimicrobial agents.

**Molecular mechanisms of resistance**

The abilities of bacterial organisms to utilize the various strategies to resist antimicrobial compounds are all genetically encoded. This can either be intrinsic or acquired. *Intrinsic resistance* is that type of resistance which is naturally coded and expressed by all, or almost all strains of that particular bacterial species. An example is the natural resistance of anaerobes to aminoglycosides and Gram-negative bacteria against vancomycin, and the lack of effect of penicillin to *Mycoplasma* which do not possess a cell wall. *Acquired resistance* lead to a change in the nature of proteins normatively expressed by the organism. Such change may lead to an alteration in the structural and functional features of the bacteria, which may result in changes leading to resistance against a particular antibiotic and is limited to selected isolates of that particular species or group of microorganisms. These changes in the bacterial genome may be through mutation or through horizontal gene acquisition via (1) transformation, (2) transduction, or (3) conjugation. For example, methicillin resistance of *Staphylococcus aureus* is primarily due to changes that occur in the penicillin binding protein (PBP) as rendered by the expression of a certain *mecA* gene in some strains of *S. aureus*, which is hypothesized to have been induced by the excessive use of penicillin. Expression of this *mecA* gene results in an alternative PBP (PBP2a) that has a low affinity for most beta-lactam antibiotics, thereby allowing these strains to replicate in the presence of methicillin and related antibiotics. Some antimicrobial resistance is brought about by multiple changes in the bacterial genome. Thus, while AMR is not a new phenomenon and can be naturally occurring in many species, the concern has been raised owing to the speed and expance by which resistance is acquired. More and more key organisms that used to be easy to treat in the past are becoming more challenging to manage.

**Antimicrobial use in animals and AMR drivers in animal production**

The introduction and use of antimicrobials in animals has brought major benefits to both animals and humans. Some of these benefits are: (1) reduction of animal pain and suffering; (2) protection of livelihood and animal resources; (3) assurance of continuous production of foods of animal origin; (4) prevention or minimizing shedding of zoonotic bacteria into the environment and the food chain; and (5) containment of potentially large-scale epidemics that could result in severe loss of animal and human lives. Clearly, the advantages generated by the use of antimicrobials for animals transcends more than just the well-being of the animals, as it has also brought about economic benefits for the food animal producers and a more secured and safer health for the general public.

However, animal production systems have evolved over the years to keep up with the continuously growing human population and shifting market demands. While needs are expanding exponentially along with human population growth, the available agricultural lands are also dramatically shrinking owing to urbanization and industrialization. In response to the increasing demand for food, the decreasing available space, as well as the increasing cost of production, most livestock and poultry are now raised in smaller spaces at the least possible cost under the shortest period of time. While good farming practices such as vaccination, biosecurity, and other measures can reduce the risk of introduction and spread of infectious diseases in farms, these do require substantial resources that many raisers do not have or are not willing to invest in. Instead, reliance on antibiotics have become the norm for many of these animal raisers for both therapeutic and non-therapeutic use in healthy animals to prevent infections (prophylaxis), to mass-treat animal population under unfavorable conditions (metaphylaxis), or to enhance growth (growth promotion). Such practices have
been shown to contribute to the emergence and spread of resistant microorganisms, which in part have ushered our global community into an era where treatment for simple infections are either becoming more difficult or completely failing.

**Prudent use of antimicrobials in animal production**

It should be noted that because of the ability of AMR genes to move between bacteria, hosts and environments, and the occurrence of spontaneous mutations, removal of the selection pressure resulting from AMU in humans and animals alone will not completely halt the emergence and global spread of AMR. Nevertheless, prudent use of antimicrobials in both sectors remain to be an important measure and preventative action as the release of large quantities of antimicrobials or resistant bacteria into the environment is a critical point for control.

While many believe that the current scientific evidence sufficiently supports mitigation to reduce the risks posed in humans and animals, others argue that such will be economically harmful to both consumers and producers and unnecessary given the ill-defined risks of inducing greater rates of antimicrobial resistance. One thing upon which all can agree is that the unnecessary or wasteful use of antibiotics should be curtailed when non-antibiotic solutions are readily available or when the use of antibiotics for a particular disease condition are clearly not efficacious. It is upon this common ground that the human medical and veterinary medical communities call for the proper and prudent use of antibiotics, and call for training of these health professionals regarding the judicious, proper and non-wasteful use of all antibiotics.

**Keywords:** Resistance Strategies, AMR Drivers, Prudent Use

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Veterinary Milestones in the Mitigation of Antimicrobial Resistance

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Summary
While it has progressively become clear that inappropriate use of antimicrobials in humans and animals lead to the increased risk for emergence of antibiotic resistance, changes have not been instantaneous. This was considered particularly more challenging in the animal health sector largely due to the long-standing norms of non-judicious use that were reinforced by promising economic gain for raisers and fueled by profit-driven market by producers and retailers. Additionally, unlike other typical infectious zoonotic diseases of concern where linkages between human and animal cases may be established, AMR is not exactly a transmissible infection with directly measurable consequences. It is a phenomenon that progressively evolves over time and while it is easy to appreciate that shared measures would be beneficial to all concerned, it is not easily demonstrable by straightforward evidence. To add to this, many countries which are thought to be at higher risk have many other competing priorities such as challenging political climate and governance, existing animal and human health issues, limited financial and human resources, and other matters of concern at the human-animal-environment interface. With opposing drivers on either end, advancing the progress on AMR mitigation in the animal health sector has been challenging to say the least.

Nevertheless, although it is undeniable that much remains to be done regarding AMR mitigation in the animal health sector, what can be appreciated is that considerable progress has at least been achieved thus far. The global issue on AMR has reiterated the critically important roles of Veterinarians in public health, and the need for approaching health issues at the human-animal-environment interface with a One Health lens. The Global Action Plan on Antimicrobial Resistance (GAP-AMR), developed and endorsed by the World Health Assembly in May 2015 underscores this, as well as the guidelines for the development of National Action Plan (NAP) on AMR. As such, various NAPs, national committees, technical working groups include the representation of the Veterinary Services and other animal health professionals. The GAP-AMR was also subsequently globally endorsed at the 83rd World Assembly of the OIE Delegates in May 2015 and the 39th Food and Agriculture Organization (FAO) Conference in June 2015. In the year that followed, these two international organizations dealing with animal health globally also released their strategies and action plan on combatting AMR following their respective mandates where animals and agriculture are concerned.

Although limited, it is encouraging to recognize some of the progress made from the Veterinary end of the AMR mitigation spectrum including those on: (1) AMR information, education and training (2) surveillance, monitoring and record-keeping; (3) reduction of infection; (4) optimization of antimicrobial use; and (5) sustainable investment to address AMR. Further contributions are clearly needed from the animal health sector to sustain this progress in support of the Global Action Plan on AMR.
Historical Background on Antimicrobial Use in Animals

*Therapeutic and non-therapeutic usage in animals.* The use of antimicrobials in animals closely parallels their discovery and usage in humans. Sulfonamide was the first antimicrobial to be introduced to food animal medicine in the 1940s. The subsequent discoveries and availabilities of newer antibiotics in the early 50's quickly led to their widespread therapeutic usage for a multitude of infectious diseases in virtually all food animal species. Antibiotics are also given to food animals for growth promotion and prophylactic medication. An unintentional discovery however, led to a whole new use for antimicrobials in animals. In 1948, animal nutritionist Robert Stokstad and biochemist Thomas Jukes of the Lederle company, were then extensively working on a variety of vitamin B12 which was believed to be the "animal protein factor" that can enhance the growth of chickens. Because the Lederle laboratories (the laboratory where the very first tetracycline, chlortetracycline, was discovered) uses vats of *Streptomyces aureofaciens* for the production of the antibiotic aureomycin, they utilized its cellular remains after the antibiotic had been extracted because they found that this contain substantial amounts of vitamin B12. They found that chicks receiving supplements of *S. aureofaciens* fermentation grow 24% more rapidly than those receiving liver extract, another source of this vitamin. They later realized that this observed growth enhancement was not because of the vitamin, but due to the minimal residues of antibiotics left in the bacterial carcasses.

*The birth of an era: antibiotic growth promotion (AGP) in animals.* This discovery opened a whole new market for antibiotics to the multibillion-dollar industry that it is today, transforming the utility of antibiotics for animal usage from being powerful medical substances to a nutritional commodity. Because the amount of antibiotic that provide growth enhancement was extremely small, the effect was regarded as largely nutritional by producers and authorities in the food industry.¹ In the years that followed, other countries also allowed the use of antibiotics in animal feeds.

Recognizing the health impact of non-therapeutic use of antibiotics in animals

*The Swann Report.* Prompted by an epidemic of *S. typhimurium* DT 29 in cattle and man in 1963-65, a Joint Committee on the use of Antibiotics in Animal Husbandry and Veterinary Medicine, chaired by Professor MM Swann, was appointed by the Health and Agriculture Ministers in the United Kingdom in July 1968. The Swann Report issued in November 1969, concluded that "the administration of antibiotics to farm livestock, particularly at sub-therapeutic levels, poses certain hazards to human and animal health"; in particular, it had led to resistance in enteric bacteria of animal origin. It further recommended that only antibiotics which "have little or no application as therapeutic agents in man or animals and will not impair the efficacy of a prescribed therapeutic drug or drugs through the development of resistant strains of organisms" should be usable for growth promotion.²

*Subsequent actions on antimicrobial use in animals.* While the Swann Report opened the discussion on the hazards of sub-therapeutic use of antimicrobials in animals, this was followed by many other similar positions from the human health sector, the scientific community, and various other relevant bodies. In 1972-74, Europe banned the use of tetracycline, penicillin and streptomycin for growth promotion; progressive ban of AGP followed, and by 2006 a complete ban of AGP in livestock was enforced in the European Union. While it was hoped by many that the years of experience following the bans on nontherapeutic use of antimicrobials in Europe would clearly signal an end to this practice, arguments continue, largely along the lines of a cost/benefit ratio and perceived deficits in solid scientific evidence.³ Globally, the World Organisation for Animal Health (OIE) reports that 74% of the 130 countries who responded to its global survey in 2015 still authorize the use of AGP in animals.⁴

Some notable progress towards AMR Mitigation in the animal health sector

While it is undeniable that much remains to be done towards AMR mitigation in the animal health sector, it also cannot be argued that considerable progress had somehow been made, particularly as ushered by the worldwide, cross-sectoral endorsement of the Global Action Plan on Antimicrobial Resistance (GAP-AMR) in 2015. Some of these are outlined below in accordance with the five GAP-AMR objectives:
Information, education and training. In 2015 the “World Antibiotic Awareness Week” was launched by WHO to raise AMR awareness on a global level and encourage actions to address this. To target the animal health sector and the Veterinary Services globally, the FAO and OIE reinforced the launch with their respective awareness initiatives for private and public veterinarians as well as other relevant stakeholders in the animal health sector. Held every third week of November each year, the global campaign in 2017 will be from 13 to 19 November. Additionally, as part of its action plan launched in 2016, the FAO is also focusing on improving awareness among food and agriculture stakeholders and pursuing to integrate AMR into policy-level discussions on food and agriculture. Likewise, the OIE is also promoting awareness through Veterinary Statutory Bodies and Veterinary Educational Establishments to encourage a professional culture that supports the responsible and ethical use of antimicrobial products in animals.

Surveillance, monitoring, record-keeping. Recognizing the need for evidence-based actions to mitigate AMR several countries have established AMR surveillance in livestock, which are often integrated with the human health sector. Denmark in 1995 was the first country to establish a systematic and continuous monitoring program of antimicrobial drug consumption and antimicrobial agent resistance in animals, food, and humans through the Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP). Recognizing its value, many others followed: the National AMR Resistance Monitoring and Surveillance (NARMS) in the US (1996), the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) (1999), the Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM) (2000), the Canadian Integrated Program for Antimicrobial Surveillance (CIPARS) (2002), FINRES-VET in Finland (2002), among others. In 2016, the FAO developed and started piloting the Assessment Tool for Laboratory AMR Surveillance System (ATLASS) to systematically assess veterinary laboratory capacities to carry out national AMR surveillance. This assists countries to identify and map existing gaps to strategically address these, reinforcing the vision of having a national AMR surveillance systems in place. Various initiatives on laboratory capacity building and networking relevant to AMR surveillance in the livestock populations are also being supported by FAO and other organizations and groups.

Reduction of infection. Development and uptake of new tools, products and methodologies to reduce the dependence of animal sectors on antimicrobials are long-standing work for many in the veterinary field. As a result, there are now a variety of alternatives in the market along this line: probiotics, competitive exclusion products, enzymes, immunomodulants and other naturally occurring substances like herbs, spices, botanicals and essential oils. Continued work on vaccine development and biosecurity improvements also addresses reduction of infection, as well as the adherence to international standards and guidelines (OIE, Codex Standards) on relevant practices recently introduced in the interest of AMR mitigation in animals. One of the focus areas for the FAO Action Plan centers on good practices in the food and agriculture systems through developing and supporting practical measures to be taken in the food and agriculture sectors to minimize the need for antimicrobials. This includes improved biosecurity to reduce infections and good hygiene practices to minimize or prevent the spread of diseases as well as AMR.

Optimization of antimicrobial use. In 2015, the OIE launched an initiative to build a global database on the use of antimicrobials in animals. Supported by FAO and WHO as part of the Global Action Plan, this initiative has established initial baseline information using a harmonized approach and will continue to measure trends over time. It will be linked to the OIE World Animal Health Information System (WAHIS), a web-based reporting system that collects, processes and avails online information about animal populations and diseases in real time, providing notifications to Member Countries of sanitary events in animals. The OIE also supports Member Countries to develop and modernize legislation governing the manufacture, marketing authorisation, importation, distribution and use of veterinary products, while FAO works on developing studies on regulatory approaches to AMU and providing assistance to countries in the development of policies relevant to AMR mitigation in food and agriculture.


**Sustainable investment to address AMR.** The launch of the GAP-AMR in 2015 transformed the way AMR is addressed globally across all sectors. The drive for all countries to develop a National Action Plan on AMR prompted inter-sectoral collaboration and underscored the need for One Health in addressing complex health issues. In September 2016, global leaders met at the United Nations General Assembly in New York to commit to fighting antimicrobial resistance together. For the first time, Heads of State committed to taking a broad, coordinated approach to address the root causes of AMR across multiple sectors, especially human health, animal health and agriculture. In the true spirit of One Health, the Tripartite FAO-OIE-WHO came together to speak with a shared message regarding combating AMR. The leaders at the UN meeting likewise called on WHO, FAO and OIE, in collaboration with development banks such the World Bank other relevant stakeholders, to coordinate their planning and actions and to report back to the UN General Assembly in September 2018. 

**Ways Forward**

It is clear that a lot remains to be done where AMR mitigation is concerned, nevertheless, progress have certainly been made and continues to be made. These initial successes have created a momentum to move the animal health forward as the veterinary force joins the rest of the One Health professionals and other stakeholders in addressing AMR and pursuing more milestones to be achieved for AMR mitigation.

**Keywords:** Communication, Surveillance, Infection Reduction, Antimicrobial Usage, Sustainable Investment

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The Global Action Plan on AMR and the Veterinary Profession

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Summary
Antimicrobial agents are crucial substances for managing and sustaining health in humans and animals. Over the years, however, their rampant misuse and overuse in human medicine and animal production have ushered the world into an era where antimicrobial resistance (AMR) has become increasingly more common and more serious. Today, AMR emergence and spread is now a recognized global challenge posing grave threat to modern medicine and to the sustainability of effective response against infectious diseases.

As a recognized complex issue, AMR is best addressed with a well-covered and well-linked force from all relevant disciplines – a One Health approach. It is thus imperative to bring together, and have an understanding of and respect for, each and every relevant expertise. This united and cohesive approach to address AMR will ensure that all areas of concern, regardless of how diverse they may be, are well-addressed by the inter-disciplinary nature of a One Health initiative in the countries. Acknowledging this need for an effective One Health approach involving coordination among numerous sectors and actors, the World Health Assembly adopted in May 2015 the Global Action Plan on AMR (GAP-AMR). The goal of this global action plan is to ensure, for as long as possible, continuity of successful treatment and prevention of infectious diseases with effective and safe medicines that are quality-assured, used in a responsible way, and accessible to all who need them.

Veterinarians are integral and essential part of this One Health approach. They have the potential to contribute to delivering synergistic solutions, together with their partner disciplines, in addressing AMR issues at the national and global levels. Following the objectives as set out in the Global Action Plan on antimicrobial resistance (GAP-AMR), Veterinary Professionals and future Veterinarians can invaluably contribute to the global issue on AMR through: (1) improving understanding of AMR and related practices through education, and sharing such knowledge with other key players in the animal health sector; (2) conducting and/or providing technical support to AMR and antimicrobial usage (AMU) surveillance; (3) observing, promoting, and supporting good husbandry practices and other animal infection prevention measures; (4) optimizing the use of antimicrobial medicines in animals; and (5) collectively pursuing sustainable mechanisms that will support and reinforce efforts to control, prevent, and manage AMR.

Antimicrobial Resistance as a One Health issue
Although the “One Health” concept was introduced at the turn of the 21st century, the core idea - that human and animal health are interdependent and bound to the health of the ecosystems in which they exist - has long been recognized. AMR embodies a typical One Health topic, being intricately linked across the human-animal-ecosystems interface. It is clearly a complex issue which goes beyond antimicrobials
and their resistance mechanisms and involve behavioral change, economics, politics and much more. Against this is a backdrop of our changing environment, evolving market chains, progressing animal and human medical challenges - just to name a few.

These complexities call for a unified and cohesive response from various stakeholders involved, bringing to the table respective areas of expertise from all disciplines and working together with a shared vision towards a common good for all.

Resolution 68.7 of the World Health Assembly in 2015 in Geneva recognized that the main impact of antimicrobial resistance is on human health, but that both the contributing factors and the consequences, including economic and others, go beyond health. It has become acknowledged that there is a need for a coherent, comprehensive and integrated approach at global, regional and national levels, in a “One Health” approach and beyond, involving different actors and sectors such as human and veterinary medicine, agriculture, finance, environment and consumers. Addressing AMR thus require whole-of-society engagement where all sectors and disciplines are engaged in the development and implementation of the action plan, and in particular in efforts to preserve the effectiveness of antimicrobial medicines through conservation and stewardship programmes.

The Global Action Plan on AMR
The Global Action Plan on antimicrobial resistance (GAP-AMR) was endorsed by the World Health Assembly in May 2015. This was later endorsed shortly after by the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE), further affirming the underscored need for an effective One Health approach involving coordination across sectors and actors.

It was expected that countries will develop their own national action plans on antimicrobial resistance in line with the global plan. Thus, all Member States around the world were urged to have at the national level, operational action plans to combat AMR that are aligned with the GAP-AMR and with standards and guidelines established by intergovernmental bodies such as the Codex Alimentarius Commission, FAO and OIE. Taking into account national and regional priorities, these national action plans should reflect whole-of-society engagement including one-health approach. As such, the national action plans as with the GAP-AMR, are expected to highlight the role of critical stakeholders from all sectors and disciplines - including the Veterinary Professionals.

The Five Strategic Objectives of the GAP-AMR and the Veterinarians
The GAP-AMR outlines five strategic objectives: (1) to improve awareness and understanding of antimicrobial resistance through effective communication, education and training; (2) to strengthen the knowledge and evidence base through surveillance and research; (3) to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; (4) to optimize the use of antimicrobial medicines in human and animal health; and (5) to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.

The invaluable contributions of public and private veterinarians in addressing this global issue on AMR can fall under any or all of the following objectives of the Global Action Plan on AMR:

Objective 1 - Improve awareness and understanding of antimicrobial resistance through effective communication, education and training: It is important to raise AMR awareness and promote behavioral change across all sectors to be able to effectively address AMR as a society. As AMR is relevant to food and agriculture, a sound understanding on the issues surrounding AMR from the animal health sector
perspective needs to be underscored to, and by, Veterinary Professionals. The topic of antimicrobial resistance should thus be a core component of veterinary education, training, certification, and continuing education for veterinarians. This should include basic and advance concepts of AMR from various thematic views under veterinary medicine - veterinary microbiology, veterinary pharmacology, veterinary public health and field practice. These perspectives can transform veterinarians and future veterinarians into well-informed practitioners and well-equipped messengers who are able to practice prudent antimicrobial use and serve as carriers of the global AMR message to other stakeholders within the animal health sector, particularly those working along the food chain.

**Objective 2 - Strengthen the knowledge and evidence base through surveillance and research:** Actions and interventions including legal tools intended for addressing AMR should be well-supported by evidence-based information that are anchored on good science. Veterinarians from the national governments, industry, intergovernmental organizations, agencies, professional organizations, nongovernmental organizations, and academia have important roles in generating such knowledge and translating these, or their derivatives, into practice. Several gaps of information on AMR in the animal health sector are still lacking in many countries and are clearly needed. This includes, but certainly not limited to: the epidemiology of AMR in livestock and aquaculture, mechanisms of AMR emergence and spread in the animal populations, social science and behavioral change of stakeholders in the animal health sector, AMR governance in Veterinary Services, and economics of AMR mitigation. AMR surveillance, particularly in high-risk countries, continues to be a major gap in which both public and private veterinarians can contribute to.

**Objective 3 - Reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures:** As with human healthcare facilities, settings where animal dwellings concentrate and where antibiotics are intensely used, can be important hotspots for the most serious and difficult-to-treat antibiotic-resistant infections. Thus, veterinarians overseeing farms, animal clinics, or hospitals, have critical roles in ensuring that adequate measures are in place to prevent and control infections in these high-risk areas. Better hygiene and infection prevention measures in these animal settings will contribute to the mitigation of the emergence and spread of antimicrobial-resistant microorganisms. Application of and adherence to international standards and guidelines relevant to AMR mitigation can help reduce infection rates and the need for antibiotics. This may include the use and implementation of: good husbandry practices, biosecurity, prudent antimicrobial usage, appropriate vaccination, and other measures on infection prevention and control in animal production and health settings.

**Objective 4 - Optimize the use of antimicrobial medicines in human and animal health:** While the emergence of antimicrobial resistance has become more and more common in the recent years, the development of new antimicrobials has dramatically slowed down in the recent years. Human and animal health care providers are thus left to face a shared dilemma of stretching this finite resources for fighting infections that are increasingly becoming more complicated. To optimize antimicrobial usage in animals, a well-trained veterinarian should be at the core of its use, exercising their professional role and responsibilities. However, most often in many countries, this is not the case. The proper utility of antimicrobials is thus hampered by the lack of access to well-trained veterinarians and poor understanding of its end-users in the field. Inappropriate antimicrobial usage in animal production settings is also a reflection of the lack of regulatory mechanisms or loose enforcement where these exist, lack of access to tools supporting good husbandry practices such as timely and accurate diagnosis, and lack of recognition that antimicrobial agents are in fact, a public good that needs to be protected in the interest of the society. While veterinarians should acknowledge and seriously pursue their responsibility to optimize the use of antimicrobials in animals, other AMR stakeholders including policy-makers, should also recognize and support this role of the Veterinary professionals to propel its success.
Objective 5 - Develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions:

While the roles of individual Veterinarians are clearly underscored in the previous four objectives, the last objective that will fuel and reinforce the other areas of work of Veterinarians on AMR could be best addressed by Veterinary associations, professional groups, and other relevant veterinary clusters. These groups should speak with one voice and cohesively work towards reinforcing the recognized accountability of the profession in support of GAP-AMR. There is a need to also ensure that while the public health is placed at the fore, the commitments of Veterinarians to ensuring food security, animal welfare and protecting animal health are also maintained.

The GAP-AMR, the Veterinarians, and the One Health Approach

As a global issue that cuts across various disciplines and sectors, AMR calls for a shared, well-orchestrated response across the world. It requires a whole-of-society engagement with all sectors and disciplines working together towards the implementation of the action plan. Thus, it should be highlighted that while some of the potential roles of Veterinarians in the GAP-AMR are mentioned above, the Global Action Plan on AMR nevertheless underscores the need for an effective “One Health” approach involving coordination among numerous international sectors and actors, including human and veterinary medicine, agriculture, finance, environment, and well-informed consumers.2

Keywords: Veterinarian, One Health, GAP-AMR

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Global Initiatives on AMR Mitigation: The Animal Health Sector

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Summary
Antimicrobial resistance (AMR) is now globally recognized as an emerging public health threat. Similar to other cross-sectoral issues, antimicrobial resistance is best viewed with a One Health lens. As a shared, borderless challenge within and across the human-animal-environment continuum, addressing this rising threat requires a holistic and multi-sectoral One Health approach. Leading the way to One Health globally, the Tripartite collaboration of the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (OIE) speak with one voice and take collective action to minimize the emergence and spread of AMR aiming to ensure that antimicrobial agents continue to be effective and useful to cure diseases in humans and animals; promote prudent and responsible use of antimicrobial agents; and ensure global access to medicines of good quality.

The Global Action Plan on Antimicrobial Resistance (GAP-AMR) has spurred and catalyzed progress in addressing AMR globally, calling on the human and animal sectors of all Member Countries to focus on the following 5 objectives: (1) improvement of awareness and understanding of AMR through effective communication, education and training; (2) strengthening of knowledge and evidence base through surveillance and research; (3) reducing the incidence of infection through effective sanitation, hygiene and infection prevention measures; (4) optimizing the use of antimicrobial medicines in human and animal health; and (5) development of the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines, and other interventions. While the GAP-AMR of WHO have provided an overarching framework, global strategies accordingly aligned with the GAP-AMR have also been developed to specifically address AMR in the animal health sector by FAO and OIE.

In addition to these, a number of global initiatives to address AMR have been put in place by various key actors across the globe. Many of these recognize and acknowledge the interconnectivity of the health of humans, animals and ecosystems and have thus fostered the ideals of a “One Health” approach. While many initiatives are focused on the human health sector, there are some which involve or specifically focus on the animal sector. These initiatives reinforce and enable the animal health sector to contribute further towards a well-orchestrated, collaborative, multi-disciplinary and cross-sectoral approach to address AMR issues that have become increasingly more complex against the backdrop of a continuously evolving world. Some of these are described here as categorized by the five objectives of the GAP-AMR.

Global Plans and Strategies to Address AMR in the Animal Sector
The GAP-AMR was launched at the 68th World Health Assembly in May 2015 and further endorsed at the 83rd World Assembly of the OIE Delegates in May 2015 and the 39th Food and Agriculture Organization (FAO) Conference in June 2015. While the GAP-AMR have provided an overarching framework, global
strategies accordingly aligned with the GAP-AMR have also been developed to specifically address AMR in animal health.

**FAO Action Plan on Antimicrobial Resistance 2016-2020.** As the global leader for food and agriculture, the FAO Action Plan identifies four focus areas of work: improve awareness on Antimicrobial Resistance and related threats (Focus Area 1); develop capacity for surveillance and monitoring of Antimicrobial Resistance and antimicrobial use in food and agriculture (Focus Area 2); strengthen governance related to antimicrobial use and antimicrobial resistance in food and agriculture (Focus Area 3); and promote good practices in food and agriculture systems and the prudent use of antimicrobials (Focus Area 4).

**The OIE Strategy on AMR and prudent use of antimicrobials.** Anchored on their existing mandate as a global leader for animal health and welfare standards, the OIE Strategy outlines the following objectives: (1) improve awareness and understanding; (2) strengthen knowledge through surveillance and research; (3) support good governance and capacity building; and (4) encourage implementation of international standards.

**The Global Health Security Agenda (GHSA) AMR Action Package.** The GHSA has a total of 11 Action Packages under the prevent, detect and respond themes. Antimicrobial resistance is GHSA Action Package Prevent-1 with a five-year target to support work being coordinated by WHO, FAO, and OIE to develop an integrated and global package of activities to combat antimicrobial resistance, spanning human, animal, agricultural, food and environmental aspects (i.e. a one-health approach). The desired national impact include: (1) decisive and comprehensive action to enhance infection prevention and control activities to prevent the emergence and spread of AMR, especially among drug-resistant bacteria; (2) nations will strengthen surveillance and laboratory capacity, (3) ensure uninterrupted access to essential antibiotics of assured quality, (4) regulate and promote the rational use of antibiotics in human medicine and in animal husbandry and other fields as appropriate, and (5) support existing initiatives to foster innovations in science and technology for the development of new antimicrobial agents.

**Select Global initiatives on AMR involving Animal Health**

It has been estimated that by 2050, 10 million lives a year and a cumulative 100 trillion USD of economic output are at risk due to the rise of drug-resistant infections if no actions are taken today. The World Bank considers AMR as a threat to our economic future and reported that AMR containment is a hard-to-resist investment opportunity. Recognizing its global significance, a wide range of initiatives and activities addressing AMR issue have been launched by international organizations, governments, and private entities around the world. Although certainly not exhaustive, some global initiatives related to or linked with AMR mitigation in the animal population is presented here, following the five objectives of the GAP-AMR:

1. **Global initiatives on communication, education and training (GAP-AMR Objective 1):** The World Antibiotic Awareness Week (WAAW) is an annual, global event to raise awareness about the serious health issue of antibiotic resistance. Held every third week of November, the WAAW will be held from 13-19 November this 2017. The annual campaign aims to increase awareness of global antibiotic resistance and to encourage best practices among the general public, health workers and policy makers to avoid the further emergence and spread of antibiotic resistance. Globally each year, the FAO-OIE-WHO Tripartite jointly observes the AAW. Relevant multi-media materials for AMR stakeholders for use by stakeholders in the animal health sector are both available from FAO (http://www.fao.org/antimicrobial-resistance/en/) and the OIE (http://www.oie.int/en/for-the-media/aml/waaaw2015/). It is also of note that various international groups and networks have been working to systematically advocate for policy solutions to address AMR and increase its visibility at the global health level. Many of these initiatives, although often broader in scope, include focus on the animal health sector: (1) The **Alliance for the Prudent Use of Antibiotics** (APUA) conducts research, education and advocacy programs to control AMR and ensure access to effective antibiotics; (2) **Antibiotic
Resistance Coalition (ARC) is a coalition across six continents working on health, agriculture, consumer and development sectors; (3) Center for Disease Dynamics, Economics and Policy (CDDEP) is a research and advocacy organization working on a range of public health threats, including AMR; (4) Consumers International (CI) is a federation of consumer groups advocating for consumer rights, as part of their work on food, they have launched the “Campaign to get antibiotics off the menu” to raise awareness about the routine use of antibiotics in livestock and agriculture as growth promoters and for metaphylaxis, and outlines the risks of widespread use in farming; (4) ReAct – Action on Antibiotic Resistance is a global network with nodes located on five continents who raise awareness on AMR to a range of constituencies, develop networks with interested parties, and develop national policy platforms with social mobilization in select countries; (5) South Centre is an intergovernmental organization of developing countries set up in 1995, and assists it 52 member countries through research policy analysis and technical advice to formulate and promote shared interests; (6) World Alliance Against Antibiotic Resistance (WAAAR) is a group of clinical, veterinary, and environmental professionals raising AMR awareness through a One Health approach.

2. Global initiatives on AMR research and surveillance (GAP AMR Objective 2): Recognizing the need to have a systematic assessment of laboratory capacities and of the organization of AMR surveillance systems to reinforce National AMR surveillance in the animal health sector, FAO has developed the Assessment Tool for Laboratory Surveillance Systems (ATLASS). The laboratory component is in part an extension of the Laboratory Mapping Tool (LMT) which are now regularly applied in animal health laboratories with good success. Pilot ATLASS missions in select countries has commenced in 2017. On the other hand, the OIE, supported by FAO and WHO within the tripartite collaboration, has taken the lead to build a global database on the use of antimicrobial agents in animals. The first annual data collection commenced in 2015 with the first “OIE Annual report on the use of antimicrobial agents in animals” reported in 2016. This report provided for the first time an overview of the global use of antimicrobial agents in animals. The WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGisAR) also established to support WHO’s effort to minimize the public health impact of AMR associated with the use of antimicrobials in food animals. In particular, the Advisory Group assists WHO on matters related to the integrated and coordinated surveillance of antimicrobial resistance across humans, animals and the environment and the containment of food-related antimicrobial resistance.

3. Global initiatives on reduction of incidence of infection through effective hygiene and infection prevention measures (GAP AMR Objective 3): The FAO-WHO Codex Alimentarius Commission develops International food standards, guidelines, and codes of practice for the safety, quality and fairness of the international food trade. Also, as the reference organization of the World Trade Organisation relating to standards to animal health and zoonoses, the OIE publishes Terrestrial/Aquatic Animal Health Codes and Manual of Diagnostic Tests and Vaccines for Terrestrial/Aquatic Animals to assure sanitary safety of international trade and provide a harmonized approach to disease diagnosis for terrestrial and aquatic animals. These standards cover specific chapters on AMR as well as relevant chapters on hygiene and infection prevention and control in animals.

4. Global initiatives on Optimization of antimicrobial use in animals (GAP AMR Objective 4): The Terrestrial Animal Health Code and the Aquatic Animal Health Code have added specific chapters on responsible and prudent use of antimicrobial agents in veterinary medicine and in aquatic animals. The OIE has also developed a listing for veterinary antimicrobials according to three categories: (1) Veterinary Critically Important Antimicrobial Agents (VCIA), Veterinary Highly Important Antimicrobial Agents (VHIA) and Veterinary Important Antimicrobial Agents (VIA). Among the VCIA in the OIE List, some are considered to be critically important both for human and animal health which should thus be: (1) not used as preventive treatment applied by feed or water in the absence of clinical signs in the animal(s) to be treated; (2) not used as a first line treatment unless justified, when used as a second line treatment, it should ideally be based on the results
of bacteriological tests; and (3) not used extra-label/off label except for reserved for instances where no alternatives are available. This List is based on expert scientific opinion and will be regularly updated as new information becomes available. In addition, the Codex Code of Practice to Minimize and Contain Antimicrobial Resistance also provides additional guidance for the responsible and prudent use of antimicrobials in food-producing animals. Its objectives are to minimize the potential adverse impact on public health resulting from the use of antimicrobial agents in food-producing animals, in particular the development of antimicrobial resistance. The International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicine Products (VICH) is a trilateral (EU-Japan-USA) programme aimed at harmonising the technical requirements for data necessary for the marketing authorisation (also called ‘registration’) of a veterinary medicinal product. There are two guidelines in the area of antimicrobial safety: (1) Guidance on Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food-producing Animals with Respect to Antimicrobial Resistance, and (2) Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food.

5. Global initiatives on developing a business case for sustainable investment (GAP AMR Objective 5):
The “Responsible Use of Medicines Report 2012” estimated that about 8% of total health expenditure or around 500 billion per year globally, could be avoided with optimized use of medicines, with 11% (US $55 billion) of the savings coming from avoidable antibiotic misuse/overuse. Several initiatives by groups, institutions, inter-governmental organizations, and other global units have thus placed emphasis on antibiotic innovations, alternatives, discovery, rapid diagnostics, and others. There are a few however that cover animal health needs. Along the lines of sustainable investment, there has been a global push for the development of National Action Plans. The World Health Assembly has urged all Member States to develop and have in place by 2017, national action plans on antimicrobial resistance that are aligned with the objectives of the global action plan. It is aimed that this national action plans are ultimately funded and implemented. A manual has been developed by WHO, in collaboration with the FAO and OIE, to assist countries in preparing or refining their national action plans. A number of supporting documents and tools have also been developed towards this end.

Keywords: Antimicrobial Resistance, Global Initiatives, Global Action Plan on AMR (GAP-AMR)

References:
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The Outcome-Based Veterinary Curriculum

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Summary
In the modern knowledge economy, information is accumulating in all scientific and professional areas at an exponential rate. If it ever was possible to include “all knowledge of an area” in a university degree programme, that age is long gone. Therefore, it is essential that core knowledge and skills for a clinical programme are defined in a “top-down” manner, so that these are effectively learned and form the foundation for “day one competence” as well as lifelong learning and sourcing of knowledge as it is required. The outcomes-based veterinary curriculum provides this framework in a way that allows the development of a coherent assessment strategy, a logical approach to learning and the prioritisation of content.

Historically, the starting point for most teachers was the content of a curriculum—what needed to be taught (2,3). The academic disciplines for inclusion, such as anatomy, physiology, medicine and surgery, were broadly defined, and responsibility for the detail of separate courses delegated to departments. In many cases, individual subject specialists were the sole arbiters of what was included and what was not. This approach makes teachers feel good in terms of discharging their duty to the learner, but it is based on two fundamental fallacies that have always hampered educational processes: teaching is not synonymous with learning (4) and the possession of knowledge of an area does not guarantee the ability to perform in that area (5).

So particularly in a professional programme, where the ability to apply core knowledge and skills to problems, and, where necessary, source additional information and support, are essential to outcomes for a graduate, curricula must go well beyond the mere delivery of information. The importance of focusing on curricular outcomes rather than inputs was first pointed out by Spady (3) in relation to North American high school and elementary programs. He proposed that, in future, course designers “work backwards” instead of adhering to conventional practice, which tends to start with teaching and content followed by an assessment related to the teaching. Spady suggested that a much more logical approach would be to look at the knowledge, skills, and behavioural competences required— the outcomes—and to work backwards from these to the required learning (6). Once the full range of outcomes appropriate to a veterinary professional curriculum is identified (7,8), valid assessment methods can be related to these outcomes both to verify their achievement and drive student learning in appropriate directions (9). In a modern curriculum (Table 1), appropriate outcomes are much more than scientific knowledge and technical skills, including communication, collaboration, management, leadership, and cultural awareness (7). From the outcomes and the assessment, it should be clear to students what they must achieve to qualify, and this helps focus both students and teachers on the support they need to facilitate appropriate learning.

A cultural problem for those advocating an outcomes-based approach is that this model is alien to those whose view is content-driven. If subject matter is seen as the most important factor in the design of a class, “learning outcomes are meaningless” (10). Over the past 100 years, this has led to a constant tussle
in clinical education between those focused on outcomes and skills development and those focused on content (11). However, this may finally be resolved in the twenty-first century with the paradigm shift created by the internet age in the sourcing and availability of information (12). An outcomes focus helps programme directors and designers to identify essential, underpinning knowledge, and helps create priorities for inclusion of content, an essential task that prevents information overload now that it is abundantly apparent that the curriculum cannot include everything (6,13).

Table 1 Overall Learning Outcomes for a Clinical Veterinary Programme
(Example given is “Curriculum 2007” from Royal Veterinary College, University of London)

1. The possession and understanding of an up-to-date body of scientific, clinical, animal industry-related, legal and professional knowledge, and critical awareness of current issues, relevant to employment in a diverse range of roles requiring a clinical veterinary degree.

2. The ability to integrate and use core knowledge, and source further information as appropriate, for the analysis of scientific and clinical problems.

3. A comprehensive understanding of and the ability to apply scientific methods, ethical reasoning skills, and clinical examination, therapeutic and surgical skills to the prevention, solution and management of complex scientific, clinical, public health and food-related problems, in the interests of animals, their owners and keepers, and people and society more generally.

4. The capacity to recognise one’s own limitations, continuously updating both knowledge and skills where needed, so that all practice remains at the forefront of the discipline of veterinary science and is executed with skill and, where appropriate, originality.

5. The ability to communicate at all levels relevant to professional practice, with clients, the public, professional colleagues and appropriate authorities, ensuring, in the interests of animal welfare, biosecurity and human health, optimal functioning of the veterinary team and maximum understanding of all involved in keeping and caring for animals.

6. The possession of a core knowledge of the organisation and management of small and medium-sized enterprises, in particular, private veterinary practices.

7. A sound understanding of the legal position and social responsibilities of the professions, particularly the veterinary profession, and the implications for ethical approaches to business practice, relationships with clients, and contributions to society, nationally and globally, in veterinary and non-veterinary arenas.

Keywords: Outcomes, Curriculum, Information Overload, Professional Skills, Technical Skills.

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What Every Educator Needs to Know About: Assessment and Feedback

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Summary

Assessment and feedback on performance are fundamental to effective and efficient student learning. Assessments should be designed to measure expected learning outcomes so that students are able to track and monitor their own progress. A well-designed assessment reinforces learning, motivates students and helps them to identify areas of strength and weakness. From an educator’s point of view an ideal assessment is a measure that predicts the likely future performance of a student. In the context of veterinary practice, assessment helps provide assurance that a student will be safe and fit to practice with a view to accountability to the public, government, and regulatory bodies. The fundamental characteristics of a well-designed assessment are reliability, validity, educational impact, cost-effectiveness and acceptability. A combination of these characteristics may be used to define the utility or usefulness of any particular assessment. Although all are important, it should be recognized that no single assessment method is likely to provide uniformly high quality information on all five characteristics. For any single assessment validity, reliability, educational impact, acceptability and feasibility are often in conflict, and a trade-off is necessary to ensure that the purpose of an assessment is achieved. Several different assessment methods are used in current veterinary education, these include: Multiple choice questions, Extended matching questions, Short answer questions, Script concordance tests, Essays, Practical skill tests, Objectively structured clinical examinations, Directly observed procedural skills, mini clinical examination exercise, and Portfolios (?). Over the years there has been a progression from assessment of single learning objectives such as knowledge, skills and attitude to a more integrative approach in which roles or competences that combine several learnings are assessed. Traditionally, assessment has focused primarily on summative tests of learning i.e. typically high stakes examinations with the main aim being to judge the level of attainment. In summative assessments students usually must achieve a minimum level in order to progress to the next stage of learning, or to obtain a qualification that completes their studies. In contrast, formative assessments are increasingly being introduced into veterinary curricula; these types of assessment provide feedback on performance. An assessment may also be both formative and summative; the student must achieve a minimum score in the assessment and additionally receives information about their performance, possibly including advice for improvement. Formative assessment promotes learning and has a critical function in enabling students to build on previously acquired knowledge and skills. The provision of feedback to students should be an essential role for the modern teacher. In addition, fellow students may give valuable feedback and other staff involved in the learning process, such as demonstrators, veterinary nurses, technicians in the lab, or the student themselves (self-assessment). Good feedback should be timely, specific, justifiable, fair, include a two-way discussion (dialogic) and encourage self-reflection by the student.

Introduction
“Assessment is defined as the measurement of achievement of progress towards meeting defined educational objectives” (1).

The purpose of assessment includes one or all of the following:
- establish the level of achievement reached at the end of a course/unit for certification.
- predict a student's likely performance level in the future.
- consolidate the work done so far before moving to the next level.
- motivate and encourage students.
- identify areas of strength and weakness.
- act as a Quality Assurance mechanism and benchmarking.
- determine whether a student can demonstrate ‘fitness to practice’ and can be accountable to the public, government, and regulatory bodies. (2).

A good assessment can drive student learning, but in practice the design and implementation of assessment is often not given adequate consideration. Another problem is that students may use assessments as the goal for their studies. Students will then narrow and restrict their efforts to learn only what they predict will be in the assessment, rather than achieving all of the learning objectives of the course and intended by the teacher. In designing an assessment it is therefore valuable to consider the purpose(s) of the assessment and how the outcomes will be used. In summary, the starting point for designing an assessment should include the concept of constructive alignment.

**Constructive alignment**

The theory and practice of constructive alignment in the context of learning outcomes and student assessment has been described by Biggs (3). First, identified learning outcomes should be developed to ensure that they are appropriate and are measurable. Then, relevant assessment method(s) should be selected to measure whether the learning outcomes have been achieved. For example, if a learning outcome for a histopathology class involves students being able to set up a microscope and recognise particular cell types, the assessment should ensure that the student can ‘do’ these tasks i.e. a practical based assessment is required, rather than an essay about microscope set-up and cell morphology.

**Assessment methods in Veterinary Education**

Learning outcomes may be in the domains of knowledge, skills and attitudes of a student, clearly assessment methods should be appropriate and matched to the identified learning objectives (4). Table 1 outlines some of the assessment tasks mapped against different domains of outcomes.

**Table 1** Assessment tasks that can be used to measure outcomes

<table>
<thead>
<tr>
<th>Outcome domain</th>
<th>Assessment task</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge and understanding</td>
<td>• Essays/reports</td>
</tr>
<tr>
<td></td>
<td>• Multiple choice questions (MCQs)</td>
</tr>
<tr>
<td></td>
<td>• Short answer papers (SAQ)</td>
</tr>
<tr>
<td>Clinical reasoning and decision making</td>
<td>• Extended matching questions (EMQ)</td>
</tr>
<tr>
<td></td>
<td>• Strip concordance tests (SCT)</td>
</tr>
<tr>
<td>Clinical and technical skills</td>
<td>• Objectively structured clinical examinations (OSCE)</td>
</tr>
<tr>
<td></td>
<td>• Directly observed procedural skills (DOPS)</td>
</tr>
<tr>
<td></td>
<td>• mini clinical examination exercise (mCEX)</td>
</tr>
<tr>
<td>Professional behavior</td>
<td>• Multisource feedback</td>
</tr>
</tbody>
</table>

In selecting the appropriate assessment method the Miller’s pyramid (5) can be used a guide. The following figure illustrates how the assessments can be mapped against the Miller’s pyramid.
Other important criteria to consider in selecting appropriate assessment tasks are:

Validity - whether a test actually succeeds in testing the competencies that it is designed to test. For example, to test the skills involved in the clinical examination of a cow what is a more appropriate assessment tool, an MCQ based assessment or a hands-on practical examination with a live cow?

Reliability – This is the extent to which an assessment method consistently and accurately measures what it is supposed to measure. For example would an assessment produce the same or similar scores on two occasions, or if measured by two assessors?

Educational impact – This refers to the content of the test and how it is embedded into the curriculum. Methods of assessment such as an MCQ may offer high reliability and reproducibility, but may have a negative educational impact on the learner by providing no formative feedback.

Feasibility – This is the capacity for an assessment to consume resources such as time, money and the time and effort of staff. Many assessment methods are resource-intensive, requiring extensive preparation and staff training (such as OSCEs).

Acceptability - An assessment must have the trust and confidence of students and of the assessors in the content and the process of the task.

None of the above criteria should be considered individually, although it is important to recognise that there may be a ‘trade-off’ between the different criteria in designing an assessment.

**Assessment ‘for’ learning and Assessment ‘of’ learning**

For the purposes of accountability and certification all assessments can be placed on a continuum between ‘assessment for learning’ and ‘assessment of learning’ (6). Accordingly, the objectives of formative (assessment to give feedback only not for grading/marking) and summative assessments (for grading and marking) may be based on ‘assessment for learning’ and ‘assessment of learning’. Formative assessment is a powerful tool that enhances learning. However, for the formative assessment to be useful tool for the student the educator must be competent in giving feedback. There are several models that can be used to structure and frame good feedback. The model of Pendleton (7) is one of the most commonly used for teachers giving feedback in clinical settings. The model adopts a strict order of delivering feedback: positive comments
before negative, and participant self-critiques before observers give feedback. Behavioural aspects are mentioned, not personal traits and specific examples are identified and targeted for feedback. The most important point is that feedback is based on what was observed rather than interpretation by the person giving him feedback. An alternative model that is frequently used is the so-called 'Agenda-led outcome-based analysis (ALOBA)'. The principle when employing this model is to identify what the learner wants help with; and then the feedback session is centered on the student’s goals (8).

Keywords: Constructive Alignment, Formative Assessment, Summative Assessment

References
What Every Educator Needs to Know About: Key Concepts Relevant to How We Learn

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Summary

Beliefs about intelligence, learner maturity, learner capacity and learner motivation all affect the nature of learning and the quality of veterinary graduates. An understanding of the implications of these four key concepts has driven the move to vertically and horizontally integrated curricula, incorporating more student-centred approaches to curriculum delivery. Teachers must recognise the need to cultivate a growth mindset in their students, so they welcome new challenges as opportunities to learn at the same time as knowing that their teachers have their best interests at heart. This maximises their opportunities to become confident and competent professionals, eager to engage in self-directed, lifelong learning.

An important first stage in deciding how best to support students in achieving the desired learning outcomes is a knowledge of how we learn. From childhood, the human brain is wired to process information of all types from all around, but it is in danger of being overwhelmed. Therefore, it becomes selective over information that is processed and retained. This depends on the information’s perceived relevance, and subsequently the amount that it is applied and reused. The sophistication and complexity of the material that can be processed also depends on prior knowledge and experience. Situations that cannot be understood may not be processed at all, or imperfectly processed and easily forgotten. It follows that four concepts are important to thinking about student learning; these are: learner beliefs about intelligence, learner maturity, learner capacity, and learner motivation and emotions more generally stimulated by learning encounters.

Learner beliefs about intelligence

As learners start to grapple with hypothetical thinking, from the age of 11 years onwards, their learning experiences themselves become increasingly important in the way they view their abilities (2). In early adolescence, based on the nature of the feedback delivered by parents and teachers, the learner is likely to have formed a view either that they are clever or not (fixed mindset), or that they are capable of a lot provided that they work hard (growth mindset). This learner view of their ability is fundamental to their learning, and will profoundly affect their motivation for learning. Those with a growth mindset are interested in mastery. They know that this will allow them to understand advanced concepts and undertake more complicated tasks. In contrast, those with a fixed mindset are much more focused on performance and avoiding exposure as incompetent. This handicaps their development, as they will avoid the new challenges which for those with a growth mindset are the beginning of learning.

Learner maturity

A significant development for all learners comes with the recognition that they have an important part to play in their own understanding. Many arrive at university viewing learning from the perspective of “basic dualism”, with the teacher having the right answers that need to be learned. As part of their education, they should then move through more relativistic positions, leading to an understanding of good and less
good explanations of phenomena, to a stage of “evolving commitments” where individual responsibility for recognising and establishing current best evidence is recognised. However, if poorly managed, even at graduation, some may still cling to dualist perspectives, or have progressed to the position of multiplicity but no further, believing that “everyone has a right to his/her own opinion”: and one person’s is just as valid as that of another (3). This maturity of thinking about the nature of knowledge proceeds alongside a change in preference for the way it is delivered. Adults are more likely to take charge of their own learning and embrace an andragogical approach (4). Rather than being subject-orientated, the problems being tackled in professional education and practice motivate the learner to seek knowledge and, where necessary, individual expert support to help with solutions to these new challenges.

**Learner capacity**

Alongside learner maturity as a limiting factor to the process of learning, the human brain is limiting in the amount of information that can be processed and integrated at any time. This relates to working memory capacity, and the limited number of “units” available for simultaneous processing of information (5). A key to understanding working memory is that these units can either be single pieces of information or “chunks” of information at various levels of aggregation. Through rehearsal and chunking, the learner is capable of solving more and more complex problems (6). The size of working memory means that it is easily overloaded if presented with too much new knowledge, or problems that are too complex for the learner stage reached. This is the basis of cognitive load theory, which helps explain the need for sequential development of concepts and problem solving skills (7). To overcome the limitations of working memory capacity, the human brain adopts two approaches to problem solving. Novel problem solving depends on extensive use of working memory (Type II processing), but recognised problems are dealt with by Type I processing (8). Type II processing is slow and demands effort, so where possible the human brain attempts to recognise patterns and retrieve memorised solutions. This is more rapid and imposes little cognitive load, ensuring the ability to negotiate busy lives and careers, without having to work through all challenges as though they were new.

**Learner motivation and emotion**

In their earliest years, learners are largely extrinsically motivated with rewards, or, in some cases, punishments dictating their behaviours. However, an important transition for those who are to go on to become effective, lifelong, self-directed learners is the development of intrinsic motivation for their discipline (9). Learners need to develop a positive attitude to subjects and for this they need to understand the relevance of learning materials and have self-belief that they can master the associated learning challenge. Subsequent mastery gives pleasure and feeds back into further interest (10). Positive emotion and a feeling of safety in the learning environment allow the learner’s mind to focus on learning as opposed to survival (11). Feeling socially challenged can lead to an individual’s entire focus being on saving face and excluding themselves from the unsafe environment. Ultimately, particularly in the presence of a fixed mindset, this leads to avoidance behaviours to prevent a recurrence of negative emotions related to perceived failure. Emotion also affects the ability to delay closure. It can be uncomfortable to deal with uncertainty and not be able to immediately identify answers (12). Some will either race to a superficial and often imperfect solution or give up. However, to be effective in reaching complex decisions, and learning complex concepts, the learner must be able to fully explore all aspects before coming to a conclusion. This will be supported by a preference for complexity and a growth mindset, and also feed into a desire for lifelong learning (9).

**Conclusions**

These four different perspectives on learning in the classroom, laboratory and clinic have profound implications for curricular design and delivery, and yet frequently they seem to be ignored in veterinary programmes which, at times, seem to be more based on what individuals and departments are prepared to deliver, rather than learner needs related to programme level learning outcomes. The need for knowledge and skills
to progress from basic levels to advanced, for learning to be really effective, emphasises the value of curriculum designs based on vertical integration of courses, rather than collections of freestanding elements that differ only in relation to their content. Certain transitions, such as that from classroom to clinic, can be particularly difficult to navigate because of the tendency for sudden changes in the way knowledge is used and acquired (13), so specific attention needs to be paid to “bridging” for these stages (14). Confronted with a disease associated with a single organ system, the learner needs to simultaneously understand the anatomy, physiology and pathologies of that organ, and, as they consider treatment, the pharmacology of the different therapeutic agents. Horizontal integration of disciplines aids this synthesis (15).

An awareness of the way learners mature has implications for the teacher. Many will arrive from first degrees or high school with dualist perspectives, believing that knowledge is black and white (16) and that they need to be taught the correct answers. Therefore, the curriculum design needs to follow the expected growth of learning maturity, over the 4-6 years of the clinical veterinary programme, with learning materials and teachers at the start acting as authority figures, giving way to teachers acting more as facilitators in later years. In the same way as learners will not be able to cope with advanced concepts before acquiring a foundation of basic knowledge, they will not be able to cope with more andragogical-type learning formats until they have matured from dependent to self-directed learners (17). Crucial to effective learning is the activity of the learner themselves (1). Reliance on information transmission approaches, requiring relatively passive involvement on the part of the learner, is a poor strategy for fostering understanding. Therefore, it is important that the teacher does not do all the work for the student in terms of the organisation of content and identification of key points. The ability to undertake both these tasks is an essential professional skill. Optimal learning will occur in classes that allow students to recall prior knowledge, and place them at their “learning edge” (in their zones of proximal development), with appropriate teacher support available to help them progress (14).

A key metaphor for the way the teacher supports active learning is the provision of “scaffolding” that can be adjusted to learner needs and removed as the learner becomes more capable (18). Students must fully participate, so it is vital that they are motivated and regard the learning environment as safe (11). This means that the teacher must foster a growth mindset-supporting culture, with experimentation and failure not seen as something to be criticised (19). Indeed, the major concern should be for those who do not participate and risk the prospect of being wrong. Understanding how these four concepts are relevant to learning means that teachers can give their students the best possible chance of graduating as confident, competent professionals who will go on learning for life.

Keywords: Cognitive Load, Motivation, Engagement, Scaffolding, Mindset, Learner Maturity, Learner Capacity

References
What Every Educator Needs to Know About: Teaching Methods for Veterinary Education

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Summary
Learning is an individual process but it is now recognized that a number of factors help most students progress and gain satisfaction from the learning experience. A good ‘teacher’ should not only impart knowledge but also create a suitable environment for learning. In addition to ‘teaching’ the teacher must also recognize and cater for different learning styles, understand the student’s needs, be supportive, motivational and inspirational. A good teacher therefore has to be multi-talented. The practical delivery of teaching must take into account the size of the class, the arrangement and format of the class, the environment, the resources available and the needs of the curriculum. The most common methods used in veterinary education include lecture based large group teaching, small group teaching and workplace based teaching. Lecture based large group teaching has the advantage of being the most cost effective when student numbers are large. Although this method is considered a somewhat passive mode of information delivery, it can be very effective when well planned and used in conjunction with appropriate pedagogical principles. The most important principles include: clearly indicating the learning outcomes at the start of the lecture, reviewing towards the end of the lecture, understanding the limitations of the students and structuring the lecture to encourage active learning. Small group teaching is more resource intensive and is appropriate for a variety learning formats, such as problem-based learning, directed learning, group practical sessions, seminars and tutorials. In addition to teaching subject specific content small group teaching is an opportunity to foster the development of a wide range of skills such as active listening, creative thinking, problem solving, leadership, communication and professionalism. Small group teaching is not an easy task and requires careful planning to lead the group towards the desired learning outcomes. The teacher requires skills in managing individual students and the group. One of the most difficult skills in small group teaching is related to questioning. Questions that lead to higher cognitive processing require careful preparation. Although it may be necessary to use simple factual recall type questions, one of the most important advantages of small group teaching is the opportunity to lead students to critically assess their own knowledge and grasp of concepts. Teaching in a clinical setting is a typical example of workplace-based teaching. A particular challenge here is formalising the learning process whilst in the presence of patients. It is easy for students who are participating in a seminar or teaching rounds to realise that they are being "taught" (and they will often take notes and come prepared for learning). Workplace teaching is often categorized as application of knowledge (for example clinical reasoning or problem-solving), practical/technical skills, and professional/non-technical skills. One of the advantages of workplace teaching is that students learn to apply their earlier knowledge and skills in the context of environmental challenges. Rather than textbook based problem-solving in a classroom environment, the workplace offers the opportunity for real-life complexity to influence and improve clinical reasoning, professional reasoning and practical skills.
Introduction

‘I never teach my pupils; I only attempt to provide the conditions in which they can learn’ - Albert Einstein (1879 -1955)

A good teacher in addition to imparting knowledge must also provide the optimum conditions for learning, these include catering for different learning styles, understanding the student’s needs, being supportive, motivational and inspirational. A good teacher therefore has to be multi-talented, how can you achieve all these? Is this feasible? You may be involved in teaching and training of clients, farmers, undergraduates, postgraduates or the general public. The teaching framework could also be different; it could be a large group, a small group, a group around an animal or in the laboratory. Whatever the target group or the setting, the expectation of the target audience is that you lead the learning process, make it easy, clear, enjoyable and inspiring! This is a challenging task even to the most outstanding lecturer. How can you face this challenge? This paper will briefly outline some evidence-based knowledge in teaching and learning to provide you with the skills that may assist you in facing these challenges.

Lecture based large group teaching

Lectures continue to be the most widely used method of knowledge dissemination despite evidence that they have less impact on learning than most other methods. Different methods of capturing lectures through the use of video recording and pod casting, have added to the wider dissemination of lectures. Even distance learning students are taught through video conferencing and mobile learning technologies. So why are lectures so popular? Lectures are effective in transmitting information, but are other methods more or less effective? There are several methods of information transfer that we use in teaching. Bligh (1) compared lectures with other methods of information transfer and the majority of comparisons showed no significant difference. These studies were undertaken before the widespread use of the Internet and the computer; therefore the results may be different today.

Table 1 Number of comparisons with other methods where acquisition of information is the main criterion
Adapted from (1)

<table>
<thead>
<tr>
<th>Teaching method</th>
<th>Lectures less effective</th>
<th>No significant difference</th>
<th>Lectures more effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discussion (various)</td>
<td>18</td>
<td>54</td>
<td>22</td>
</tr>
<tr>
<td>Reading and Independent study</td>
<td>10</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Inquiry (I.e. projects)</td>
<td>6</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Other (audio, TV, computer based)</td>
<td>27</td>
<td>57</td>
<td>2</td>
</tr>
</tbody>
</table>

What happens during a lecture? Factors affecting student attention or the ability to concentrate have been studied using changes to heartbeat. The heart rate is taken as a measure of arousal and motivation. Figure 1 outlines what happens to the heart rate of students during a lecture.

![Figure 1](image_url)  
**Figure 1** Student heart rates were measured during an uninterrupted lecture.
Note: The data are for 16 students in four lectures with measures taken every 5 seconds. Thus each dot is the mean of 960 readings in a five-minute period. Heart rates stabilized before teaching began. Each lecture was eighty minutes (longer than most). From: (1), redrawn and adapted by the Royal Veterinary College, London, UK.

There was a sharp decline of the heart rate during the first 30 minutes of the lecture and a further gradual decrease up to 80 minutes. Although it is unusual to have lectures for over a 50-60 minute period, the experiment clearly shows the decline of the arousal and motivation.

**Limits to learning and structuring a lecture**

Assimilation of information is limited by the short-term memory (also called working memory). Limits to learning during a lecture are mostly due to the limitations of short-term memory. Short-term memory is the memory that is active in an on-going basis. This memory and can only hold up to 7 +/- 2 facts at any one time. This is the memory or attention span that is available to the teacher during a teaching session. Overloading the short-term memory will lead to information overload, therefore should be avoided.

Structuring the lecture based on pedagogical principles can make a lecture more effective. Several factors affect the effectiveness of a lecture, these include the content to be covered, the time available, the size of the group and even the cultural attitudes towards the teacher-learner relationship. But as a general guide the following are most useful:

- Develop a clear teaching plan for the time available
- Ensure that students are comfortable
- Try and make the students feel at ease, use examples and ‘story telling’ to engage
- Clearly indicate the learning outcomes at the start of the lecture
- Review towards the end of the lecture, to see whether the lecture has achieved those outcomes.
- Be confident, passionate and demonstrate knowledge
- Introducing an activity every 15-20 minutes improves the attention and helps to maintain focus.
- Different activities can be used to break up a lecture and to energise and motivate the learners.

Among the most widely practiced are: Questioning, Brainstorming, Buzz groups, Snowballing, Interactive voting systems.

Additional aids such as anatomical models, bones, feed samples, surgical instruments can be used to foster engagement. All these interventions to a lecture have to be carefully planned and timed.

**Small group teaching**

Small Group Teaching (SGT) is widely used in veterinary education. The most commonly used methods of SGT are tutorials and seminars. Other forms include practical classes, clinical rounds, specific teaching methods that include directed learning, case-based learning and problem-based learning. In all these SGT, a tutor or facilitator will be present with the group. There are also tutor-less and online-based SGT sessions. Common to all SGT is group work. The use of group work is congruent with the main objective of SGT, which is to foster the thinking and self-directed learning skills of students through engagement with others (1). Small group teaching has generated more interest and research during the last three decades. As a result, there is a considerable amount of information related to SGT. In this lecture some key characteristics that will enable to be an effective SGT facilitator will be addressed.

Teaching in small groups has the unique potential to promote learning at an individual level. It is a challenging and a dynamic teaching and learning method for both the tutors and learners. The participatory nature of small group teaching enables the learners to discover their own abilities and skills. One of the main objectives of small group teaching is to foster self-regulated learning.

Developing conscious awareness of skills and attributes is another main objective of small group teaching. Skills such as those below are considered as learning outcomes through small group teaching in addition to discipline-based knowledge (2). Some of these are Critical thinking, Creativity, Problem solving, Decision-making, Personal effectiveness, Personal competencies and Team working.

The basic principle in small group teaching is ‘group work.’ The group members therefore become ‘learning
tools’ for each other. It is vital that group members feel safe in the group environment. The facilitator must ensure that this condition is met before group work begins. Successful small group teaching requires skill and planning. There is an assumption that it is unstructured teaching and therefore can be conducted in a loose, open-ended and informal manner. But there is a clear framework as well as spoken and unspoken rules, which must be adhered to. Skills for effective SGT are questioning, listening, answering and maintaining silence (or feigning ignorance) and managing difficult, dominant and quiet students.

Teaching in the workplace
One of the advantages of workplace teaching is for students to learn to apply their earlier knowledge and skills in the context of environmental challenges. Rather than textbook problem-solving in a classroom environment, the workplace offers the opportunity for real-life complexity to influence clinical reasoning and professional reasoning and practical skills. The level of complexity should be gradually increased with students’ experience (their first physical examination of a live animal should ideally not be in front of a demanding owner and on a patient who wants to bite/ kick them!). However clinical teaching is under much criticism for its variability, lack of intellectual challenge, and unpredictable nature (3). Some cases are considered ‘imperfect’ for student learning. As students develop their knowledge and gain experience at the workplace, they will benefit from being exposed to “imperfect” cases. There is an abundance of literature about workplace teaching and learning in relation to professional education. In addition to learning the more easily definable problem-solving and practical skills, there is important tacit knowledge that is gained from exposure to the workplace. An investigation of students’ perceptions of their learning during clinical rotations revealed that many students recognised they had acquired and improved technical skills during rotations, but fewer had grasped the more subtle or complex learning outcomes, such as approaches to clinical problem-solving, or understanding client needs (4).

Keywords: Lecture Based Large Group Teaching, Small Group Teaching, Workplace Teaching

References
Developing Veterinary Professional Skills

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Summary
In addition to scientific knowledge and technical skills, it is important that a modern veterinary curriculum develops the broader professional skills of its graduates, for the variety of different roles that veterinarians pursue, to meet the needs of the client. Historically, veterinary curricula have been efficient in delivering information, but much less effective in developing technical skills, although huge efforts have been made to address this over the last 20 years. However, until recently, curricula tended to pay lip service to broader professional values and behaviours, including communication, collaboration, clinical and ethical reasoning, and the development of professional identity. Mistakenly, it was assumed that these were picked up when learning took place in the workplace. It is now thought that a distinct approach to developing professional skills is needed that has some similarities and some differences from teaching of the more technical aspects of veterinary science. As with more traditional areas, professional studies modules need to provide frameworks to act as the foundations of skills development in this area. However, it is important that they acknowledge the prior learning and experience of students, and the way in which their personal identity is engaged with their developing professional identities. Unlike technical and skills knowledge, professional skills bring with them a prominent affective dimension, and effective learning requires the development of skills in reflection involving both appreciative and critical inquiry, as well as the management of emotions. Employers and clients regard these skills as among the most important when becoming involved with young veterinarians.

The education of the “consummate professional” (May 2015) involves the development of a range of knowledge, skills and attributes essential to thriving in modern veterinary practice. Historically, curricular descriptions and legal requirements (e.g. Directive 2005/36/EC, Annex 5.4.1) focused on the knowledge required, often in terms of the scientific disciplines that had a relevance to veterinary science. However, this led to continued concerns by employers of a lack of technical skills of graduates, and attempts by professional bodies to remedy this perceived deficit (e.g. Royal College of Veterinary Surgeons 2001, NAVMEC 2011). Increased attention to technical skills over the last 20 years has exposed another deficit; that of the non-technical professional skills. These include areas such as communication, empathy, collaboration, clinical and ethical reasoning, the human-animal bond and professional identity. With the apprenticeship model of clinician development, through clinical rotations in university hospitals and externships in private practice, it was assumed that students acquired professional skills and business knowledge through the process of enculturation (Heath 2006). However, it is now clear that many struggle to recognise learning opportunities without clear objectives that direct their attention (Woods et al 2011), and it is difficult for individuals to articulate objectives in the absence of conceptual frameworks that form the foundations for these. The situation is further complicated by the fact that many of their teachers have gained their professional skills and expertise experientially and therefore have difficulty in explaining these aspects of their skills. They may recognise when students are struggling but it can be difficult to provide the kinds of behavioural feedback that are required to support student learning and improvement.
For over 100 years, the formal curriculum has provided frameworks for understanding pathological processes and the medical and surgical therapies required to address these. In contrast, curricula have provided few frameworks for the support of informed ethical debate, or understanding clinical reasoning and professional identity formation, as students navigate the challenging environment of the clinics and deal with clients and their animals. In order to support meaningful professional skills development, it is important that this deficit is addressed from the start of veterinary education.

A distinctive feature of the so-called professional skills is that all students already have these developed to a greater or lesser extent. They know how to communicate, although not necessarily appropriately for the consulting room and other professional contexts, and they know how to reason, although not necessarily in the disciplined, inductive way that assures high quality clinical decision-making. Therefore, it is important not to patronise students and to acknowledge their foundational abilities, at the same time as indicating that such skills are not innate but can be developed in all of us.

Custom and practice in veterinary education arguably means that too much attention is paid to the scientific foundations of clinical practice (Thompson et al 2016). However, this means that there is often little space in traditional curricula for the professional skills foundations that are required. These include an introduction to ethical systems of thinking, and the way in which mature judgements are made by balancing competing stakeholder interests and ethical perspectives. An understanding of the nature of scientific reasoning and how it is distinguished from clinical reasoning, and, in particular, a first principles, inductive, logical approach to clinical cases is important if students are going to be able to recognise their own development as clinicians and that of others (May 2013). They can thus appreciate the strengths of pattern recognition and the biases to which it is prone and how to guard against these.

In the same way as clinical skills laboratories bridge classroom theory and clinical practice, and sequentially develop technical skills, problem-solving involving paper-based cases of increased complexity can support student clinical and ethical reasoning separately and in combination (May and Silva-Fletcher 2015). This cognitive approach and development will not predict how students will perform as they are given greater responsibility for real cases, but it will provide them with frameworks and ways of thinking that can help them deal with the particular circumstances of individual cases and their emotional, and often financially challenged, owners. Recognising that these challenges are all part of their professional identity formation, with professional values, the clinical culture and the personal identities all requiring a degree of alignment, and that this process is not without its own stressors, aids the reflexivity and self-awareness that is needed for students to make the most of clinical learning opportunities and enter practice as confident, competent graduates. A part of this is to understand how to communicate and collaborate with the whole veterinary team in a way that maximises the quality of services provided to the client (Kinnison et al 2015).

Repeated surveys have demonstrated that professional skills are among the most important of the attributes of the modern veterinary graduate (Cake et al 2016). Employment interviews often assume knowledge and technical skills, and focus primarily on establishing the expertise of the graduate in the non-technical skills. Despite graduate misgivings about their level of knowledge, this is rarely a cause of complaint. Most complaints in the UK to the Veterinary Defence Society and Royal College of Veterinary Surgeons relate to poor communication skills (Radford et al 2003). As graduates recognise that their theoretical knowledge, by itself, is not the answer to cases that they are treating, they become increasingly stressed by the day-to-day challenge of working in practice, and those who do not rapidly acquire the deficient skills experientially may drop out of the profession or even commit suicide. Therefore, the incorporation of professional skills strands into the modern curriculum and the development of these skills in graduates is not only essential to the quality of services they deliver to the public, but also their personal wellbeing and their ability not just to survive but also to thrive in veterinary practice and achieve their long-held career ambitions.
Keywords: Curriculum, Outcomes-Based, Active Learning, Curriculum Integration, Veterinary Pedagogy, Scaffolded Learning, Content Overload

References
Clinical Skills Development

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Summary

Although a set of ‘clinical skills’ (CS) may mean different things to different people a definition has been established in the medical education sector. These include the skills associated with physical examination, practical clinical procedures, communication and treatment, therapeutic practice and patient management. Common to all these CS are essential underlying knowledge domains, namely: procedural knowledge, declarative knowledge such as anatomy and physiology, verbal and non-verbal skills and higher cognitive skills such as reasoning and decision-making. In developing a CS curriculum the educator/instructor must pay attention to all these sub domains and ensure that the student is actively involved in utilizing the knowledge required for practical application. A number of teaching models are in practice today that is designed to enable the educator to teach and support the students in developing these skills. Of these the ‘Novice to Expert model’ and ‘Peyton’s four-stage model’ are useful in developing appropriate technical CS in veterinary students. In order to teach clinical reasoning several models are in current use, these include the ‘Hypothetico-Deductive’ or ‘First Principle/Analytical’ model, the ‘Pattern Recognition’ or ‘Inductive reasoning/ Intuitive model’ and the ‘Logical approach’. These models are used to underpin problem-based clinical reasoning. The development of clinical reasoning in students requires the ‘ability to think, understand, and form judgments by a process of logic.’ It is increasingly recognized that communication skills are also fundamental to all professional practice and the development of verbal and non-verbal skills in students is now given considerable attention in modern veterinary curricula. Several models are used in teaching and developing communication skills. The most common are the ‘Calgary-Cambridge model’, ‘The Four Es model’ (engagement, empathy, education and enlistment) and the ‘Four Habits model’. All these provide a good framework for developing communication skills and may be adapted to suit the needs of the year group, clinical situation and the social-cultural nature of the country. Non-verbal communications, knowingly or unknowingly transmit feelings and attitudes. Especially in emotional situations non-verbal behaviours are likely to be influential in determining the success or otherwise of an encounter. Therefore, teaching and learning about non-verbal behaviours and how to manage them is important. A structured and progressive approach to teaching CS allows integration of different techniques and skills to form a more holistic competence. An essential feature of all CS development and teaching models is summarized in ‘Kolb’s continual cycle of learning by doing’ (observation, thinking, action, experience). In developing CS it is also of course necessary for students to practice repeatedly. The concepts based on ‘deliberate practice’ developed by Ericsson can be useful here to make the student confident in CS. The clinical skills laboratory plays a valuable role in the teaching and practice of technical skills and this kind of facility is growing in popularity in veterinary teaching institutes. The safe environment created by a CS lab facilitates learning, offers the opportunity to practice a skill and may even be used to support peer teaching. The CS lab can also be used for assessment, enabling and supporting students to gain competence and confidence.
What are clinical skills?
Clinical skills may range from simple to complex; from straightforward procedural tasks such suturing to advanced and complex clinical reasoning. For the purpose of this lecture a CS is defined as follows:

Clinical skills (CS) contribute to competences and as more and more veterinary teaching institutions are moving towards competency/outcome-based curricula it is necessary to understand how to guide our students or trainees to develop these skills. A body of underlying knowledge is essential to perform an effective CS and both educators and students should be fully aware of this before starting to teach and learn a CS.

The essential points to consider in the process of learning a CS are:
- Developing procedural techniques/skills in a safe learning environment, so that the students can eventually perform these procedures
- Developing Reasoning skills with a clinical, scientific and ethical perspectives (including experience and reflection)
- Repeatedly practicing the CS (deliberate practice)

Teaching Technical Clinical Skills
The old adage, See one, Do one, Teach one can be detrimental to patients (2) and has now been modified with newer and more complete models to teach CS. These models are underpinned by theory and evidence and are also useful in developing the ability of teachers to successfully impart CS.

The main focus of teaching models for CS use a sequential and staged approach to skill acquisition. Of these the ‘Novice to Master’ model (3) is used in teaching of many skills including playing musical instruments to more mental games such as chess. The principles and the mental functions involved in developing from beginner to more advanced stages in this model can be applied in CS development.

<table>
<thead>
<tr>
<th>Mental Function</th>
<th>Novice</th>
<th>Competent</th>
<th>Proficient</th>
<th>Expert</th>
<th>Master</th>
</tr>
</thead>
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<tr>
<td>Reflection</td>
<td>Non-situational</td>
<td>Situational</td>
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<td>Situational</td>
<td>Situational</td>
</tr>
<tr>
<td>Recognition</td>
<td>Segmented</td>
<td>Segmental</td>
<td>Holistic</td>
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<tr>
<td>Decision</td>
<td>Analytical</td>
<td>Analytical</td>
<td>Analytical</td>
<td>Intuitive</td>
<td>Intuitive</td>
</tr>
<tr>
<td>Awareness</td>
<td>Monitoring</td>
<td>Monitoring</td>
<td>Monitoring</td>
<td>Monitoring</td>
<td>Absorbed</td>
</tr>
</tbody>
</table>

*Figure 2 Novice to Expert model (based on 3)*
The four-stage approach (4) is another very useful model in developing CS. In this model the student is led through four stages as outlined in Figure 3.

**Figure 3** four-stage approaches to technical skill development (4)

### Developing clinical reasoning

Several models that are used for the teaching of clinical reasoning skills will be briefly described here.

1. The ‘Hypothetico-Deductive’ or ‘First Principle’ model involves the generation of hypotheses based on clinical data and knowledge followed by the testing of these hypotheses through further inquiry, and a combination of inductive and deductive reasoning (5).

2. The ‘Pattern Recognition’ or ‘Inductive reasoning or ‘Intuitive’ model involves remembering diseases that fit a with a pattern of clinical signs (6).

3. The ‘Logical approach’ model involves using problem-based clinical reasoning; each significant clinical problem is assessed in a structured manner before being related to other problems that the patient may present with. This ensures that one's mind remains open to more diagnostic possibilities than might seem initially the most obvious and helps prevent errors caused by pattern-based tunnel vision.

The first and third models above use orienting frameworks, that are analytic (i.e. conscious, controlled), in contrast the second model uses non-analytic (i.e. unconscious / automatic) reasoning strategies. It is important to stress that evidence is important for both forms of reasoning. All three models are designed to enable students to marshal reasoning processes in a flexible and context-specific manner (7).

### Development and teaching of communication skills

Communication is considered a core competency for the veterinary profession and is listed in the day-1-skills of several institutional, national, regional and international accrediting bodies (see RCVS, EAЕVE, AVMA and OIE). In teaching communication skills, engagement in experiential role-playing, with feedback, seems to offer the most potential for transformative learning (8). Several models have been proposed to capture the elements of communication skills that are required in a typical patient-client consultation. One of the most widely used is an adaptation of the ‘Calgary-Cambridge’ model (9) that is used in medical education. This is just one of a number of clinical consultation models developed for use in the health profession,
others include, the ‘Four Es model (engagement, empathy, education and enlistment) (10) and the ‘Four Habits’ model (11). Experience has shown that there are advantages if the elements of a consultation exercise are divided into two components; activities directed towards furthering the clinical side of the case, and those that are aimed at building the relationship with the client. Verbal and non-verbal communication may play a critical role depending on the situation. Non-verbal behaviours can be described as tone of voice, facial expression, eye contact, physical touch, appearance, body/posture, proximity and physical gestures, hand and body movements (12). In conclusion, to develop the ability of students to competently and confidently deploy the CKs required by their profession it is important to provide dedicated curriculum time, specially designed CSs labs and a wide array of CSs assessment techniques and procedures.

Keywords: Skills and Competences, Deliberate Practice, Clinical Reasoning and Communication

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How Should We All Develop Ourselves as Educators?

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Summary
This paragraph from a paper by Boud (1) is a good reminder why we should develop ourselves as educators. In the world of today's higher education the title of ‘educator’ should be reserved for those who have undertaken professional training and have by doing so developed the appropriate knowledge and necessary skills. Theoretical knowledge from the discipline of ‘education’ should underpin how we perform as educators (or pedagogues). Although the knowledge and the skills that we deploy in educational practice today have been developed over centuries it is only relatively recently that empirical studies have provided evidence to support the value of these approaches. Ideally educators should take an active approach to explore this knowledge and evidence base to develop a framework for their own development. Understanding the internal and external drivers that influence one’s development, as an educator must be explored. This exploration will illuminate the strengths and areas of development needed to follow the personal journey to become a professional educator. At the outset it is important to recognise and define the role of ‘professional educator’ as most veterinary educators, at least at the beginning of their careers consider themselves primarily as scientists or clinicians rather than educators. It is also important to explore the key competencies of a veterinary educator and to identify one’s own strengths, weaknesses and areas to develop. To achieve this goal there are a variety of formal and informal approaches and methods. The formal approaches include qualifications in teaching and learning, which can be, based on professional standards frameworks. Informal approaches include participation in short training workshops, seminars, conference attendance, reading, journal clubs, webinars and online discussion forums. Getting involved in educational research also promotes scholarship in teaching and learning but this may require training in methodologies that are more appropriate for educational research. One of the most important activities in professional development is reflection. Developing a critical reflective approach to all activities related to the role of educator will help the practitioner to take an evidence-informed approach to their own development.
As we monitor and carry out our developmental programmes it is ultimately the students that we teach and our teaching peers who provide the best feedback to ourselves as educators.

Definition of educator
Who is an educator? This is a fundamental question that needs to be defined before we can start developing ourselves as educators. The Merriam-Webster dictionary defines the educator as i) skilled in teaching ii) a student of the theory and practice of education and iii) an administrator in education. Do you identify yourself with these definitions? Educators are almost always experts that have studied and been trained in one or more disciplines. In the veterinary sector this may be a discipline in the basic sciences that underpins clinical work or a discipline that underpins disease and treatment processes such as pathology, microbiology or a clinical discipline such as medicine and surgery. Disciplinary experts may however not have studied or had any formal training in the knowledge (theory of education) and skills (practice) of education. Given
that many, and possibly most, veterinary graduates and disciplinary experts will at some stage in their career become involved directly or indirectly in education it is recommended that they should receive at least some training in the theory and practice of education.

Training in education
In many teaching institutions throughout the world educator development in higher education is managed through postgraduate teaching programmes and other short courses. Some training is ‘generic’, offering training in general theory and practice whilst other training is ‘discipline-specific’ (2). Although ‘generic’ training is usually adequate to gain theoretical knowledge of the elements of teaching and learning, in the veterinary sector it is necessary to impart specific skills and the ability to teach in diverse environments such as around a cadaver, in barns, stables and outdoors. In educational theory there is increased recognition that methodologies to address subject-specific teaching must be adapted and developed within particular disciplinary contexts (3). Each discipline has its own traditional pedagogies and methods that influence the characteristics and approaches to learning within their context and student community. To help distinguish between generic and discipline specific learning the term ‘pedagogical content knowledge of a discipline’ has been coined. It is clear that teaching and training in a discipline must utilize the ‘pedagogical content knowledge’ that have been developed over decades (or centuries) based on the experiences of teaching in that discipline (3).

The Educator as a learner
Before one can become an expert, professional educator it is necessary that one accepts and embraces the need to once more become a ‘learner.’ Being a conscious learner needs a mental adjustment and the use of mental faculties that most discipline experts may have more or less neglected. The experience of learning in the context of higher education is valuable and my lead to novel insights and understanding. Being a learner’ once again offers opportunities to evaluate and reflect on the new understanding regarding theories of learning. For example the concept and practice of ‘student-centred learning’ that is now at the core of most higher education may not have existed when you were a learner. Through reflection the learner can better understand how learning happens, the power of feedback, self-assessment skills, and learning through self-regulation. The insights and skills gained in turn will help trainee educators in developing their future students. Figure 1 illustrates how becoming a conscious and aware learner can help educators to develop themselves.

Figure 1 The experiential learning for a teacher as a learner (4)
Understanding the drivers that affect the educator

If you wish to become an educator it may help to consider what drives you and what are the factors that influence and motivate you? Figure 2 some of the outlines major factors that have been identified as influencing an educator. An awareness and thorough understanding of these influences may help you to critically assess your own limitations and strengths and then to identify the specific training needs that you require to be a more effective educator.

Of these factors the one described as professional identity is possibly the most important. Educators in the veterinary and paraveterinary sectors can be scientists, veterinary clinicians, veterinary nurses, or other para-veterinary professionals. Their primary external and self-perceived identity is usually not as an educator. Their professional identity is often viewed in the context of their disciplines and how their position is ranked in their discipline. It is important to realise that many educators in the veterinary sector do not consider teaching as their primary role. In this context three tension points that members of veterinary and other science departments may encounter in deciding whether or not be involved in changing and improving the quality of their teaching (5):

- Training should be focused primarily on a research identity and not a teaching identity
- Scientists are afraid to "come out" as teachers
- The professional culture of science considers teaching to be of a lower status than research.

To develop a cadre of highly skilled and enthusiastic educators it is therefore important to acknowledge and promote the ‘educator identity’.

![Diagram](image)

**Figure 2** The internal and external drivers that influence an educator (adapted from 6)

**Scholarship and research in teaching and learning**

As professionals with a science-based background we are accustomed to studying the natural world using inquiry-based quantitative research methodologies. However, when conducting research into teaching and learning it is often necessary to employ qualitative methodologies that are more usually seen in the social sciences. This can represent a challenge of understanding and culture for educators in the veterinary profession who are trained in the natural sciences (7). To be good educators we need to adapt our approach to scholarship, we need to be open to the insights and findings obtained from qualitative methods of research. Similarly, if as educators we see the need to engage in research we must be aware of the inherent constraints
and limits imposed by our subject matter and when appropriate consider the use of qualitative research methodologies.

**Self-Assessment through Reflective Practice**

Reflection for an educator is not ‘just thinking’ and not just introspection. Structured and critical reflection is a fundamental tool in successful development of an educator’s knowledge and teaching skills. Reflection in this context is ‘active, persistent, and careful consideration of any belief or supposed form of knowledge in the light of the grounds that support it and the further conclusions to which it tends.’ (8). Using a reflective approach is often the key concept in educator development in higher education (9). Developing a reflective approach requires the fostering of metacognitive skills (thinking about thinking) and an iterative approach. An outline of how to develop reflective skills and how to utilize the insights gained from reflection is given in Figure 3 Although this may look simple it is not an easy task. As an educator we can use 4 different lenses to evaluate and improve our practice and continue to develop as educators:

1) Our own perspective (reflection) 2) Our students’ eyes 3) Our colleagues’ eyes 4) Educational theories and literature

It is the combination of all these that we must use to develop ourselves as educators

**Keywords:** Educator Competence, Reflective Practice, Self-Assessment

**References**

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Summary
Integrated curriculum design, the modes of its delivery and the faculty development required to successfully support high quality student learning all require expertise in evidence-based, educational development. In medicine and veterinary medicine, various models now exist of academies that support core teams and reach out to the broader scientific and clinical veterinary community to support them in their collective education endeavours. One such model, used as a case study here, is the LIVE Centre for Excellence in Teaching and Learning at the Royal Veterinary College (RVC), University of London, UK. Since 2005, LIVE's purpose has been to provide leadership in pedagogical developments and to act as an “incubator” to help support all those interested in veterinary teaching and learning, and associated research. This has involved a multi-layered strategy, with core cross-curricular themes, initiatives supporting the revision of individual classes and courses, and faculty development through a portfolio of formal taught programmes and short course CPD. LIVE and similar centres elsewhere are becoming catalysts for the new breed of clinical teachers and educational researchers that are needed for modern veterinary curricula appropriate for the 21st century. Empowered by innovative teaching and learning methods, relevant to both medical and veterinary education, these teachers are starting to fulfil the need for educators who can truly meet the needs of modern society by helping students become rounded, confident and competent professionals.

Society's increasing demands and the responses of governments and professional bodies around the world are once more reshaping our profession. For instance, in the United Kingdom, the 21st century has seen formal recognition of veterinary nursing as a sister profession, related to the expansion of the veterinary team in recent years; the modernisation of the disciplinary processes for both veterinary professions; and an emphasis on professional skills alongside technical skills in the Royal College of Veterinary Surgeons’ new Certificate in Advanced Veterinary Practice. These developments parallel those taking place in the United States and in other parts of the world. With globalisation, as Brown and colleagues note, "international veterinary medicine has taken on a much broader definition and involves a variety of career paths in a number of disciplines." To cope with this reality, and respond to the remarkable changes which are happening in our world, it is imperative our graduates are equipped with "intercultural competence so that scientific advice and decisions can be compatible with the demands of a global marketplace.”(2)

In parallel with societal changes, the growth of knowledge and development of new technology, in all scientific fields continue to accelerate. The problem faced by any individual is no longer one of access to information but rather how to remain well-informed and up to date, without becoming overwhelmed, and the intelligent and effective application of that knowledge. Clients seek professionals capable of analysing and solving problems based on the sound judgment that must accompany sophisticated techniques. This change has implications for education generally, in terms of curricular design and delivery, but it particularly affects a discipline like veterinary medicine, in which, by definition, the broad comparative approach has facilitated the movement of knowledge between species and extrapolation from individuals to populations and from
one context to another.

In responding to these external pressures, educators face the additional challenge of assisting an increasing number of students who neither understand nor engage with the educational processes of higher education. Medical (3) and veterinary students (4) who are generally very well motivated and academically capable, seem paradoxically troubled, from their first day at university onward, by more challenging teaching and the pressure to adopt more adult learning styles. They are frequently resistant to changing learning habits that have previously been successful (at least in terms of grades); as a result, they experience demotivation, which may result, at its worst, in examination failure and even termination of their studies. Bender et al. have already recognized that young veterinary students learn best in a didactic environment, memorizing facts (5). Some students find it hard to adapt their learning styles to cope with new, interactive teaching methods and feel fundamentally challenged by small-group and team activities, problem-solving techniques, and dealing with “grey areas” of knowledge. Educationalists such as Marton and Säljö (6) have pointed out that there is a threshold that marks the difference between a learner reproducing knowledge and a learner seeking meaning. Even when facilitated by excellent teachers, it is a true challenge for learners to master this transition, which makes the difference between individuals functioning as “technicians” and individuals functioning as professional practitioners.

These three challenges – societal demands, the information explosion, and the need to deliver integrated, professional curricula that optimise skills development during the clinical veterinary programme – mean that it is incumbent on all universities to develop expertise in curriculum design and teachers who are experts in the delivery of modern veterinary curricula. One way of achieving this is through a clinical educational academy structure that supports the shift from a teaching-centred pedagogy to a learner-centred, andragogical approach, a concept on which general educational research has been focusing during recent years (7). One model for this is the RVC’s LIVE Centre which has as its mission the transformation of the veterinary and para-veterinary (or veterinary-related) professions by educating professionals who are capable and committed lifelong independent learners from induction to retirement. The clinical portions of veterinary and related programmes are of particular interest to LIVE faculty, because at this stage the learner needs to recognize serendipitous learning opportunities while at the same time contributing to a service team. For those who have been well prepared, this is an educationally rich and rewarding environment. Veterinary students are given the chance to acquire and apply knowledge and skills, in a relevant setting; if well prepared, as extensively discussed in Kolb’s work on experiential learning theory, they will be able to make use of concrete, “here-and-now” experiences and to test ideas, and will also be given feedback to change their practices (8).

LIVE acts as both a virtual and a physical meeting place for teachers, educational researchers, veterinary practitioners, and students who are jointly passionate about improving learning opportunities at all levels in the profession. Core LIVE faculty are veterinarians with expertise in education, adult learning, simulation technologies, and e-learning. They provide a dedicated team focused on educational development through approaches that are evidence based and delivered in a language that should be compelling for the full range of teachers, from basic scientists to specialist clinicians. This team is responsible for a number of “flagship projects” in recognition and reward of teachers, clinical and professional skills development, lifelong learning, work-based learning, and teacher development of faculty and staff at all levels. These are major educational themes of relevance to the RVC and to veterinary schools internationally. In all these developments, LIVE tries to address the real problems of veterinary education in its work - for example, the difficulties veterinary students face when “learning to think with their hands,” or the constraints on active learning created by large group sizes; in both of these examples, LIVE continues to exploit computer technology to develop and explore interactive teaching tools. Clinical practical skills are an intrinsic part of a veterinarian’s capability, and it is important that all students graduate competent in required “Day One” skill sets, as well
as possessing a degree of mastery of “Year One” and more advanced skills. Clinical skills centres incorporating simulations, ranging from simple models for intubation and catheterization to more sophisticated haptic devices, are facilitating the transition from classroom to clinic by developing a basic level of manual dexterity, creating confidence and reducing anxiety when the technique is first applied to a patient, reducing animal use (a welfare consideration) by inexperienced trainees, improving efficiency of learning in the clinics, and permitting, in some cases, more valid and reliable assessments to be reproducibly created for a whole student cohort. Broader professional skills are finally achieving the prominence that they deserve - in particular, communication skills, and clinical and ethical reasoning – and, in parallel with other veterinary schools in the UK and US, LIVE has steadily created and evaluated high-quality, ready-to-use educational materials for both undergraduate and post-graduate groups. However, more development in this area is imperative.

In the broader field of veterinary and medical education, it has become clear that LIVE is not, and should not be, an isolated example of a team and facility focused on educational development for clinical learners. In the United States, academies of medical education, with a range of different dedicated budgets and varying emphasis on staff recognition and reward, educational developments, and research, have been established in a number of medical schools, including those at Harvard Medical School; the Mayo Clinic; and the University of California, San Francisco Medical School. All these developments have led to the creation of a Medical Educators’ Academy Network (9). Unfortunately, progress seems to have been slower in the world of veterinary education than in the medical profession over the past few years. As Eyre states, colleges of veterinary medicine could be described as communities of academicians “doing their own thing,” loosely guided by a variety of committees and boards that are generally too preoccupied with rules and regulations to develop a collective vision or collaborative strategies (4). However, in meeting their responsibilities to all stakeholders – students, clients and broader society – it is important that the value of clinical educational academies, working collaboratively on integrated curricula in the interests of learners, is fully recognized embraced and celebrated.

**Keywords:** LIVE (Lifelong, Independent Veterinary Education), Educational Research, Medical, Academy, Innovative, Clinical Skills, Professional Skills, Faculty Development

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Where Do We Go from Here? Potential Changes for Veterinary Practices

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Summary
As with most businesses, veterinarians need to compete strongly on the basis of their least-substitutable service, which is their knowledge of veterinary medicine and their ability to advise pet owners on the basis of this expertise.

When examining practice revenue opportunities, the best prospects are associated with outpatient care in terms of preventive care (wellness), earlier detection of disease and pre-disease states, better management of chronic conditions, improving communication with clients (and thereby compliance), and enhancing customer service initiatives and making it easier for clients to do business with us.

The Current Situation
While many veterinarians focus on the impact of the current economy on financial metrics for their hospitals, it is clear that while the economy might play some role in the current lackluster performance of many veterinary practices, the root cause of these problems likely preceded the economic downturn by many years.

Some of the most profound findings of these marketplace studies have been the following:

- Pricing of veterinary services may be inappropriate given the ultimate value delivered, and with clients desiring more predictability in veterinary expenses
- There is a discrepancy between preventive care initiatives that practices say they recommend and what actually takes place
- Practices attribute poor compliance to client’s unwillingness to pay whereas noncompliance is typically associated more with the practice’s failure to make a firm recommendation or to convey the value or importance of a recommendation
- There is opportunity to increase demand for veterinary services, especially in preventive and chronic care, with cats being particularly underserved
- Veterinarians lack the business skills that could result in more appropriate economic success
- The supply of veterinarians does not closely match demand
- The economy impacts veterinary spending, but primarily by exacerbating existing issues

Predicting the Future
If the contention is correct that the economy is not the root cause of the current veterinary marketplace situation, then it is likely also correct that things will not automatically self-correct as the economy improves, even though any increase in discretionary spending will ultimately benefit small businesses, including veterinary practices.

There are a few situations that need to be considered when attempting to predict the future. Most of the
trends are evident from indicators already evident, projected cautiously into the future. Others can be predicted through foresight analysis.

In all likelihood, the factors that will have the most impact on the future of the profession are changes to specific aspects of the marketplace.

**Changes to the Economy**
It is not possible to predict the future of the economy with any precision, but some features seem to be enduring and are likely to continue. This includes the ongoing situation of income inequality and the fact that many Americans carry a significant amount of personal debt and often have constrained access to credit.

One of the challenges for the future will be the likely demographic cliff that we should be experiencing in the next few years. This is the point at which our prime customers, the baby boomers, will be leaving their peak spending years behind, but the next big generational wave, the Millennials, will not yet be ready to take up the slack in their pet healthcare purchases.

To help counteract some of these situations, veterinarians will likely need to concentrate of their valuation proposition, making it very clear where they provide value in the pet health care equation, they will need to promote standards of care as well as consistency of care, and they are going to need to be able to counsel pet owners in different risk management strategies (such as pet insurance).

**Changes to Technology**
When there is often a common refrain of “there’s an app for that” for almost any need, veterinarians are going to need to come to terms with the ubiquitous internet connectivity of clients today. This is likely to spur the need for new “virtual” business models for veterinarians to remain competitive in the marketplace.

Veterinarians and veterinary hospitals will likely struggle with how to meet client needs on a 24/7 basis, and manage their expectations accordingly.

**Changes to the Client Base**
The veterinary client of today and tomorrow may be significantly different than was the case when many veterinary hospitals initially opened their doors. While many practices were built on the expectations of the Baby Boomer generation, the average client of tomorrow will tend to be younger, more culturally diverse and more digitally skilled than most current hospital owners.

Since much veterinary and peer advice is available for free online, veterinarians might find it difficult to stay ahead of the curve, compared to clients with specific information needs. In fact, crowd sourcing of medical information has been commonplace in both human and veterinary medicine for many years.

Most of today’s clients could rightly be considered “omnichannel” consumers – they often collect information to help them determine a short list of what their needs are and the products or services that are most appropriate, they then collect additional information online from trusted sources, and they then make their decision of what, when and how to purchase. Might this affect how consumers also shop for health care services?

**Changes to the Profession**
The profession itself has not changed considerably over the years, with a few notable exceptions. Over the past forty years, the number of women veterinarians has blossomed so much that they represent the majority of veterinary students and, if this trend continues, they will constitute the majority of practicing veterinarians as well. Other than gender, though, there is very little cultural diversity within the profession,
unlike the situation in the general population.

Another concern is that with each passing year, it appears that fewer new graduates appear interested in owning and managing veterinary clinics. Given that there is an appreciable number of practices owned by baby boomers who will be retiring in the next several years, this could constitute both a challenge and an opportunity for the profession.

**Changes to Hospital Services**

It appears clear that veterinary practices need to take more of an interest in business practices, especially reviewing financial data with some regularity, improving client communication skills, and focusing on customer service initiatives. We also need to re-visit our pricing, our marketing, and our payment options to better articulate our role as “solutions providers” rather than just purveyors of veterinary services.

One revenue center that has seen more than its share of outside competition is dispensing. Many of the medications used by veterinarians are now available at less client cost from retail outlets and pharmacies, so also would lead to less foot traffic in veterinary practices. As long as veterinarians choose to preferentially dispense products for which substitutes are readily available at substantially lower prices, such client defections will likely continue unabated.

In a future with more marketplace competition, veterinary hospitals need to stock, recommend and dispense premium differentiated products. While not every product or service needs to be profitable, if it is not profitable on its own, it will need to drive other goods and services that are profitable.

**Keywords:** Omnichannel Consumer, Demographic Cliff, Preventive Medicine

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Pet-Specific Care: A Lifetime of Personalized Pet Care

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Summary
Lifelong Care is a practice philosophy in which veterinary care is transformed from a reactive model to a more proactive version in which veterinarians provide solutions rather than just services, and pet owners become more engaged with veterinarians in the pet care process. Lifelong Care encourages active, ongoing veterinary care throughout a pet’s life as a continuum of care, rather than just a passive transaction-based process. The approach can result not only in happier, healthier pets, but also healthier families, practices and communities. The strategy of Lifelong Care depends on three main areas of engagement – prevention, detection, and treatment – preventing problems consistently in practice; detecting problems earlier, preferably when they are still subclinical and when there are typically the most options for intervention, and; managing problems appropriately given their anticipated treatment course. Standards of care can then be created, reflecting the particular goals and aspirations of each individual veterinary practice.

The Need
All veterinarians intend to practice the highest quality of veterinary medicine possible, but compliance studies demonstrate that this is not always the case. Animals continue to contract infectious diseases, even when highly effective vaccines exist, they get parasites despite the widespread acceptance that all pets should have year-round parasite control, diagnoses are often not made until a pet has overt clinical signs of an illness, and even well-understood chronic diseases like atopic dermatitis and osteoarthritis are sometimes treated with an on-again, off-again treatment regimen, despite their lifelong timeline.

Accordingly, it makes sense for veterinarians to embrace a lifelong care model of prevent-detect-treat, in which diseases that can be prevented are prevented, especially through comprehensive vaccination and parasite-control protocols, diagnostic testing is done as part of a sensible surveillance system, preferably even identifying animals in a subclinical state when there are the most options available for management, and finally, treating animals over an appropriate time period. In this manner, acute diseases may be treated over days or weeks, while chronic problems, such as osteoarthritis, atopic dermatitis, diabetes mellitus and many others are managed on a continuum of care over the remainder of the pet’s life.

To be successful in this regard, standards of care are critically important for hospitals. While personal freedom for veterinarians to treat as they wish is aspirational, it does not allow practices to deliver consistent quality of care to pets and their owners. At least when it comes to basic preventive care, there is value in determining protocols for vaccination and parasite control, and care pathways for the best evidence-based approaches to the most common chronic conditions managed by the practice, such as osteoarthritis, atopic dermatitis, periodontal disease, etc. Diligence is particularly important for these chronic diseases, since how these conditions are managed when an animal is young will greatly affect its quality of life as it gets older.
Hospitals should endeavor to codify best practices that are common to all veterinarians in a practice and based on the most current standards of care available (www.lifelongcare.com). These standards need to be periodically reviewed and updated as new evidence becomes available. Pet owners want veterinarians to provide them with health guidelines in accordance with their pets’ actual needs, so adopting and implementing guidelines, protocols, and evidence-based care pathways allows the veterinary practice team to satisfy this desire of pet owners while simultaneously better meeting practice revenue objectives. A suitable starting point is to consider what risk factors might influence the decision-making process through use of health risk assessments, which involve client-focused questionnaires, taking a thorough medical history, and performing a skilled physical examination. The process can then continue by evaluating which strategies should be employed for prevention, detection, and treatment for an individual pet. Ongoing monitoring of the process is critical to determine when and where gaps in compliance are compromising the anticipating quality of care provided by the hospital.

THE FOUNDATIONS OF LIFELONG CARE

Prevention
Preventive medicine is a core competency of the veterinary profession. Our major preventive strategies include vaccination, screening for early indicators of disease, parasite control, optimal nutrition and physical activity for each life stage, behavior counseling, sensible exercise programs, breeding recommendations (to help prevent hereditable conditions), optimal spay/neuter timing, oral hygiene, and even counseling on pet selection to minimize the risk that a pet will later be relinquished to a shelter, abandoned, or euthanized for non-medical reasons.

Currently, many pet owners only associate the need to see a veterinarian with vaccination or serious illness. This failure to grasp the true value of preventive medicine and regular lifelong care and the positive impact both can have on pets and pet owners can adversely affect the health of pets and the financial health of veterinary practices.

Another troubling trend is a belief held by many pet owners and some veterinarians that certain preventive practices, such as vaccinations or parasite preventives, may be inherently risky or unsafe. Vaccination is actually associated with minimal risk and should be part of routine preventive health care.

Each year, despite the availability of highly effective parasiticides and treatments, thousands of cases of parasitism are still diagnosed, with many more not identified or reported. Fleas, ticks, and other parasites may be regarded as a “nuisance” by the pet owner, but some can also transmit diseases between pets and people. In addition, heartworm infection and disease is certainly not a new threat, and yet, despite recommendations from the American Heartworm Society and Companion Animal Parasite Council, many dogs and cats still fail to receive appropriate prophylactic medications for this entirely preventable infection. Year-round protection encompassing both internal and external parasite control is now recommended for all dogs and cats. From a cost perspective, preventing the problem is always less expensive for a pet owner than treating a parasite infection or infestation; hence, the old adage “an ounce of prevention is worth a pound of cure.”

Detection
The second pillar of lifelong care is the early detection of a disease or disorder while the condition is still subclinical and the pet is “well.” A comprehensive history, physical examination, and appropriate periodic diagnostic screenings are the key components of detection. Diagnostic screening might also include genotypic (e.g., DNA testing) and phenotypic testing (e.g., using actual physical expression, such as radiographic assessment for hip dysplasia) for heritable medical issues. A good patient history is important not only
as a diagnostic tool, but also to discern the client’s needs and expectations.

Diagnostic screening tests can provide baseline values and facilitate long-term monitoring to establish trends that may help to identify subclinical disease. Without early detection and management, many of these conditions can lead to a significant decrease in a pet’s quality of life. Clearly, such periodic testing of otherwise healthy animals is indicated to help identify affected individuals before there are clinical manifestations evident. Selecting appropriate tests can be facilitated by performing health risk assessments periodically and screening for conditions that might be considered at higher risk because of breed predisposition, family history, lifestyle, or geographic considerations.

Treatment
The third component of lifelong care centers on the medical management of conditions. Early therapeutic intervention has been shown to offer the best chance of successful long-term management of many conditions. Clearly distinguishing between curing a medical condition and long-term management is important when discussing the many benefits of intervention and management of disease states with pet owners.

One component of health management is the systematic and organized approach to providing care for such chronic conditions as osteoarthritis, atopic dermatitis, and diabetes mellitus, to name a few. If the veterinarian has not communicated the benefits of lifelong care or nor established an ongoing relationship with the pet and owner, care may not be sought until late in the course of a disease.

Early intervention in primary conditions can also reduce the risks of secondary problems. Periodontal disease is among the most common condition affecting dogs and cats, yet it is often ignored by pet owners or under treated by veterinarians. When existing periodontal disease is comprehensively managed, the risk of debilitating sequelae is often reduced.

In addition, prevention, detection, and treatment of pain should be provided to all patients. Through this process, patient quality of life, pet owner satisfaction, and perceived value of veterinary care are more likely to improve while patient stress, recovery time, and potential for exacerbation of co-morbidities will likely decrease.

Keywords: Client-Centric Care, Lifelong Care, Early Detection

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Achieving Hospital Goals Together - The Importance of Alignment

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Summary
Running an efficient and effective veterinary hospital doesn't happen by accident. It takes all staff members working collectively to achieve hospital goals. Perceptions sometimes differ between staff members and management about the direction in which the hospital needs to go, but hospital goals only get achieved when attention is paid to the appropriate factors.

Alignment is so critical for a variety of reasons not the least of which is that without alignment, it is impossible deliver on a unified mission and vision to pet owners. Also, in most veterinary hospitals, profit margins are so tight that there is no room for misalignment --- salaries and jobs are at risk.

The Hospital Situation
Most veterinary owners are “reluctant leaders”. They like the concept of “being the boss”, but not necessarily leading. Accordingly, many veterinary hospitals do not have established reporting structures and care becomes inconsistent when there is not a senior “champion” for ensuring a level of care is delivered for every client visit. When there is a lack of clear leadership, the typical result is that the hospital relies on gimmicks (such as production or bonuses) to motivate employees to be doing what they are supposed to be doing.

If staff are to believe that their practice is different than the others in the area, then differentiation becomes an important concept to embrace. The hospital must first define and then celebrate these distinctions, realizing that the differentiated practice may not be ideal for every client. In the real world, consumers have lots of choices, such as the type of car they drive, the type of restaurants they prefer, where they shop for clothes, etc., and it is quite possible that the newly-differentiated practice might be a better fit for some clients, but not necessarily for all of them. However the practice is differentiated, it will be important to be able to measure and illustrate the value that the practice offers its clients. To fully embrace this concept, it is important to understand the “core” market sought by the practice, and not to panic when clients who don’t want that type of practice seek a better fit elsewhere. If hospitals don’t provide this differentiation, then clients are likely to differentiate themselves, and they most often do this based on price.

To have a well-defined hospital vision and mission is fine, but to reflect this to staff and clients requires the practice to have shared goals and expectations. To make this work, staff need to appreciate that it’s not just about the medicine – it’s about the total client experience and clients receiving value for their money spent. It’s not just about individual excellent doctors, technicians and receptionists – it’s about individuals functioning as a team to deliver excellent care and ensuring that things don’t fall through the cracks. It’s also not just about meeting client needs – it’s about delivering care in a way that meets hospital goals as well. Like any successful business exchange, there needs to be a commitment to the concept of “win-win”.

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Accountability is a critical attribute in the functioning of a veterinary hospital. While it might at first appear that it is the doctors that are responsible for the perception created by a practice, careful review of experiences both positive and negative show that all staff – doctors, technicians, receptionists and managers all have an important role to play in how the practice is perceived by the world outside the walls of the clinic.

For a hospital to function at its best, every employee must be accountable not only for their own duties, but to deliver on implied promises to all stakeholders regarding the mission and vision of the practice. Without accepting “ownership” of all responsibilities in this regard, a practice will need to rely on only its medical skills. With competition for veterinary services increasing regularly, and with the very high cost of delivering those services, being second best can be a costly demotion.

While it is easy to brush off the rants of a single client, in veterinary practice this can have a decidedly bad cascading effect. Happy clients tell a few of their friends about a good experience, but unhappy clients share their displeasure with many more. Clients will only accept so many negative stories before they consider their other options.

It's impossible to guard against any negative experience, but staff that are empowered and accountable are much less likely to create scenarios that get blown out of proportion, and can usually easily document their good-faith efforts to make things right. That's really all that anyone can hope for.

It's important to avoid artificial boundaries within the practice so that the practice identity, vision and mission won’t become clouded. There might be distinctions between departments, issues between shifts, tension between front-office and back-office staff, and many other instances in which personal accountability is lost or camouflaged. This cannot be allowed to fester in a practice, or it can have long-time deleterious consequences on business potential.

To some extent, personal accountability tends to lapse in any organization in which anonymity is possible. So, the challenge with veterinary hospitals is to make accountability a scalable attribute. People voluntarily must maintain their accountability even as staff numbers grow, and everyone must tie their collective success on each other stepping up to always do the right thing … and then be prepared to ask what else they can do to keep things moving in the right direction.

As was mentioned from the very start, running an efficient and effective veterinary hospital doesn’t happen by accident – it takes planning. The fundamental design of a strategic planning initiative is to deliver exceptional medical care and client service in a team-based fashion, pay staff appropriately for the value they deliver, pay employee veterinarians appropriately for the value delivered (and owners a fair return on investment), and then charge clients appropriately for the value they receive. In some cases, this might mean adding services, in others in might mean changing the way services are delivered, and in other cases in might suggest discontinuing services that cannot be delivered profitably (or those for which clients aren't prepared to pay).

In a well-designed strategic plan, there is a division of duty between the management team (the trained practice management consultant, owners, and hospital manager/administrator) and the hospital staff that will be implementing the changes.

Once a core group of actionable missions have been created, the value of which is evident to all stakeholders, then specific plans for implementation should involve staff. In many cases, teams are the most effective way to ensure the success of the process. In many ways, this is more like acting as captain of a ship rather than the dictator of a small empire. Management determines the destination for the voyage, and
the staff explore options for the best way to get there, acting on smaller pieces of the strategy.

In any strategic action plan, it is important to regularly determine whether the plan is still on target, whether the stakeholders are all happy with the program, and whether the target is still desirable. Strategic plans are fluid and flexible, and some plans may not be able to be successfully implemented. The most important aspect is to be responsive to the needs of clients and to alter programs accordingly.

**Keywords:** Alignment, Accountability, Strategic Planning

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Is Practice Ownership Worthwhile?

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Summary
A veterinary practice is a place where dedicated staff members work to provide important health care for pets, but it must also be a going business concern and provide a sufficient return on investment to justify the considerable investment. If pet owners want to receive the most current and appropriate care for their pets, and if veterinary staff expect to be compensated fairly in return for their expertise and efforts, then everyone needs to be concerned with a hospital's sustainability and return on investment. The good news is that profitability and the delivery of great medicine are both achievable.

Veterinary practices are places where medicine is conducted, and so it is sometimes difficult to view them as investments... and yet they are. Sometimes, practice owners can even try to convince themselves that veterinary practice is different than every other business on the planet, and doesn't conform to the same rules of business and economics. There is also, sometimes, the wishful thinking that as long as there is enough revenue to ensure that everyone gets paid that the hospital is doing just fine. Are any of these true?

Investing in a Veterinary Hospital
For anyone to invest in a veterinary clinic, it should pay back the investor as least as much as the investor could have gotten from another investment of similar risk (opportunity cost). In fact, the investment value of any business equals the amount of the return (profit) that the business provides to its owner(s), divided by the rate of return expected on such an investment. We expect that a very safe investment does not provide much return, but as investments get riskier, we expect a higher potential return, commensurate with the risk we are prepared to take.

\[ \text{Value} = \frac{\text{Benefit Stream}}{\text{Rate of Return based on risk}} \]

So, if a veterinary clinic is a business like any other, it needs to generate profits commensurate with the risk of actually realizing those profits. This is not only important when the investment is initially made, but when the owner is contemplating selling the business and wants to recoup some measure of investment value from the next potential buyer. Accordingly, some sellers are in for a rude awakening when they go to sell their practices and realize those practices are worth less than they thought...typically because of inadequate earnings.

Owners of veterinary clinics must strive to maintain profit in their practices, to preserve the value of their investment. It is not enough just to be able to pay the bills. The owner of a veterinary practice is certainly entitled to fair compensation of the basis of their production, but also for management duties, and a variety
of perks associated with ownership. However, they are also entitled to a Return on Investment (ROI) that should be every bit as much and hopefully more than an investment in alternate opportunities with similar risk profiles.

**Veterinary Practice – An Expensive Undertaking**

Veterinary practices often concentrate on revenue, but understanding where the money goes is also important, since the difference between the two is profit. Given the inherent risks in a small business such as a veterinary practice, the expenses can eclipse the needed profit potential if practice owners and managers aren’t careful.

A large part of offering veterinary services is the staffing component for the hospital. While most veterinary staff are continuously busy throughout the day, it is still almost impossible that every minute is directly billable to one client or another. There are many “non-billable” gaps in the day, including communicating with clients on the telephone, cleaning, and time between appointments or procedures and the time needed to complete medical records. It is not unusual that less than 50% of a veterinarian’s time in any given day is directly billable to specific clients, and typically about half that for technicians. It is also critical to remember that many staff members are not directly billable (e.g., receptionists, managers), and so a pro rata share of all non-billable labor must be carried by those providing billable services.

The overhead for a veterinary practice includes all the basic expenses associated with the facility (such as rent, utilities, insurance, etc.). Overhead can be calculated for the entire practice, or there can be individual overheads for each profit center in a hospital. Another approach is to set a defined profit margin, and given billable and non-billable labor rates, equipment, and materials, overhead can be calculated as a “plug”, the amount left over from total expenses when all other expenses have been calculated.

The purpose for acknowledging overhead is that it can be an invisible expense for both doctors and staff. When performing procedures within the hospital, it is important to remember that along with products and direct services provided, charges must also include an amount suitable for covering such fixed expenses as rent, utilities, telephone service, insurance, and many others.

Materials that are used up in the process of offering a service must be included in the pricing, together with the “invisible” indirect costs of the products. The indirect costs are often significant and likely average about 20-45% of the direct costs in most practices. The indirect costs include ordering costs (employee’s time spent buying the product, receiving and unpacking the orders, and putting the product on the shelves) and holding/carrying costs (the value tied up in inventory, personal property taxes paid on the inventory, wastage, insurance costs, theft, etc.). In some practices, product theft or diversion (often known as “shrinkage”) can be significant enough that the indirect costs surpass 50% of the direct costs.

**Profit in Margin Pricing**

There are many definitions for profit, but in margin (cost-plus) pricing, the profit is the actual value (often expressed as a percent) that is added to the equation as the desired return on investment. It is important to note that the profit margin must be “off the top” or the amount remaining will not cover the underlying costs. The same holds true for production-based compensation. If it is not calculated correctly, the profit margin will not yield the desired return.

To claim a fixed percentage profit of the final client charge (C) the equation needs to be set up as

\[ C = \frac{P}{1 - \text{Margin}} \]

The true costs of providing veterinary services, is often shocking, given that most veterinary practices undercharge for their services. This is a chronic problem and translates not only to the relatively low wages that veterinarians earn, and trying to capture profit from retail services such as pharmacy, but also affects
what they can afford to pay paraprofessional staff, receptionists and practice managers.

Calculating fees with costs and profit in mind will help veterinarians charge appropriately, or to stop performing services for which they cannot be adequately compensated to cover costs and make a fair return on investment.

**Is an Emphasis on Profit Wrong?**
It’s important to keep the concept of profit in perspective. The main purpose of any business is to serve its customers. Clearly, a veterinary practice does this by providing pet health care services to clients. Profit comes from doing this well and charging appropriately. Herein lies the problem – veterinary practices typically focus on delivering excellent health care services, but not necessarily on charging appropriately to ensure adequate profit.

Success in veterinary practice requires being mindful of client needs, but also having systems in place to ensure that as those needs are being met, adequate profit is also being generated for the practice.

Focusing on profit is like playing a sport by looking at the scoreboard. This is obviously not a good strategy. So, what needs to happen is that veterinary hospital owners need to understand what it costs them to deliver the services they provide and price them appropriately so that if the level of care is delivered as intended that profit is as well. So, to be able to do this, hospitals need to know their cost structures and where they are making adequate profit... and where they are not. Veterinary practices have the potential to be great investments if these principles are considered and applied.

**Keywords:** Return on Investment, Valuation, Profitability

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Alterations of Perfusion Parameters in a Canine Septic Shock Model

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Purpose
In order to define useful monitoring criteria and resuscitation goals suitable for canine septic shock, hemodynamic values and perfusion factors were continuously monitored from canine polymicrobial septic shock model.

Materials and Methods
Five purpose-bred Beagles were anesthetized with zolazepma/tiletamin induction (10 mg/kg IV) and isoflurane maintenance (1.5%). Under complete anesthesia, septic shock was induced by autologous fecal infection. Perfusion factors including oxygen extraction ratio (O₂ER), central venous oxygen saturation (SvO₂), lactate, and base excess (BE) as well as hemodynamic (cardiac and volumetric) parameters were analyzed. All animal procedures were approved by the Institutional Animal Care and Use Committee of Chonnam National University (CNU-IACUC-YB-2013-47).

Results
Low systemic vascular resistance index and high nitric oxide level were observed at early distributive shock. In the terminal stage, cardiac output decreased and cardiac troponin-I increased at the same period. O₂ER gradually increased following progress of septic shock. SvO₂ and BE had a strong correlation (r=0.923), but lactate was not correlated with SvO₂ and BE. Main causes of BE reductions have been identified as non-lactate unmeasured anion and chloride by physicochemical analysis.

Conclusion
Hemodynamic and perfusion alterations in septic shock were observed from the canine polymicrobial peritonitis model. Increased vascular permeability affected the hyperdynamic state in the early stage, whereas myocardiac dysfunction was linked to the refractory hypodynamic state in the terminal stage. BE with non-lactate unmeasured anion can be a more useful diagnostic marker and therapeutic goal than lactate in canine septic shock.

Keywords: Septic Shock, Canine, Base Excess

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Balloon Technique of the Intra-Abdominal Hypertension in Conscious Dog Model

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Purpose
To evaluate the effect of intra-abdominal pressure (IAP) on cardiovascular, respiratory, and arterial blood gas values in a conscious dog model that used a balloon technique to generate intra-abdominal hypertension (IAH).

Materials and Methods
A new balloon device designed for this study using a Foley urinary catheter and latex balloon was placed in the intra-abdominal cavity. Consecutive IAP were measured by measuring the intravesicular pressure. The abdomen was inflated with air to IAP levels of 10, 15, 20 and 25 mmHg. Heart rate, respiratory rate, systolic arterial blood pressure, and arterial blood gas were evaluated at baseline and at 15, 30, 45, 60, 120, 240, and 300 minutes after IAP elevation.

Results
The air insufflated into the intra-abdominal balloon device significantly increased the IAP and sustained the IAH. Respiratory rate increased significantly (p<0.05) when IAP was increased to 15, 20, and 25 mmHg. Although heart rate, systolic arterial blood pressure, PaO₂ and PaCO₂ did not show statistically significant differences between baseline and post-treatment values over time, the dogs with increased IAP showed a distended abdomen, discomfort, and 4/6 (67%) vomited. After measurement of IAP, air was removed. There were no side effects after removal of the balloon device.

Conclusion
The balloon device was successfully insufflated and sustained IAH in conscious dogs. This balloon technique does not require general anesthesia for instillation or removal of gas after installment. An acute IAP increase in normal conscious dogs induced discomfort and vomiting, as well as increased respiratory effort.

Keywords: Balloon Technique, Intra-Abdominal Hypertension, Intra-Abdominal Pressure

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Comparison of Thromboelastography and Prothrombin Time for Therapeutic Monitoring of New Oral Anticoagulant (NOAC), Rivaroxaban, in Dogs

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Purpose
To suggest clinical alternative methods for determining rivaroxaban concentrations, thromboelastography (TEG) values using various activators and point-of-care prothrombin time (PT) were compared to plasma rivaroxaban concentration using chromogenic anti-Xa assay after oral rivaroxaban administration in a healthy Beagle dogs.

Materials and Methods
Four different dosages of rivaroxaban (0.5, 1, 2, 4 mg/kg) were administered orally to six healthy Beagle dogs. Citrated blood was collected after 3h of administration and analyzed within 2hours of collection. Plasma rivaroxaban concentration was determined by chromogenic anti-Xa assay. Point-of-care PT and TEG with 4 activators were performed: Rapid TEG; Ca²⁺ with 1:100 tissue factor (CTF100); Ca²⁺ with 1:3700 tissue factor (CTF3700); Kaolin.

Results
Anti-Xa determined rivaroxaban concentration and point-of-care PT had a strong correlation (r=0.884). R time in Rapid TEG-TEG (r=0.812), ACT value in Rapid TEG-TEG (r=0.812), and R time in CTF100-TEG (r=0.782) showed a significant correlation with rivaroxaban concentration measured by anti-Xa assay.

Conclusion
TEG using strong activator and point-of care PT can be used for therapeutic monitoring of rivaroxaban in dogs. Oral rivaroxaban administration has predictable anticoagulant effects and is well tolerated by healthy dogs.

Keywords: Rivaroxaban, Thromboelastography, Prothrombin Time, Anti-Xa, Dog

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Ionized Serum Magnesium in Hospitalized Dogs
- Prevalence and Clinical Implications

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Purpose
To describe the incidence of abnormalities in ionized serum magnesium levels of hospitalized dogs at a veterinary teaching hospital and to determine whether altered magnesium levels is related to specific diseases, electrolyte disturbances, or mortality.

Materials and Methods
Data of 537 dogs of ionized serum magnesium levels was retrospectively analyzed to determine prevalence rate, associated disorders and electrolyte abnormalities, and survival rate.

Results
Two hundred and thirty-six out of 537 hospitalized dogs had ionized serum magnesium abnormalities. The prevalence of hypermagnesemia in the study population was 5.2% (28 of 537 dogs) and the mortality rate was 64% (18 of 28 dogs). Hypermagnesemia was associated with abnormal calcium, and was found in dogs with renal disease, hemolytic disorders, thrombolytic disorders, neoplastic diseases or pancreatitis. The incidence of hypomagnesemia was 38.7% (208 of 547 dogs) and the majority of dogs had renal and gastrointestinal disorders but the diseases were not specific. Results from 208 hypomagnesemia cases indicate that the most significant risk factors of hypomagnesemia were serum potassium level (p<0.001; OR = 0.75) and serum calcium level (p<0.001; OR = 0.05).

Conclusion
Ionized serum magnesium abnormality is common in hospitalized dogs. Hypermagnesemia is associated with certain diseases and related to high mortality. Meanwhile, hypomagnesemia is related to electrolyte disturbances but there are more than generally known disorders related to magnesium alteration. Routine measurement of ionized magnesium in hospitalized dogs is recommended and may imply prognostic information to clinicians.

Keywords: Ionized Magnesium, Electrolyte, Dogs, Hypermagnesemia, Hypomagnesemia

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Nonsteroidal Anti-Inflammatory Drug (NSAID) Use to Reduce Behavioural Signs of Pain in Piglets Undergoing Surgical Castration

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Purpose
Millions of boar piglets in North America are surgically castrated each year, to minimize boar taint and aggression. EU legislation and Canadian guidelines require that piglets be provided with appropriate analgesia to control post-procedural pain. NSAIDs are the most practical analgesic to administer on-farm; however there is little evidence that they sufficiently mitigate castration pain. The objective of this study was to assess the efficacy of meloxicam (Metacam; Boehringer Ingelheim, Burlington, ON, Canada) and ketoprofen (Anafen; Mérial Canada, Baie d’Urfé, Quebec) in reducing pain in castrated piglets, using validated behavioural scoring techniques.

Materials and Methods
Fifteen litters of 5 day-old piglets (n=80) were used and boar piglets were randomly assigned to one of eight possible treatments: 0.4mg/kg meloxicam (castrated or uncastrated), 1.0mg/kg meloxicam (castrated or uncastrated), 6.0mg/kg ketoprofen (castrated or uncastrated), saline-castrated, or sham-castrated (n=10 piglets/treatment group). Piglets were video recorded for 1h pre-procedure, immediately post-castration for 8h and for another 1h, 24h post-procedure (10h total). Twenty-one behaviours were scored continuously for the first 15mins of every hour. Data was analyzed using a mixed model ANOVA with repeated measures and a post-hoc Tukey test.

Results
Castrated piglets were significantly less active 3h and 4h post-castration (p= 0.0007 and p<.0001, respectively) while uncastrated piglets had unvarying levels of activity throughout the observation period. Castrated piglets also displayed significantly more tail wagging and pain-related behaviours than uncastrated piglets (p<.0001). None of the treatments significantly reduced pain behaviours.

Conclusion
Meloxicam and ketoprofen are not effective in alleviating castration-associated pain in neonatal pigs.

Keywords: Analgesic, Animal Welfare, Piglet Castration, Pain, Refinement

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Perioperative Use of Low-Dose Intravenous Acepromazine in Dogs: 1068 Cases

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Purpose
To evaluate the perioperative effect of low-dose intravenous acepromazine in clinically healthy dogs.

Materials and Methods
The anesthetic records of dogs receiving intravenous acepromazine for general anesthesia from September 2007 to September 2015 were reviewed at the Veterinary Medical Teaching Hospital of Seoul National University. A total of 1068 dogs with American Society of Anesthesiologists physical status classification I-II were included. The dosage was titrated through repeated intravenous bolus injections of 0.01 mg/kg. A dose of less than 0.01 mg/kg was used in large breed dogs, geriatric patients, or dogs with mild coexisting disease. Groups were established according to the administered dose (G0, G1, G2, G3; <0.01, 0.01, 0.02, >0.02 mg/kg, respectively). When necessary, benzodiazepines (midazolam or diazepam) were added for stable anesthesia with minimal cardiovascular depression.

Results
Acepromazine was used with dosages ranging from 0.0025 to 0.05 mg/kg, and the low-dose (≤0.02 mg/kg) acepromazine was used in approximately 98% of all cases (G0 = 25%, G1 = 61%, G2 = 12%). Benzodiazepines were added in 18% of cases, and hypotension requiring the use of inotropic agents was observed in 5% of cases.

Conclusion
Acepromazine has been commonly used at various doses (0.05-0.2 mg/kg) because of its relatively large safety margin. However, titration was required due to the possibility of dose-dependent cardiovascular depression and the lack of an available antagonist. This study demonstrated that lower doses (≤0.02 mg/kg IV) could be sufficient for stable general anesthesia in clinically healthy dogs.

This research was supported by the Basic Science Research Promotion program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2011-0007777, NRF-2016R1D1A1A09919546), and the BK21 PLUS program and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University.

Keyword: Acepromazine, Dog, Lower Dosage, Perioperative Effect, Sedation

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Acute Postoperative Hypertension after Operation of Cervical Disc Disease in Two Dogs

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Purpose
To present a case regarding the development of acute postoperative hypertension after surgery for cervical intervertebral disc disease in two dogs.

Materials and Methods
Dog 1 (19.5 kg, 15-year-old, intact male Beagle) and dog 2 (5.2 kg, 8-year-old, castrated male Pekingese) visited for paresis caused by cervical intervertebral disc disease, and received the ventral slot technique at the second and third intervertebral space, respectively. Dog 1 was anesthetized with intravenous diazepam (0.1 mg/kg), fentanyl (4 μg/kg/hr), and alfaxalone (1 mg/kg), and dog 2 with intravenous midazolam (0.2 mg/kg), fentanyl (4 μg/kg/hr), and propofol (6 mg/kg). Both dogs were maintained with isoflurane in oxygen and there were no abnormalities during both surgeries.

Results
After extubation, systolic BP abruptly increased to greater than 250 mmHg in both dogs. Even though the dose of fentanyl was increased and other analgesics (hydromorphone [0.025 mg/kg IV] in dog 1, ketamine [0.6 mg/kg/hr] and lidocaine [3 mg/kg/hr] in dog 2) were added, BP could not be controlled. Subsequent use of vasodilators (sodium nitroprusside [1 μg/kg/min] in dog 1, hydralazine [0.4 mg/kg IV] in dog 2) resulted in a gradual decrease in BP. The acute postoperative hypertension was regulated 14 hours after extubation in dog 1 and 23 hours in dog 2.

Conclusion
Acute postoperative hypertension, which can be refractory to analgesic treatment, may occur after ventral slot surgery, and subsequent use of vasodilators may help correct the hypertension. Since chronic hypertension (systolic BP greater than 180 mmHg) can induce end organ damage, vasodilators can be administered when the analgesic treatment has no effect on BP control.

This research was supported by the Basic Science Research Promotion program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2011-0007777, NRF-2016R1D1A1A09919546), and the BK21 PLUS program and Research Institute of Veterinary Science, College of Veterinary Medicine, Seoul National University.

Keywords: Cervical Disc Disease, Dog, Postoperative Hypertension, Vasodilator, Ventral Slot

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Muscular Rigidity after Administration of Butorphanol in a Dog with Pre-Existing Intracranial Disease

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Purpose
To describe the cause of muscular rigidity after administration of butorphanol in a dog with intracranial disease.

Materials and Methods
A 13-year-old, castrated male Yorkshire terrier with a history of seizures was referred to the Veterinary Medical Teaching Hospital of Seoul National University. The extracranial causes of seizure were ruled out and magnetic resonance (MR) scan of the brain was scheduled. The patient was pre-medicated with intravenous butorphanol (0.1 mg/kg) and midazolam (0.2 mg/kg). Anesthesia was induced with propofol (6 mg/kg IV) and maintained with isoflurane in 100% oxygen. Heart rate, direct blood pressure, respiratory rate, pulse oximetry and end-tidal carbon dioxide were monitored.

Results
Injection of butorphanol resulted in forelimb muscular rigidity and lateral recumbency. In addition, a seizure was observed 20 minutes after the onset of rigidity, which stopped after injection of midazolam (0.2 mg/kg IV). No additional events were observed during the maintenance and recovery periods. The MR scan revealed severe ventriculomegaly, a mass on the left frontal lobe and syringohydromyelia at the cervical level.

Conclusion
Muscular rigidity is a rare adverse effect of \textmu-oid receptor agonists, and patients with neurological conditions are more vulnerable to this complication. Since recent reports have suggested that butorphanol may also function as a \textmu agonist, and it was the only drug that was injected prior to the event, the muscular rigidity observed in this patient was suspected to be caused by butorphanol. Therefore, careful monitoring is required when using butorphanol in patients with neurologic compromise.

This research was supported by the Basic Science Research Promotion program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2011-0007777, NRF-2016R1D1A1A09919546), and the BK21 PLUS program and Research Institute of Veterinary Science, College of Veterinary Medicine, Seoul National University.

Keywords: Butorphanol, Dog, Rigidity

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Hypertensive Crisis During Functional Carotid Body Tumor and Thyroid Carcinoma Resection in a Dog

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Purpose
To present a case regarding the development of hypertensive crisis during carotid body tumor resection in a dog.

Materials and Methods
A 12-year-old, castrated male Beagle dog presented for cervical masses. Thyroid neoplasia and submandibular tumor were diagnosed by computerized tomographic imaging. Right thyroidectomy and submandibular mass resection were planned. The dog was premedicated with intravenous (IV) cefazolin (22 mg/kg), diazepam (0.1 mg/kg), and a constant rate infusion (CRI) of remifentanil (6 ug/kg/hr). Arterial catheterization was performed before induction for blood pressure (BP) monitoring. Upon external manipulation near the submandibular mass, systolic BP increased to greater than 200 mmHg. Anesthesia was induced with alphaxalone (2 mg/kg, IV) and maintained with isoflurane in oxygen.

Results
Right thyroidectomy was unremarkable, but intraoperative manipulation of the submandibular mass resulted in hypertensive crises followed by episodes of tachyarrhythmias. A CRI of sodium nitroprusside and IV esmolol were administered to control the hypertension and tachyarrhythmias, respectively. Although esmolol was effective in managing the tachyarrhythmias, BP was difficult to control with sodium nitroprusside and could only be managed by discontinuing manipulation. Surgery was resumed with vigilant monitoring for hemodynamic instability. The dog recovered from anesthesia without any evidence of end-organ damage. Histopathology revealed a mixed thyroid carcinoma and carotid body tumor.

Conclusion
Although rare, carotid body tumors may secrete excessive catecholamines. In cases where preoperative tumor manipulation results in severe hypertension, a catecholamine-secreting tumor should be suspected and the possibility of intraoperative hypertensive crisis should be considered.

This case has been accepted by the Journal of American Veterinary Medical Association.

Keywords: Carotid Body Tumor, Catecholamine, Hypertensive Crisis, Dog

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**Influence of Open Fractures Management on Bacterial Contamination in Dogs and Cats at the Emergency Unit**

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**Purpose**
To identify an influence of open fractures management on bacterial contamination in dogs and cats at the emergency unit.

**Materials and Methods**
Samples were obtained from an open fracture wound at two different time points for bacterial identification; immediately before wound cleaning (a), and before surgery (b). Emergency wound cleaning and intravascular broad spectrum antimicrobial injection were performed as routine protocols.

**Results**
There were 25 dogs and 13 cats with open fractures enrolled in this study. Seventeen bacterial species were identified; the majority of bacterial contamination was gram-negative bacteria. *Pseudomonas sp.* was the most common bacteria found in both dogs and cats. In dogs, 76% (19/25) were positive at time point “a” and 40% (10/25) were positive at time point “b”. In cats, 53.85% (7/13) were positive at time point “a”. However, the bacterial contaminations in cats increased to 64.54% (8/13) at time point “b”; the majority were gram-negative bacteria.

**Conclusion**
In dogs, routine wound cleaning and open fractures management at the emergency unit were potentially effective in decreasing bacterial contamination. Although the same protocols were established for both dogs and cats, the bacterial isolates from open fractures in cats increased before surgery. This result indicated that the routine wound cleaning and open fractures management protocol at the emergency unit was not adequate for cats. Further effective management of open fractures in cats should be studied.

**Keywords**: Open Fracture, Emergency, Bacterial Identification

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Anesthetic Effect of Different Ratio of Ketamine and Propofol in Dogs

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Purpose
This study was aimed to compare cardiorespiratory variables and quality of induction, intubation and recovery in dogs induced anesthesia with propofol alone and two different ratio of ketofol.

Materials and Methods
Previous studies using ketofol combined with propofol and ketamine in one syringe during anesthesia used fixed ratio (1:1 mg/mL). When ketofol was used, cardiovascular suppression was alleviated, but respiratory-related side effects were not different from or increased when propofol alone was used. In this study, quality of ketofol anesthesia and changes in cardiopulmonary function according to ratio of ketamine to propofol were evaluated. The experimental group consisted of propofol alone (P group), 3:7 ketofol group (PK 1 group) and 1:1 ketofol group (PK 2). Each group was given intravenous injection of 0.8 mL/kg at constant rate until intubation was possible. After induction of anesthesia, animals were maintained with isoflurane for 120 minutes.

Results
There was no significant difference in quality of anesthesia among three groups and there was no difference in respiratory rate, tidal volume, end-tidal CO₂ partial pressure, and oxygen saturation. In P group, heart rate (HR) during anesthesia was maintained, but arterial blood pressure (ABp) decreased, whereas both HR and ABp showed significant increase in PK2 group. In PK 1 group, HR and ABp during anesthesia remained similar to pre-anesthesia level.

Conclusion
Use of 3:7 ketofol has been shown to be effective in patients with severe cardiovascular changes. Additionally, 1:1 ketofol may be used as alternative to propofol in induction of anesthesia in patients with hypotension or hypovolemia.

Keywords: Anesthesia, Dog, Ketamine, Propofol, Ketofol

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The Ratio of Blood Ionized Calcium Per Magnesium in Rats with Experimental Cardiomyopathies

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Purpose
However magnesium ions (Mg\textsuperscript{2+}) are pivotal in the regulation of more than 325 enzyme systems in mammals, most clinical laboratories only assess the total magnesium, which consists of all three Mg\textsuperscript{2+} fractions (ionized, complexed and protein-bounded form). In the present study, we attempted to determine the values of plasma ionized Ca\textsuperscript{2+} (iCa\textsuperscript{2+}), iMg\textsuperscript{2+} and the ratio of iCa\textsuperscript{2+}/iMg\textsuperscript{2+} in experimental myocardial infarction and cardiac hypertrophy in rats.

Materials and Methods
Myocardial infarction induced by coronary artery ligation and cardiac hypertrophy induced by transverse aortic clamping of rats. Blood samples were obtained after 3, 7 and 30 day after operation and were analyzed whole ions using Nova Stat Profile 8 CRT including the plasma pH, blood gas compositions and the concentrations of ionized Na\textsuperscript{+}, Cl\textsuperscript{-}, K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+} and lactate.

Results
In rats with myocardial infarction, the iMg\textsuperscript{2+} was decreased at 3, 7 and 30 days. Although the iCa\textsuperscript{2+} at 3 and 7 days were decreased, it was recovered at 30 days. So the ratio of iCa\textsuperscript{2+}/iMg\textsuperscript{2+} at 30 days was elevated. In rats with cardiac hypertrophy, although plasma iCa\textsuperscript{2+} was not statistically different from control, iMg\textsuperscript{2+} was decreased and the ratio of iCa\textsuperscript{2+}/iMg\textsuperscript{2+} was elevated.

Conclusion
Our results point to important uses for iMg\textsuperscript{2+} and the ratio of ionized iCa\textsuperscript{2+}/iMg\textsuperscript{2+} during the diagnosis and treatment of cardiac disease in medicine.

Keywords: Ionized Mg\textsuperscript{2+}, Myocardial Infarction, Cardiac Hypertrophy

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Development of Image-Based Diagnostic System of Bovine Pulmonary Lesion

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Purpose
Bovine respiratory disease complex is one of the most costly cattle diseases world-wide. Most slaughterhouses operate slaughter check for food inspection in South Korea, however, the data provides only sporadic and insufficient information to monitor the health issues of the producing industry. Therefore, a better system for the slaughter check is required to establish a system to collect continuous animal health data. This study is designed to propose a novel, image-based slaughter check system for bovine lungs.

Materials and Methods
At slaughter house, cattle lungs with or without gross lesions were photographed, and subjected to the lesion mapping and analysis of the images. Simultaneously, samples for the histopathological and microbiological tests of the lesions were collected and processed according to routine laboratory protocols. Collected histopathological, and microbiological data were integrated to the labeled image for training data set of deep learning-based classification software.

Results
From the result of histopathological and microbiological tests about approximately 200 lung tissue samples, a database of training set for deep learning-based classification was established, culminating in a prototype of the image-based diagnosis system.

Conclusion
A database of training set for deep learning-based classification and a prototype of image-based diagnosis system are novel and innovative systems, however, are still in infant stage of the development that warrants further studies.

Keywords: Image-Based Diagnosis, Bovine Respiratory Disease, Deep Learning

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Optimized Immunocytochemistry Using Lymphocyte and Tissue Markers on Previously Stained Slides from Dogs and Cats by Two Commercial Diagnostic Laboratories

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Purpose
The intent was to optimize a protocol for manual immunocytochemistry on pre-stained cytologic material for use in two commercial diagnostic laboratories involving detection of CD3e, CD20, PAX5, cytokeratin, vimentin, and Melan-A expression in canine and feline cases.

Materials and Methods
Clinical specimens (blood, tissue aspirates, effusions) submitted for cytologic evaluation were stained by a methanolic Romanowsky method and examined by board certified pathologists. Additional slides from fluid and extra unstained aspirate smears were used for comparison with pre-stained materials. Antigen retrieval involved citrate buffer at 95°C for 25 minutes in a decloaking chamber (Biocare). Following peroxidase and casein protein blocking, CD3e (Dako) and CD20 (ThermoFisher) rabbit polyclonal antibodies were applied. Negative controls for specimens lacked primary antibody. Concurrent positive controls involved samples of known reactivity. Signal amplification consisted of polymer antibody (Biocare/Dako) with horseradish peroxidase. The chromogen (3,3’diaminobenzidine) was used with hematoxylin counterstain. Signals were compared between unstained and pre-stained slides; reactions graded by intensity and percent stained cells. Lymphoid cases were phenotypically previously confirmed.

Results
Optimized protocols for ICC staining of pre-stained material (minimum 3.5 hrs) were developed for tissue origin identification and phenotyping lymphoma. Antibody dilutions ranged 1:100-500 (CD3e) and 1:100-200 (CD20) with 30 min incubation. Unstained and pre-stained slides had similar membrane/ cytoplasm graded reactions. Multiple antibodies with negative control were interrogated on a single cellular slide.

Conclusion
Effective manual immunocytochemistry of pre-stained cytologic specimens was achieved in a commercial diagnostic laboratory setting.

Keywords: Immunocytochemistry, Lymphoma, Canine, Feline, Cytology

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Histological Study of Integument in Hedgehog

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Purpose
The Hedgehog (Erinaceus europaeus) is a small mammal of the family Erinaceidae. More recently, as a pet is a lot of attention. The skin has complex structure and many of the components. Hedgehog integument has many apparent differences with other mammals and thus we study histological structure of integument of this animal.

Materials and Methods
In this study two hedgehog (one male and one female) with weight between 312 - 400 gr were used. Hedgehogs are euthanized. Weight, Length and diameter of spins were measured. For histological study, skin samples were fixed in 10% neutral buffered formalin and then histological slides were prepared.

Results
Hedgehog skin has two types of Integuments. In the dorsal areas of the body are spines and in ventral areas, face and limbs wearing of the hairy skin. Spiny follicles inserted in different depth of dermis. The skin of dorsal surface lock of sweat gland. In the skin area of face and nose, besides the normal hair has tactile hair. Deep part of spine is solid but near the skin surface, spine shaft contains very small empty spaces that created with keratinized septum.

Conclusion
Hedgehog has very individual and special security system as it is rolling its body and become hemispherical during confrontation with enemy then surface body spins erect in different direction. Du to special and individual characteristics of hedgehog integument and its important roles in security mechanism and other this animal's physiological activity, other studies is necessary for our knowledge enhancement.

Keywords: Hedgehog, Integument, Histology

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A Comparative Study Between Fine Needle Aspiration Biopsy (FNAB) Findings and Histopathology in the Evaluation of Canine Skin and Skin Adnexal Tumours

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Purpose
The aim of this study was to compare the accuracy and the efficiency of a cytology technique with histopathological diagnosis, and to examine the sensitivity of cytology in the diagnosis of canine skin tumours.

Materials and Methods
Fortythree skin tumour samples were evaluated. Cytologic specimens were prepared by Fine Needle Aspiration Biopsy (FNAB) Technique and stained with May-Grünwald Giemsa (MGG). For histopathologic examinations tissue samples were obtained by surgery. Samples were fixed in neutral formalin solution and embedded in paraffin by routine procedures and stained with Hematoxylin-Eosin (H&E).

Results
Cytopathologically; 69.76% were malignant epithelial tumours, 11.62% benign epithelial tumours, 13.95% suspect epithelial tumours and 4.65% few malignant epithelial tumours were diagnosed. Histopathological diagnosis determined 27.9% squamous cell carcinoma, 20.9% hepatoid gland tumours, 16.3% basal cell carcinoma, 16.3% sweat gland tumours, 11.6% sebaceous gland tumours, 4.7% papilloma and 2.3% malignant pilomatricoma. The diagnoses were in agreement cytopathologically and histopathologically in 86.05% of cases.

Conclusion
Although cytopathological examination has not yet come into extensive use in veterinary medicine for the diagnosis of skin tumours, the results obtained in the present study suggest that cytopathology is a practical tool for the early diagnosis of skin tumours. Therefore, it is suggested that cytopathology can be used for the diagnosis of skin tumours in dogs and should be confirmed by histopathology.

Keywords: Cytopathology, Dog, Skin Tumours

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Ductal Carcinoma with Osseous Metaplasia within Skin as a Consequence to Metastatic Mammary Ductal Carcinoma: A Case Report in a Bitch

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Purpose
Reports on Invasive ductal carcinoma in bitches are scarce, compared to those in humans. Metaplastic bone formation due to mammary ductal carcinoma has not been shown previously in animals or in humans.

Materials and Methods
We described in detail the histology of a mammary ductal carcinoma of a bitch with metastatic characteristics and metaplastic osteoid formation within the mammary gland and the skin utilizing the routine Haematoxylin & Eosin stain and Immunohistochemical markers.

Results
The mammary tumor was poorly defined and consisted of irregular tubular structures that were embedded in a fibrous connective tissue. Neoplastic cells that forming the tubules were present in more than 2 layers surrounding a narrow slit-like Lumina. Some tubular and nests proliferations were seen crossing tumor stroma and infiltrating the adjacent skeletal muscle. The mitotic index was high (>28/10 HPF). Interestingly, a mature bone was observed occupying a wide area of the tumor. The tumor in the skin of axillary area showed tubular formations and bone differentiation occupying a large portion of the tissue. Immunohistochemistry using anti-Cytokeratin 18 and anti-Estrogen receptor-α immunoglobulins, revealed the presence of neoplastic cells with epithelial origin and a high-grade carcinoma with a poor prognosis.

Conclusion
To the best of our knowledge, this is the first report of a mammary ductal carcinoma with metastatic features and osteoid metaplasia in the primary (mammary gland) and secondary site (skin).

Keywords: Invasive, Metastasis, Bone Metaplasia, Mammary Gland, Cutaneous, Carcinoma, Duct Formation

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**Dysbiosis of Intestinal Microbiota Aggravate Atopic Dermatitis in a Mouse Model**

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**Purpose**
This study aimed to investigate the effects of gut dysbiosis in early life using a mouse model (ovalbumin-induced) of AD.

**Materials and Methods**
AD phenotypes (clinical score and skin barrier function) and systemic immune response (serum IgE using ELISA) as well as the gut immune response (intestinal innate lymphocytes-3 by flow cytometry) including metabolites were evaluated.

**Results**
Mice who received antibiotics without oral healthy feaces had significantly aggravated phenotypes (clinical score, transepidermal water loss, and histopathology). Total IgE production and skin Th2-cytokines were significantly increased in antibiotic group mice compared to the antibiotic with oral healthy feaces mice. In the gut, interleukin-17 and group 3 innate lymphoid cells were increased and the production of the short chain fatty acids was significantly suppressed by antibiotics.

**Conclusion**
Intestinal microbiota could play a crucial role in development of AD. Further studies are needed in animals and humans.

**Keywords:** Microbiota, Atopic Dermatitis, Mice

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Occurrence of Skin Diseases in Cats in Jatinangor Education Area, West Java, Indonesia and Their Zoonotic Importance

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Purpose
The research aims to determine the prevalence of skin diseases in cats of Jatinangor Education Area and their zoonotic significance.

Materials and Methods
The research was done in Jatinangor Education Area, West Java, Indonesia, during the period of January 2016 to December 2016 with total 222 cats examined.

Results
The results showed that there are 4 type of diseases were recorded such as mycosis, scabiosis, demodecosis, and tick. The prevalence of every type of disease are 52.70% of mycosis, 31.98% of scabiosis, 1.35% of demodecosis and 13.96% tick. All of the skin disease has a high rate of incidence from January to March and from September to November, except demodecosis which has high rate of incidence only in August to October.

Conclusion
Some of the incidence has transmitted to the cat owner but already can be addressed and cured. The research showed that there was a high risk of transmittable zoonotic disease from cats in Jatinangor Education Area, so that suggested hygiene and sanitation for cats and the owners to prevent and control the risk of zoonoses.

Keywords: Skin Diseases, Cats, Jatinangor Education Area

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Comparison of Thermographic Imaging and Other Diagnostic Techniques in Diagnosis of Cattle with Laminitis

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Purpose
Aim of the study, compared with thermographic imaging (IRT) and radiographic, magnetic resonans (MR), computer tomography (CT), histopathological analysis on the diagnosis of laminitis.

Materials and Methods
Twenty Holstein-Fresian foreleg feet were used as a materials. Thermographic examination were performed the animals after routine clinical examination. Increased temperature on the dorsal side of feet were considered as a laminitic groups and five of them were not shown any changes as a control group, after thermographic examination. The marked claws were evaluated for radiographic, MR, CT imaging and laminar tissues histopathologic analysis. Tissue samples taken 10% buffered formaldehyde solution after establishing a routine laboratory procedure following the prepared paraffin blocks 5 mm thick sections taken with hematoxylin-eosin dye method according to the staining and light microscopy were examined.

Results
As a results, thermographic examination may have potential as a detection tool for laminitis. MR transversal images provided excellent depiction of anatomical structures and many biometric research in the bovine hoof can be easily investigated. The usefulness of IRT, MRI, CT in evaluating laminitis in the acute patient remains stil open.

Conclusion
The usefulness of thermography, MRI, CT in evaluating laminitis in the acute patient remains stil open this study evaluates the use of thermography, MRI, CT during the initial active phase of laminitis as a means to increase the understanding of the disease and also serves as a justification for the development of an experiment involving live cows induces laminitis.

Keywords: Bovine, Thermography, CT

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Interpretation and Evaluation of Cat Thoracic Radiography (2013-2016)

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Purpose
The aim of this study was to evaluate thoracic radiographs of cats pathological, etiological, diagnosis and treatment and complications between year 2015-2017 which were obtained to surgery department of Small animal Clinics, Faculty of Veterinary Medicine

Materials and Methods
Totaly, 62 cats were used as materials. Following clinical examination of the patients radiological examination were performed ventro--dorsal, latero-lateral and dorso-ventral positions in all cases.

Results
Distribution of the cases are traumatic pneumothorax (13), hydrothorax (12) and hemia diaphramatica (15) has due to the high falling any hight. Thirty cats has pneumonia and pulmoner oedema with the lower respiratory tract problems. In these cases, the alveolar lung model was determined. While many cases of lung nodules in 2 cases, it has been diagnosed with lung metastasis.

Conclusion
Radiographic evaluation and interpretation of thorax requires experience. First of all, the correct position and the right technique should be selected. The expiration period of the cats could make misinterpretation. Thorax region should be divided into four regions for diagnosis. In the present study, the etiology of thoracic lesions was the most common cause of high-fall syndrome. In addition to these, tumoral formations include traffic accidents and metabolic diseases. In conclusion, thorax radiography is a rapid, non-invasive technique used for evaluating structures within surrounding the thorax. Because the changes seen on thoracic radiographs are often indicative of systemic disease, the clinician needs to keep the patient, signalment, physical examination, and other laboratory findings in mind when prioritizing the differential diagnosis.

Keywords: Cat, Thorax, Radiography

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Thoracolumbar Transitional Vertebrae (TTV) with Extra Rib on L1 Formation of Korean Wild Raccoon Dogs

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Purpose
This study aimed to describe Thoracolumbar Transitional Vertebrae (TTV) with extra rib on L1 vertebrae and to report its prevalence of Korean Wild Raccoon Dogs (KWRD).

Materials and Methods
We evaluated 82 Korean wild raccoon dogs (36 males, 46 females) rescued by the Wildlife Rescue Center of Kangwon National University in Kangwon province, South Korea. Morphology of extra rib on L1 vertebrae was used to check TTV using ventrodorsal radiographs.

Results
Of the 82 evaluated cases, 29 (35.4%) were found to have TTV with extra rib on L1. Of the 29 cases, 11 were male and 18 were female. Of the 29 cases of TTV with extra rib on L1, 20 were bilateral and 9 were unilateral. Of the 9 unilateral cases, 4 were observed to have TTV on the left and 5 were on the right.

Conclusion
The present study shows a high prevalence of TTV (35.4%) with extra rib on L1 vertebrae in KWRD rescued in Kangwon province. However, a future study with more cases is required to estimate a nationwide prevalence of TTV (35.4%) with extra rib on L1 vertebrae of KWRD. The finding of this study may need to be considered for surgical planning.

Keywords: Transitional Vertebrae, Extra Rib on L1 Vertebrae, Korean Wild Raccoon Dogs

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Situs Inversus Totalis in a Dog: A Case Report

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Purpose
Situs inversus totalis (SIT) is a rare congenital malformation with complete reversal of organ placement within the body, producing an anatomical mirror image of normal placement. SIT is also termed Situs Inversus with dextrocardia. This case report describes radiographic findings of a dog with SIT without reversal placement of the descending colon.

Materials and Methods
An 11-years-old, 9.3kg, mixed breed, intact male dog was referred to the Veterinary Medical Teaching Hospital at Kangwon National University because of a suspected spleen cancer. SIT without reversal placement of the descending colon was observed during radiographic and ultrasonographic examinations.

Results
On ventrodorsal thoracic radiograph, cardiac apex was on the right of the midline, confirming the presence of dextrocardia. On ventrodorsal abdominal radiograph, the fundus of stomach and head of spleen were on the right side of midline. The pylorus of stomach and liver were located in the left side. The right kidney was more caudal than left kidney. However, the descending colon was on normal position. Based on radiographic characteristics of the patient, a diagnosis was SIT without replacement of the descending colon.

Conclusion
To our knowledge, this is the first report of SIT without replacement of the descending colon in a dog.

Keywords: Situs Inversus Totalis, Descending Colon, Dextrocardia

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Abdominal Aortic Mineralization in a Hyperadrenocorticism Dog

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**Purpose**
Common radiological findings in Hyperadrenocorticism include hepatomegaly, enlargement of adrenal gland and dystrophic mineralization of soft tissue structures. Although rare, there is a report of abdominal vessel calcification in a patient with Hyperadrenocorticism. However, we are unaware of any published radiographic studies on blood vessel mineralization among Hyperadrenocorticism patients with thrombosis and its ultrasonographic features.

**Materials and Methods**
A thirteen-year-old, intact male Shih-tzu dog weighing 7.3 kg presented with one week’s history of progressive paresis of hindlimb. He had a history of uncontrolled hyperadrenocorticism. At the time of presentation, generalized scale, alopecia and cold pelvic limb and weak pulse was noted. Physical, laboratory tests and radiography, ultrasound examination was done.

**Results**
On lateral and ventrodorsal radiograph, mineralized vessels were seen from level of 5th lumbar vertebra to distal femur level and its branches were also seen. Ultrasonographic exam revealed bilateral adrenomegaly, hyperechoic liver, suspected thrombus in the aortic lumen. Parts of the vessel had thickened hyperechoic wall with posterior acoustic shadowing. The patient was euthanized at the owner’s request and mineralization of tunica muscularis was identified on histopathological exam of abdominal aorta.

**Conclusion**
Arterial calcification can be identified in patients with renal failure generally. However, this patient’s serum calcium concentration was within the normal range. Thus, abdominal aortic mineralization appears to be dystrophic calcification. Mineralized abdominal aorta can be identified on ultrasonographic scanning with focal hyperechoic, thickened aortic wall with posterior acoustic shadowing.

**Keywords:** Chronic Hyperadrenocorticism, Abdominal Vessel Mineralization, Histopathological Exam

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Electromagnetic Tracking Based Fusion Imaging of Ultrasonography with Computed Tomography in Canine Head

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Purpose
Fusion imaging of real-time ultrasonography with computed tomography (CT) can improve diagnostic value for undetermined lesions and navigation for interventional procedure. Image fusion requires precise registration of the target organ. In this study, electromagnetic tracking technique using external fiducial markers was applied to canine head to investigate the applicability of this technique.

Materials and Methods
Under general anesthesia, contrast enhanced CT images of head were obtained with placing external fiducial markers over the frontal region and both side of forepaw in 7 beagle dogs with sternal recumbency. Without position change, ultrasonography was performed under magnetic field with installing a position sensor in the linear probe. The position of external fiducial markers was adjusted and matched based on CT images. Execution time of co-registration and the distance between regions of interest and the co-registration points; the frontal bone, eye ball and optic nerve were estimated.

Results
External fiducial markers were properly recognized about 60% in all dogs. After adjusted, all external fiducial markers were precisely matched. The execution time of co-registration was less than 1 min. The distance between regions of interest and the co-registration points was less than 3 mm in all dogs.

Conclusion
Electromagnetic tracking technique using external fiducial markers was a simple and applicable method for fusion imaging of real time ultrasonography with CT of canine head. This technique was considered to be useful for an interventional procedure of the retrobulbar and peri-orbital lesions.

Keywords: Fusion Imaging, Electromagnetic Tracking Method, External Fiducial Markers

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Radiation Exposure in C-Arm(Fluoroscopy) During Small Animal Orthopedic Surgery

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Purpose
The purpose of current study is investigating the radiation exposure level of the surgeons in C-arm-guided small animal orthopedic surgery using Thermoluminescent dosimeters (TLDs) locating at personnel shielding inside and outside in major body parts.

Materials and Methods
A prospective study was conducted to measure radiation exposure dose of three designated positioned persons (first assistant, operating surgeon, anesthesiologist) using TLDs. The lead equivalent protective devices included panorama mask, thyroid shield, apron, and arm shield. TLDs were placed on the inside and outside of the protective gears in five different anatomic sites (eye, thyroid, breast, gonad, and arm).

Results
Radiation exposure was measured in 14 surgical procedures with mean kVp(51) and mean mAs(1.6). The equivalent doses of the first assistant's thyroid, breast, and gonad were 1.75mSv(outside)/0.58mSv(inside), 2.01/0.13, and 3.03/0.11 respectively. The dose of operating surgeon of thyroid, breast, and gonad were 1.46/1.69, 4.82/0.35, and 5.25/0.22. The dose of anesthesiologist of eye, thyroid, breast, gonad, and arm were 0.51/0.61, 0.35/0.3, 0.67/0.34, 0.72/0.29, and 0.62/0.35.

Conclusion
The exposure dose to gonad outside lead protection marked the highest values in all participants. With lead protection, the reductions of exposure dose to gonad were significant (first assistant: 96%, operating surgeon: 96%, anesthesiologist: 60%). The result suggests the radiation shield in veterinary surgery with C-arm is essential, particularly for gonad protection.
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Keywords: Small Animal, C-Arm, Radiation Exposure

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Computed Tomographic Findings of Adenocarcinoma of the Esophagus and Gastric Cardia in a Pekingese Dog

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Purpose
In human, adenocarcinoma commonly occur in esophagocardia region, but it has not been reported in veterinary medicine. This report describes computed tomographic findings of adenocarcinoma of the esophagus and cardia in a Pekingese dog.

Materials and Methods
A 6-year-old female Pekingese dog, weighing 3.85 kg was presented with regurgitation, ptyalism, and weight loss. Radiography, ultrasonography and CT were performed to evaluate gastrointestinal tract. Definite diagnosis was established by endoscopic biopsy. The sites of the biopsy were esophageal mass and cardia.

Results
On physical examination, the dog was cachexic and severely dehydrated. On the lateral thoracic radiograph, the caudal one third of the esophagus was visible as a soft tissue band. CT revealed oval-shaped, soft tissue attenuating mass in size of 2.3 X 1.0 X 1.1 cm arised from caudal one third of the esophagus. The mass was protruding from esophageal wall to lumen and irregular margined with heterogenous contrast enhancement. The wall of cardia and fundus was focally thickened. Gastric lymph node was enlarged with contrast enhancing. On histopathologic examination, the esophageal mass was mildly to moderately pleomorphic, ranging from columnar to cuboidal, forming irregular short papillary structures, or occasional small glands. Esophageal mass was confirmed as papillary adenocarcinoma, and the gastric sample showed same result.

Conclusion
Tumor of the esophagus is rare and accounts for less than 0.5% of all tumor in the dog. Other neoplasia, not squamous cell carcinoma and sarcoma, rarely occur in the esophagus. This case is the first report that presents computed tomographic features of adenocarcinoma of the esophagocardia region.

Keywords: Papillary Adenocarcinoma, Esophageal Mass, Computed Tomography

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Hyyperglycemic, Hyperosmolar Syndrome in a Cat with Atrial Septal Defect

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Purpose
Hyperglycemic, hyperosmolar syndrome (HHS), also known as nonketotic hyperosmolar diabetes, in cats and dogs is a life-threatening presentation of diabetes mellitus. This often has other severe systemic disease and should be closely monitored for signs of crisis. And it is known that the mortality rate for HHS cats and dogs is high. This report describes a HHS cat accompanied by congestive heart failure secondary to atrial septal defect (ASD).

Materials and Methods
A 13-year-old castrated male cat was referred to Seoul National University Veterinary Medicine Teaching Hospital February 2017 for emergency situation of lethargy and anorexia. Physical examination, blood analyses, thoracic and abdominal radiography, abdominal ultrasonography, and echocardiography were performed.

Results
On physical examination, the patient had depression, dehydration, hypotension, and plantigrade stance. Blood analyses showed increased serum BUN, glucose, and fructosamine level. But there is no identified ketones in urine. And on radiographs, cardiomegaly, pleural effusions, and slightly decreased serosal detail of the abdomen are identified. Abdominal ultrasound and echocardiography scan showed dilated caudal vena cava, ascites, dilated right side heart, and atrial septal defect (ostium secundum type). High serum osmolality (382.5 mOsm/kg) and absence of urine ketones were criteria for the diagnosis of HHS. After that, hospitalization, close monitoring, and fluid and insulin therapy were started, but convulsion occurred and the patient was expired.

Conclusion
This case was thought that HHS in a cat with ASD had poor prognosis, because of diabetes and congestive heart failure concurrently.

Keywords: HHS, ASD, Cat

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Effect of Injection Rate of Contrast Agent on Maximum Slope Based Computed Tomography Perfusion Values in Normal Canine Kidney

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Purpose
Computed tomography perfusion (CTP) values using maximum slop method are estimated with assumption that no outflow occur during arterial inflow into the interrogated region of interest (ROI). The duration of contrast injection needs to be as short as possible. Herein, the effect of injection rate of contrast agent on maximum slope based CTP value was investigated in normal canine kidney.

Materials and Methods
Five dogs underwent CTP using three different injection rate of contrast agent; 1.5 ml/s, 3.0 ml/s, and 4.5 ml/s at the level of left renal hilus. CTP began at 10 s after the initiation of 300 mgI/kg of iohexol (300 mgI/ml) injection for 40 s. Peak time of renal artery and initiation time for enhancement of renal vein were measured from the time intensity curve. CTP values including blood flow and blood volume were calculated using the maximum slope method.

Results
In all injection rates, renal artery reached to peak enhancement before or simultaneously the initiation of renal venous output. Peak time of renal artery, initiation time of renal venous output, and median values of CTP values were not significantly different according to injection rates.

Conclusion
Main assumption of maximum slop method was satisfied regardless of injection rate when using 300 mgI/kg of contrast agent for CTP study. The lower injection rate was practical for CTP in small breed dogs without underestimation of CTP values.

Keywords: Injection Rate, Kidney, Computed Tomography Perfusion

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Measurements of Pulmonary Vasculature on Thoracic Radiographs in Healthy Cats and Cats with Hypertrophic Cardiomyopathy

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Purpose
This study investigated the normal range of pulmonary vein (PV) and artery (PA) in healthy cats and cats with hypertrophic cardiomyopathy (HCM).

Materials and Methods
Total 62 cats (36 healthy cats and 26 with HCM) undertaken thoracic radiography were reviewed. The diameters of the right cranial PA and PV were compared with the diameter of R4. The diameters of the right caudal PA and PV were compared with the summated shadow of R9 made by the caudal vessels.

Results
Cranial PV diameter and PV to R4 ratio was significantly greater in cat with HCM (diameter; 2.4 ±0.85 mm, ratio; 0.9 ±0.32) than normal cats (diameter; 2.0 ±0.45 mm, ratio; 0.7 ±0.19). Caudal PA was significantly larger in cat with HCM (diameter; 4.3 ±1.3 mm, PA to R9 ratio; 1.7 ±0.5) than normal cats (diameter; 3.3 ±0.9 mm, ratio; 1.2 ±0.35). Caudal PV was significantly greater in cat with HCM (diameter; 4.0 ±0.8 mm, PV to R9 ratio; 1.54 ±0.29) than normal cats (diameter; 3.3 ±0.35 mm, ratio; 1.18 ±0.27). A cut off value of 1.45 when applying the caudal PV to R9 ratio had 86% specificity and 63% sensitivity to differentiate between normal cats and cats with HCM.

Conclusion
Caudal PV to R9 ratio can be used to differentiate normal cats from those with HCM for screening test and the cut off value of the criterion was 1.45.

Keywords: Cats, Hypertrophic Cardiomyopathy, Pulmonary Artery, Pulmonary Vein, Radiography

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Computed Tomography Cholecystography with Ultrasound-Guided Percutaneous Contrast Injection in Dogs

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Purpose
This study is to evaluate feasibility and safety of computed tomography (CT) cholecystography with ultrasound-guided percutaneous contrast injection in normal dogs.

Materials and Methods
In this crossover design, 10 healthy beagles were used to evaluate 4 contrast formula of cholecystography; 2 dilution ratio (1:1 vs. 1:3) and 2 total volume (8 ml vs. 16 ml) of 300 mgI/kg iohexol. After ultrasound-guided percutaneous contrast injection into gallbladder, CT images were obtained at 3, 10, and 30 min. CT cholecystography images were assessed qualitatively and quantitatively.

Results
In all contrast formula, CT cholecystography showed gallbladder, the intra- and extrahepatic bile ducts. Size of intra- and extrahepatic bile duct was significantly different according to total volume of contrast agent regardless of dilution ratio. Patency of bile duct was effectively assessed using 16 ml volume with 1:3 dilution formula. Beam-hardening artifact deteriorated CT image quality for visualizing biliary system 1:1 dilution compared with 1:3 dilution. There was no significant complication except for minor leakage of contrast agent in 50% of all studies immediately after injection.

Conclusion
Ultrasound-guided percutaneous cholecystography is a relatively non-invasive, effective method to visualize the biliary system. A total volume of 16 ml of a 1:3 contrast and saline mix provided optimal CT cholecystography.

Keywords: Percutaneous Cholecystography, Dog, Computed Tomography, Contrast Formula, Ultrasound-Guide

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Evaluation of Cardiac Function in Dogs with Heartworm Disease Using Tissue Doppler and Strain Rate Imaging

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Purpose
Cardiac function was evaluated in dogs with heartworm disease using tissue Doppler and strain rate imaging to determine change of cardiac function in heartworm disease and compare between different echocardiographic indices.

Materials and Methods
Conventional echocardiographic values including RV end-diastolic area (RVDA), RV end-systolic area (RVSA), RV fractional area change (RVFA), peak early and late diastolic velocities, RV isovolumic relaxation time (IVRT), and Tei index were obtained. TDI and strain and strain rate values such as ventricular wall velocity, systolic strain and systolic strain rate were obtained at basal and middle segment of right ventricle free wall and interventricular septum.

Results
RVFA, Conventional E/A ratio, IVRT and TCO of HW group were lower compared to control group, which was not significantly different. Tei index of HW group was significantly higher than control group (HW: 0.73±0.12, control: 0.46±0.09, P:0.037). TDI E/A ratios were decreased in every segment, but none of those were significantly different. Systolic velocity, strain, strain rate presented no difference between groups.

Conclusion
In heartworm disease, conventional echocardiography and TDI values showed no huge difference between HW group and control group. Tei index was significantly higher in HW group and might be the evidence of the decrease of global cardiac function.

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project title: Functional feed material development and efficacy evaluation for detector dog’s ability improvement, Project No: PJ011989)” Rural Development Administration, Republic of Korea.

Keywords: Dog, Echocardiography, Heartworm, Tissue Doppler, Strain Imaging

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Computed Tomography Features of Atherosclerosis in a Dog

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Purpose
Arteriosclerosis is rare in dogs, but atherosclerosis can be diagnosed by computed tomography (CT). Therefore, features of atherosclerosis seen on CT scan are described.

Materials and Methods
A 10-year-old, neutered male Schnauzers suffering from pancreatitis was referred with thrombocytopenia, anorexia and lethargy.

Results
At first admission, systemic hypertension (160 mmHg) and elevation of alkaline phosphatase and amylase concentration, and positive reaction of canine pancreas-specific lipase were found. Multiple hypoechoic splenic nodules were found on ultrasonography and CT and determined as nodular hyperplasia. Mineralization of iliac arteries, about 3 cm length, was found and considered as an incidental finding. After immunosuppressive therapy, the dog recovered from thrombocytopenia but persistently showed anorexia. Pituitary-dependent hyperadrenocorticism was ruled out through low-dose dexamethasone suppression test. Elevation of C-reactive protein (CRP) level and hypertriglyceridemia were found and systemic hypertension persisted in spite of medication. On CT recheck, multiple mineralization of arteries including coronary artery, aorta, pulmonary vessels, celiac artery, iliac artery, renal artery, cranial mesenteric artery, lumbar artery, carotid artery, lingual artery, and maxillary artery were identified. Multiple subcutaneous and soft tissue mineralization were also detected. The diffuse arterial mineralization was characterized as atherosclerosis and soft tissue mineralization was secondary to atherosclerosis.

Conclusion
Although atherosclerosis is rare in dogs, atherosclerosis should be considered as differential diagnosis for multiple mineralization of arteries and soft tissue on CT images, particularly in dogs with risk factors of atherosclerosis including hypertension, hypertriglyceridemia, and elevated CRP level. CT can detect and quantify coronary calcium, a marker of atherosclerosis.

Keywords: Atherosclerosis, Computed Tomography, Dog, Artery, Mineralization

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Computed Tomography of Ductus Diverticulum in Three Dogs

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Purpose
Ductus diverticulum is a focal bulge on the aortic isthmus. It is detected relatively common in human. However, there are no reports of ductus diverticulum in dogs. This study introduces the computed tomography (CT) characteristics of ductus diverticulum in dogs.

Materials and Methods
Three dogs (2 female malteses and 1 male mongrel dog) underwent CT for heart murmur. CT was performed using 32-slice CT scanner under general anesthesia. CT angiography was performed 20 seconds after starting of the administration of 900 mgI/kg of iohexol at speed injection of 1 ml/sec. All CT images were evaluated with three cross-sectional images and three-dimentional images.

Results
Ductus-like structure without communication with pulmonary trunk was detected in all 3 dogs. Sagittal and three-dimentional image was useful to reveal an outpouching from the aortic isthmus with smooth margin. In one dog, pulmonic stenosis was also observed with ductus diverticulum.

Conclusion
Computed tomography was useful for diagnosing ductus diverticulum in dogs. Ductus diverticulum should be considered in the differential diagnosis for young dogs with heart murmur.

Keywords: Angiography, CT, Dog, Ductus Diverticulum

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**Purpose**
The current study was designed for comparisons of cytokines between healthy dogs and dogs with acute pancreatitis.

**Materials and Methods**
Three healthy dogs were included as a control group and three dogs with acute pancreatitis were included as an experimental group. Serum samples were collected in both groups. For analysis, canine cytokine microarray was utilized to comparison analysis of Interleukin (IL)-2, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), monocyte chemotactic protein 1 (MCP-1), receptor for advanced glycation endproducts (RAGE), stem cell factor (SCF), transforming growth factor- alpha (TNF-α) and Vascular endothelial growth factor (VEGF).

**Results**
Based on the results, IL-2, IL-6, IL-10, GM-CSF, and TNF-α were not detected with cytokine microarray in both groups. On the other hand, IL-8 (p=0.035), MCP-1 (p=0.0138), RAGE (p=0.0079) and SCF (p=0.034) were significantly increased in the experimental group. However, VEGF (p=0.6971) was not significantly different in both groups.

**Conclusion**
This study demonstrated that the difference of cytokines inolved in the pathogenesis of acute pancreatitis in dogs compared to normal control.

**Keywords**: Acute Pancreatitis, Antibody Array Analysis, Cytokine

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Evaluation of Iris-Pupillary Ratio as a Non-Invasive Biomarker of Higher Sympathetic Activity in Hypertensive Canines and Felines

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Purpose
Pupillary diameter is exclusively regulated by sympathetic and para-sympathetic system under constant light conditions. Hence we hypothesised iris-pupillary ratio can be a non-invasive biomarker of elevated sympathetic activity.

Materials and Methods
Hypothesis was tested by comparing iris-pupillary ratio of normotensive and hypertensive dogs and cats presented to outpatient clinics. Digital images of the eyes were captured and analysed independently using NIH Image-J software. Blood pressure of each patient was recorded during the clinical examination using a digital sphygmomanometer.

Results
The ratio of iris-pupillary area, perimeter and integrated density was significantly lower (* p<0.05, ** p<0.01, *** p<0.001, n=8-10) in hypertensive dogs (3.39 ± 1.14*, 1.85 ± 0.14* and 3.29 ± 1.63**) and cats (1.23 ± 0.07***, 1.14 ± 0.06** and 1.41 ± 0.08**) compared to normotensive dogs (5.76 ± 1.81, 2.22 ± 0.35 and 9.64 ± 4.33) and cats (3.05 ± 0.04, 1.77 ± 0.03 and 4.42 ± 0.28) respectively. The blood pressure in hypertensive dogs and cats was 179.30 ± 36.69 mmHg and 205.50 ± 14.85 mmHg respectively while blood pressure in normotensive dogs and cats was 103.50 ± 12.09 mmHg and 115.15 ± 15.36 mmHg respectively. Thus, lower ratio of iris-pupillary area, perimeter and integrated density is a reflection of dilated pupil, possibly due to higher sympathetic activity in hypertensive dogs and cats.

Conclusion
The data from this study supports our hypothesis that iris-pupillary ratio can be used as a non-invasive biomarker of higher sympathetic activity.

Keywords: Hypertension, Sympathetic Activity, Stress

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Vector-Borne Hemoparasitic Pathogens in Feral Cats in Jeju Island, Republic of Korea

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Purpose
This study was designed and conducted to detect the vector-borne hemoparasitic pathogens in feral cats in Jeju island.

Materials and Methods
Blood samples were collected from 120 feral cats in Jeju. The genomic DNA was extracted from 0.3 ml of whole blood sample anticoagulated with EDTA. To detect vector-borne pathogens (Babesia spp., Theileria spp., Hepatozoon spp.), DNA fragment inside 18s rRNA of these organisms was amplified through PCR.

Results
Of the 120 samples, there were no cats infected hemoparasitic pathogens. Pathogens (Babesia spp., Theileria spp. and Hepatozoon spp.) were not detected by PCR.

Conclusion
In this study, we could not confirm that infection of vector-borne hemoparasitic pathogens is present in feral cats in Jeju. Although the infection is not detected, considering the importance of the diseases, further study is required to attain more accurate and reliable data by increasing the sample size and regular screening.

Keywords: Vector-Borne Diseases, Hemoparasitic Pathogens, Feral Cat

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The Wireless Electrocardiogram and Photoplethysmogram Detection in Animal

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Purpose
Demands on personal healthcare monitoring have been growing explosively not only by patients but also by general public, while demands on medical information services which allow health maintenance through continual monitoring also have been increasing steadily. In this study, we proposed noninvasive and continuous measurement of blood pressure pulse transit time (PTT), obtained by electrocardiogram (ECG) and photoplethysmogram (PPG).

Materials and Methods
The detection system is composed of the monitoring part and measurement part which is composed ECG and PPG measurement part. The wireless sensor measured physiological signal such as the ECG, SPO₂, pulse rate, heart rate, skin temperature and 3D posture.

Results
By wireless gateway, the signal data measured at the ECG sensor and the PPG sensor of this system were sent to and stored in a PC in real time. ECG / SPO₂ signals were processed statistically in real time and displayed in graphs. HR, skin temperature and BP values were displayed as numeric data. Based on user data, the system generated various alarm messages according to situations such as bradycardia, tachycardia, hypothermia, or cardiac arrest. In the derived equation, the experimental systolic pressure result is 111mmHg and at the same time the measured blood pressure result by sphygmomanometer is 115mmHg.

Conclusion
By measuring physiological signals with the wireless sensor, it was possible to monitor in real time and to minimize restrictions imposed to everyday activities of animal.

Keywords: Wireless, Electrocardiogram, Photoplethysmogram

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Mg$^{2+}$ Homeostasis and Therapeutic Potential of Mild Hypothermia in Heart

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**Purpose**
Severe deep hypothermia (4°C) is widely used in conjunction with cardioplegia to minimize neurologic and cardiac damage during cardiac surgery. However, during the subsequent rewarming and reperfusion many destructive processes result in inevitable post-ischemic myocardial injuries due to Ca$^{2+}$ overload. Therapeutic mild hypothermia (core temperature 30-35°C) or Mg$^{2+}$ has a protective capacity against the reperfusion injuries. Our aim was to investigate Mg$^{2+}$ homeostasis in heart during mild hypothermia.

**Materials and Methods**
Intracellular Mg$^{2+}$ concentration ([Mg$^{2+}$]i), intracellular Na$^+$ concentration ([Na$^+$]i) and total Mg efflux ([Mg]e) were measured in isolated papillary muscle and perfused heart from guinea-pig by ion-selective microelectrode technique and atomic absorbance spectrophotometry.

**Results**
In beating papillary muscle of the guinea pig, [Mg$^{2+}$]i was significantly increased by hypothermia (34°C and 30°C), accompanied by an increase in [Na$^+$]i, a large positive inotropic effect, depolarization of membrane potential and prolongation of action potential duration. In addition, mild hypothermia enhanced the increase in [Mg$^{2+}$]i induced by high extracellular Mg$^{2+}$ concentration. The rate of [Mg]e in perfused heart was significantly attenuated by hypothermia.

**Conclusion**
These results suggested that the therapeutic mild hypothermia could produce an increase in [Mg$^{2+}$]i in guinea-pig heart by stimulation of Mg$^{2+}$ influx and attenuation of Mg$^{2+}$ efflux.

**Keywords**: Mg$^{2+}$, Mild Hypothermia, Therapeutic Potential

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Anti-Inflammatory Effects of Feline Adipose Tissue-Derived Mesenchymal Stem Cells by PGE2 Pathway

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Purpose
Mesenchymal stem cells (MSCs) have immunomodulatory functions and differentiation capacity, conferring them with various clinical uses. Although MSCs have been applied in the treatment in various inflammatory diseases, mechanistic research on feline MSCs is lacking. Accordingly, in this study, we aimed to analyze the immunomodulatory mechanisms of MSCs isolated from feline adipose tissue (fAT-MSCs).

Materials and Methods
Cytokine expression in RAW264.7 murine macrophages and allogeneic feline peripheral blood mononuclear cells (fPBMCs) was measured using quantitative real-time polymerase chain reaction (qRT-PCR) and compared according to the presence of fAT-MSCs. We also extracted RNA and collected supernatants from fAT-MSCs for measurement of soluble factor expression levels by qRT-PCR and enzyme-linked immunosorbent assay.

Results
The levels of pro-inflammatory cytokines, e.g., tumour necrosis factor-α (TNF-α), inducible nitric oxide synthase and interleukin (IL)-1β, were significantly decreased in coculture of mitogen-stimulated RAW264.7 cells with fAT-MSCs compared with that in RAW264.7 cells. Additionally, the expression of immunomodulatory factors from fAT-MSCs, including cyclooxygenase-2 (COX-2), transforming growth factor (TGF)-β, indoleamine-2,3-dioxygenase (IDO) and hepatocyte growth factor, increased in the presence of RAW264.7 cells. Expression of pro-inflammatory TNF-α, interferon-γ and IL-6 decreased, and expression of anti-inflammatory IL-10 increased during coculture of mitogen-stimulated allogeneic fPBMCs with fAT-MSCs. TGF-β, COX-2 and IDO expression and prostaglandin E2 production from fAT-MSCs increased in the presence of allogeneic fPBMCs.

Conclusion
Our data suggested that soluble factors secreted from fAT-MSCs play an important role in the immunomodulatory effects of these cells.

Keywords: Feline Mesenchymal Stem Cells, Immunomodulation, Prostaglandin E2

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A Case Study of Pathological Bali Local Dogs That Infected by Distemper Disease

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Purpose
A case study was conducted on twenty local dogs aged 1-3 years who died with common signs: fever, weak, lethargic, dull hair, loss of appetite; and with the clinical symptoms were observed such as: thick mucopurulent on the nose area, diarrhea, with some cases observed pustule on abdomen area and thickening of skin on the soles of the feet.

Materials and Methods
RT-PCR laboratory test performed and the results showed positive for canine distemper. Necropsy in the dogs conducted to observe the changes that occur in their organs, and several organs were collected and fixed for making preparations of histopathology. The preparations made with Hematoxylin Eosin staining, then the preparations were observed under binocular microscope to observe the histopathological changes.

Results
The pathological changes that have been observed showed that the brain has suffered redness, the heart seemed swollen, lungs observed with dark red color, and the part of vesica urinaria lumen looked suffer by redness and thickening. Histopathology inspection showed that the brain has suffered congestion and gliosis, the septa of lungs alveoli experienced thickening, the infiltration of inflammatory cells, and vesica urinaria suffered hemorrhage and epithelial thickening.

Conclusion
The pathological changes in organs of local dogs that infected by canine distemper virus in Bali observed mainly suffered from inflammation.

Keywords: Bali Local Dogs, Distemper, Pathology

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Genetic Absence of ClC-2 Hastens Progression of Colitis-Associated Colorectal Cancer via Dysregulation of Adherens Junction

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Purpose
Unlike sporadic colorectal cancer that has a well-defined genetic etiology, the risk of colitis-associated colorectal cancer (CAC) appears to be related more to local gastrointestinal mucosal homeostasis. The adherens junctions, which includes E-cadherin and several catenins, are required to regulate cell adhesion and differentiation and restrict the development of neoplasia. We have previously identified a novel protein target for regulating adherens junctions called chloride channel protein 2 (ClC-2). We hypothesized that the absence of ClC-2 would result in dysregulation of the adherens junctions, leading to progression of CAC.

Materials and Methods
Colonic homeostasis and tumorigenicity were examined in CIC-2+/+ and CIC-2−/− mice or control and CIC-2 knockdown HT-29 cells.

Results
Colonic tissues and colonoids from ClC-2−/− mice had reduced differentiation with altered distribution of E-cadherin and β-catenin as compared with ClC-2+/+ mice. ClC-2−/− mice with CAC induced by azoxymethane/dextran sulfate sodium had an increased tumor number as well as increased incidence of high-grade dysplasia. ClC-2−/− CAC mice had increased re-arrangement of adherens junctional proteins E-cadherin and β-catenin during CAC development. ClC-2 knockdown HT-29 cells showed increased tumorigenicity associated with disruption of adherens junctions.

Conclusion
Collectively, these results suggest that the absence of ClC-2 in the gut plays a critical role in promoting the early stages of cancer due to a lack of regulation of adherens junctional proteins E-cadherin and β-catenin.

Keywords: Colitis-Associated Colorectal Cancer, CIC-2 Chloride Channel, Adherens Junctions

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Increased Metanephrine and Normetaneprine in a Dog with Suspected Pheochromocytoma and Hepatocellular Carcinoma

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Purpose
Ante-mortem diagnosis of pheochromocytoma is difficult in small animal practice. The increased level of metanephrines can be a valuable tool for the diagnosis of pheochromocytoma.

Materials and Methods
A fifteen-year old, spayed female Shih-Tzu dog with exercise intolerance was presented. On physical examination, the dog had severe hypertension (systolic 180 mmHg), tachycardia (180 bpm) with grade 4/6 systolic murmur (PMI: M area). Abdominal radiography and ultrasonography followed by computed tomography revealed a solitary hepatic mass with heterogenous multicystic lesions at the caudate lobe and enlarged right adrenal gland compressing the caudal vena cava.

Results
The hepatic mass was tentatively diagnosed as hepatocellular carcinoma by fine-needle aspiration cytology and elevated serum alpha fetoprotein levels. Plasma free catecholamines and metanephrines were measured for further evaluation of adrenal mass. Both metanephrine and normetanephrine were increased in this dog. Based on fine needle aspiration and laboratory results, hepatocellular carcinoma with pheochromocytoma was diagnosed. Due to difficulty in surgical removal, α-adrenergic antagonist with diuretics, angiotensin-converting-enzyme inhibitor and antibiotics was prescribed. The dog’s blood pressure was reduced and the dog has lived well with improved clinical signs.

Conclusion
In this case, the dog was non-invasively diagnosed with pheochromocytoma by measuring plasma free metanephrines. Measuring plasma free metanephrines is a useful alternative diagnostic tool in early detection and management of pheochromocytoma, which can be difficult to diagnose without biopsy.

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Keywords: Dogs, Metanephrines, Pheochromocytoma

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Fluoxetine-Induced Cardiac Functional Abnormalities

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Purpose
Fluoxetine is a widely used antidepressant, but there are many case reports on dysrhythmias, such as atrial fibrillation or bradycardia. An intracellular Mg\textsuperscript{2+} concentration ([Mg\textsuperscript{2+}]_i) should be maintained within a relatively narrow concentration range in order to ensure the proper functioning of the heart. Our objective was to investigate an effect of fluoxetine on cardiac functions and to investigate alteration of ionic homeostasis.

Materials and Methods
Left ventricular development pressure (LVDP), maximum velocity of the change in pressure (dP/dt\textsubscript{max}), minimum velocity of the change in pressure (dP/dt\textsubscript{min}), heart rate (HR) and Mg\textsuperscript{2+} efflux ([Mg\textsuperscript{2+}]_e) were measured simultaneously in isolated rat hearts with Langendorff’s perfusion system. [Mg\textsuperscript{2+}]_i was measured using Mag-fura 2 AM in H9c2 cell. The activation of ERK 1/2 was analyzed by Western blot. Electrocardiogram in anesthetic rats was recorded using a BIOPAC® system.

Results
Fluoxetine produced reversible dose-dependent decreases in the LVDP, dP/dt\textsubscript{max} and dP/dt\textsubscript{min} in the perfused hearts of rat. After wash out, [Mg\textsuperscript{2+}]_e was significantly increased, accompanied by decreases in HR. In H9c2 cells, fluoxetine produced significant activation of ERK 1/2 and a dose-dependent increase in the [Mg\textsuperscript{2+}]_i, which were inhibited by pretreatment of PD98059, an ERK 1/2 inhibitor. Phosphorylated-ERK 1/2 in isolated hearts were increased by treatment of fluoxetine. Fluoxetine caused change of ECG including prolongation of PR, R-R and QT interval.

Conclusion
Fluoxetine can produce cardiac depression partly through an increase in [Mg\textsuperscript{2+}]_i and [Mg\textsuperscript{2+}]_e accompanied by the activation of ERK 1/2.

Keywords: Fluoxetine, Cardiac Function, Mg\textsuperscript{2+}

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Prevalence of Severe Fever with Thrombocytopenia Syndrome Virus from Single Tick in the Republic of Korea

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Purpose
Severe fever with thrombocytopenia syndrome (SFTS) is viral infectious disease caused by SFTS virus (SFTSV), and ticks are thought to be a potential vector of this novel virus. This study was carried out to investigate the prevalence of SFTSV in each individual nymph/adult ticks from Republic of Korea.

Materials and Methods
Ticks were collected from March to November 2016 in Ganghwado, Sukmodo and Deogyusan National Park by flagging and sweeping methods. Each collected tick was identified by species and developmental stages following taxonomic identification keys using stereomicroscope. After identification, each single of nymph or adult tick was homogenized, and the viral RNA was extracted from supernatant of the homogenates. One-step reverse transcription (RT)/nested polymerase chain reaction (PCR) was performed to detect small (S) segments of SFTSV.

Results
In total, 3,301 nymph and adult ticks were collected from Ganghwado, Sukmodo and Deogyusan National Park. The most collected ticks were Haemaphysalis longicornis (97.27%), followed by Haemaphysalis flava (1.94%), Ixodes nipponensis (0.76%), and one Ixodes ovatus, respectively. S segment of SFTSV was detected from H. longicornis, H. flava and I. nipponensis ticks, and the infection rates were 7.75% (231/2,980), 1.59% (1/63) and 13.64% (3/22), respectively. The infection rate of SFTSV in total collected ticks was 7.12% (235/3,301).

Conclusion
This study presents the prevalence of SFTSV infection in individual nymph and adult ticks, and shows difference in infection rate with prior researches.

Keywords: SFTS, Tick, Prevalence

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Isolation of Severe Fever with Thrombocytopenia Syndrome Virus from Dogs in Korea

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Purpose
The aim of this study is to investigate the prevalence of severe fever with thrombocytopenia syndrome virus (SFTSV) in large dog breeds and attempted to isolate SFTSV from positive dog sera.

Materials and Methods
Canine bloods were collected from 103 dogs in Gangwon Province (Korea) from June to October 2016. Sera were separated from whole bloods by centrifugation and were used for viral RNA extraction. One-step RT-nested PCR was performed to detect viral RNA gene from serum samples. To isolate SFTSV from positive canine serum, Vero cells were used. After Vero cell monolayers formed, positive serum was added into 12 well plates. The plates were then incubated at 37°C with 5% CO₂ for 5 - 7 days. For identification of isolated virus, the SFTSV S, M, and L segment genes were amplified using RT-PCR. Additionally, indirect immunofluorescent antibody assay (IFA) was performed for confirming.

Results
SFTSV was detected in 3 samples using RT-nested PCR targeting the S segment. Of the three SFTSV positive sera, only one serum was positive in the virus isolation test. The S, M, and L segments of the viral isolate were sequenced. Using Vero cell infected with the isolated virus, IFA was conducted. Until 1,600-fold dilution, infected Vero cell was positive against SFTSV antibody.

Conclusion
This is the first report of SFTSV isolation from dogs in Korea.

Keywords: Severe Fever with Thrombocytopenia Syndrome Virus, Dog, Korea

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Triple Different Synchronous Tumors of Sinus, Kidney and Penis in a Dog

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**Purpose**
In the case, ‘Triple different synchronous tumors of sinus, kidney and penis’ in a dog was defined as clinically and pathomorphologically.

**Materials and Methods**
Male-mongrel-dog from an animal shelter was brought to Surgery-Department with suspected sinus tumor. After euthanasia, dog was sent for necropsy. Frontal, maxillary and ethmoidal sinuses fully were covered with mass. Another mass was encountered in medulla of the right kidney. Also, tumor was observed on the penis. Then they were stained with hematoxylin-eosin(H&E) and Masson’s Trichrome.

**Results**
Histopathologically, in the mass of sinus, neoplastic epithelial cells were arranged in acinar shape in large areas. They had oval or round, large, normo/hyperchromatic nuclei, eosinophilic cytoplasm and different shapes and sizes. In some places the lumen was completely filled with neoplastic cells. These cells were accompanied by mitotic figures. Uniform smooth muscle cells are elongated with eosinophilic or occasional fibrillar cytoplasm and distinct cell membranes in tumor of the kidney. They were arranged in fascicles and interlacing bundles and separated by well vascularized connective tissue. Atypical polygonal neoplastic cells were encountered in the form of solid areas or thin bands in the mass of the penis. They usually had oval/round hyperchromatic nuclei, a distinct eosinophilic nucleolus and narrow cytoplasmic borders. Therefore, the tumours were respectively diagnosed as ‘Acinar type adenocarcinoma, leiomyoma, transmissible venereal tumor’.

**Conclusion**
As a result, there are not much articles in animals related to triple different synchronous tumors. So, this case has been seen as worth sharing.

**Keywords:** Dog, Pathomorphology, Synchronous

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Purpose
Understanding the appropriate use of veterinary medical devices is important for the animal's health and also veterinarian's diagnosis and treatment. The purpose of this study was to investigate awareness of safety and adverse events in veterinary medical devices in Korea.

Materials and Methods
The questionnaire survey on awareness and the adverse events of veterinary medical devices was conducted to veterinarians and providers. The questionnaire was designed to assess: the classification of veterinary medical devices; the purchase, usage and inconvenience of medical devices; awareness of safety and effectiveness of these devices; and finally, occurrence of adverse events associated with veterinary medical devices.

Results
A total of 146 questionnaires including 22 from veterinary providers and 124 from veterinarians were returned. Most veterinary providers (90%) recognized the classification. However, only 44% of users were aware of current classification of veterinary medical devices. Among the responded veterinarians, 47% had been experienced adverse events associated with veterinary medical devices. 30% of the events had moderate to severe effects to the patients, and 7% of the adverse events caused death of the patients.

Conclusion
This study revealed that the needs for establishing the systematic management program to ensure the safety and effectiveness of medical devices for animals. Also the system was not established in our country, and this is the first effort to investigate adverse events in veterinary medicine.

Acknowledgment
This research was supported by a Grant from the Animal and Plant Quarantine Agency, republic of Korea(Z-1543072-2015-16-02).

Keywords: Veterinary Medical Devices, Safety, Adverse Events

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IM-0171

Uterine Primitive Neuroectodermal Tumor (Ewing’s Sarcoma, PNET) in a Boxer

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Purpose
In the case, ‘Uterine primitive neuroectodermal tumor’ in a boxer was defined as clinically, pathomorphologically and immunohistochemically.

Materials and Methods
A-Boxer-breed-dog was taken to referred with vaginal bleeding to Department of Obstetrics and Gynecology. After clinical examination, ovariohysterectomy was performed. For histopathological examinations, the samples were stained with Haematoxylin-Eosin(H&E) and Masson's trichrome stains. Immunohistochemically, single and double staining were made by using Avidin-Biotin-Complex Peroxidase method (ABC-P). P53, cytokeratin(CK)8 and 19, progesteron, eostrogen, nestin, CD99 and Syntaptosin as primary antibodies were used single staining method. For double staining method, were matched with Vimentin/actin and P63/CK19 combinations.

Results
Histopathologically, neoplastic cells often had ovoid/round hypochromic distinct nuclei, large prominent nucleoli, large eosinophilic or clean cytoplasm. Numerous mitoses were seen. In addition, neutrophil leukocytes, plasma cells, lymphocytes and macrophages and necrotic areas with erythrocytes were found among these cells. Immunohistochemically, while P53, CD99, and Syntaptosin antibodies were positive, CK8,CK19, eostrogen, nestin and progesteron were negative for single staining. P63/CK19 was negative for double staining. But for Vimentin/actin; vimentin was positive in cytoplasm of tumor cells, actin was positive only at muscles. Thus, the case was diagnosed as ‘Uterine PNET’.

Conclusion
Primitive neuroectodermal tumor (PNET), derived from neuroectoderm, is a rare malignant neural crest tumor. The tumor is seen rare in human uterine and was mostly recorded in brain parts in cattle, monkey, dogs or cats. As result, it is thought that the case would be first report of ‘Uterine PNET’ only in uterus of a dog at veterinary literatures.

Keywords: Dog, Primitive Neuroectodermal Tumor, Uterus

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Immunity Analysis Kit for Canine: A Novel Tool for the Analysis of NK Cell Activity Using Whole Blood

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Purpose
NK cells perform critical roles in the innate immune response against pathogens and tumors. Functional impairment and a low activity of NK cells were observed in many disease condition including cancer and viral infection. Thus NK cell activity has been suggested a surrogate marker of general immunological functions. In this study, we developed a simple assay to determine canine immunity using a small amount of whole blood.

Materials and Methods
Eighty healthy beagle dogs and twenty seven unhealthy dogs from veterinary hospital were used in this study. All dogs were diagnosed based on histologic and/or biochemical evaluation. NK cell activity was determined using the canine NK activity kit (ATGen).

Results
Our assay system was designed on the premise that more potent NK cells secrete higher levels of IFN-γ when activated. Using a proprietary stabilized immunomodulatory cytokine, Promoca, NK cells were stimulated in whole blood. After their activation a quantitative sandwich ELISA was used to determine the levels of IFN-γ. As expected, Promoca induced IFN-γ production and its levels were significantly higher in healthy canine, than in unhealthy ones with severe diseases

Conclusion
These results suggest canine NK cell activity may be a potential tumor biomarker for dogs. Also this novel assay is suitable for high-throughput monitoring of immunity and health condition of dogs.

Keywords: Canine, Nk Cell, Immune Potency

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**Purpose**
Extraskeletal osteosarcomas are defined as osteoid-producing mesenchymal tumors without primary bone engagement. Extraskeletal osteosarcomas are highly malignant and rare enough to be comprised 1% of all osteosarcomas excluded from mammary origin. In contrast to humans, most canine extraskeletal osteosarcomas are arising from visceral organ rather than soft tissue. So we report a case of extraskeletal osteosarcoma which occurs in soft tissue of a Maltese dog.

**Materials and Methods**
A 10-year-old Maltese dog had a 4x3x3-cm mass in subcutaneous of shoulder. Excised mass was submitted in 10% formalin, embedded in paraffin, sectioned at 4μm, and stained with H&E and alcian blue for microscopic examination. Immunohistochemical staining was performed with antibody for pan-cytokeratin, vimentin, osteocalcin, S100 and C-kit.

**Results**
Microscopically, the mass consists of malignant mesenchymal cells producing a chondroid matrix and osteoid. Neoplastic cells display moderate anisokaryosis and variable numbers of mitotic figures. There was positive staining of chondroid material with Alcian blue. Immunohistochemically, neoplastic cells were positive for vimentin and osteocalcin, negative for pan-cytokeratin, S-100 and C-kit.

**Conclusion**
Base on histopathologic and immunohistochemical features, this case was diagnosed as extraskeletal chondroblastic osteosarcoma. We report a rare case of extraskeletal chondroblastic osteosarcoma in dog and in our best knowledge this is first report of extraskeletal chondroblastic osteosarcoma of soft tissue in a Maltese dog. The authors declared no potential conflicts of interest with respect to the research. This research was supported by the Bio&Medical Technology Development Program of the NRF funded by the Korean government, MSIP(2016M3A9B6903437).

**Keywords**: Dog, Extraskeletal Osteosarcoma, Immunohistochemistry

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Purpose
Perianal gland tumor is common skin tumor in the dog, but little is known about its immunohistochemical features. It is generally accepted that perianal gland tumor is affected by sex hormone, androgen. The aim of this study is to investigate expression of other sex steroid hormone receptors, ER-α, ER-β, PR. We also analyzed p63 and PTEN known as homologue of p53 and tumor suppressor, respectively.

Materials and Methods
Total 68 perianal gland tumors were examined and classified as adenomas (n=36) and carcinomas (n=32) on H&E sections. Immunohistochemistry for each markers was performed and analyzed. All markers except p63 were scored based on percentage of labeled cells and intensity. Expression of p63 was evaluated by the numbers of cells which showed nuclear staining. Chi-square test for PTEN and Student's t-test for all the other markers were conducted to analyze expression.

Results
Expression of ER-α (P=0.0018), ER-β (P=0.003) and PR (P=0.000) was higher in perianal gland adenomas than in carcinomas. There were no differences between adenomas and carcinomas in p63 and PTEN expression level.

Conclusion
Canine perianal gland tumor may be influenced by progesterone and estrogen, not only by androgen. Complex interactions of these hormones would be the key for proliferation of perianal gland tumors. The author(s) declared no potential conflicts of interest with respect to the research. This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2016M3A9B6903437).

Keywords: Perianal Gland Tumor, Immunohistochemistry, Sex Hormone

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An in Vivo Immunomodulatory and Anti-Inflammatory Study of Dendropanax Morbifera Leveille

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Purpose
Dendropanax morbifera Leveille, an economically and medicinally important subtropical tree, is endemic to Korea and have anti-complement, anti-thrombotic, anti-diabetic, anti-atherogenic, anti-cancer and anti-oxidant activity. However, there is no documentation on the in vivo immune activity of the plant. Hence, this study was designed to determine the immunomodulatory activity of D. morbifera extract in mice.

Materials and Methods
A five weeks old female BALB/c mice, arranged in six groups, were used for the 21 day experiment. Splenocyte count was measured with a hemocytometer by the trypan blue dye exclusion method and splenic lymphocyte proliferation assay were determined using MTT assay. Flow cytometry was performed for phenotyping T-lymphocyte. The cytokine and immunoglobulin quantitation was done using sandwich ELISA.

Results
Spleen cells were higher in mice treated with D. Morbifera. In both, in vivo and ex vivo experiment, proliferation of splenocytes by concanavalin A is twice higher than that of LPS. Phenotyping of T-cells indicated that treated groups showed higher CD8α+ and CD3+ T-cell expression. However, treatment groups IL-1α, IL-1β, IL-4 and IL-9 levels and immunoglobulins (IgA, IgG1, IgG2A, IgG2B, IgG3 and IgM) were suppressed.

Conclusion
Dendropanax morbifera Leveille is important for T-cell proliferation and has immunomodulating activity. However, they also suppressed immunoglobulins.

Keywords: Dendropanax Morbifera Leveille, Immunomodulation, T-Cell Proliferation

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Pharmacokinetics of Mixture of Florfenicol and Tylosin After Single Intravenous and Intramuscular Administration to Beagle Dogs

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Purpose
The study was designed to determine the pharmacokinetics parameters of florfenicol and tylosin following administration of an injectable solution containing mixtures of florfenicol and tylosin tartrate in dogs.

Materials and Methods
A randomized crossover design was conducted in two groups of dogs with three animals each. An injectable solution (FTD-inj, Shinilbiogen Co., Korea) containing florfenicol (100 mg/ml) and tylosin tartrate (50 mg/ml) were used to treat the animals. Animals received 5 mg/kg (10 mg/kg) florfenicol and 2.5 mg/kg (5 mg/kg) tylosin through the jugular vein, whereas similar doses of the product were administered to the other group through the inner thigh muscle. Blood samples were taken at different time point and serum drug concentrations were determined by a validated high-performance liquid chromatography.

Results
A rapid and nearly complete absorption of both drugs with a mean bioavailability of 103.9% (florfenicol), 92.6% (tylosin), and prolonged elimination half-life with steady-state volume of distribution of 2.63 L/kg (florfenicol) and 1.98 L/kg (tylosin) were found in dogs treated with 15 mg/kg of the product. However, dogs treated with 7.5 mg/kg of the mixture showed lower bioavailability, reduced volume of distribution and shorter elimination half-life of florfenicol as compared with the findings at 15 mg/kg dose.

Conclusion
Pharmacodynamic and toxicological studies are required before recommendations can be made regarding the clinical application of the product in dogs.

Keywords: Florfenicol, Pharmacokinetics, Tylosin

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Antitumor Effects of SB Injection, Extract from Phytopharmaceutical Preparations, in Canine Malignant Cell Lines

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Purpose
The purpose of this study is to evaluate the effects of SB injection, an antitumor agent obtained from natural extracts, on canine cells

Materials and Methods
The antitumor effect of SB injection in D17 (canine osteosarcoma cells) and LMeC (canine melanoma cells) was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, cell cycle analysis, and annexin-V assay.

Results
Cell viability was proportional to the concentration of chemical agents. The cell cycle of the affected cells was arrested in the G2/M phase that led to an anti-proliferation effect. SB increased the rate of apoptosis dose-dependently. It is also used in combination with chemical drugs to reduce its dosage. SB showed no effect on the viability of PBMC (peripheral blood mononuclear cells) regardless of concentration, which suggested that SB does not suppress the activity of normal cells.

Conclusion
This study suggests that SB can be considered as a relatively effective and safe alternative medication for animal cancers in veterinary medicine.

Keywords: Antitumor, SB Injection, Canine Tumor

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Flowcytometric Analysis of Th1/Th2/Treg Cells in a Dog with Focal Myasthenia Gravis

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Purpose
The purpose of this study is to establish the possible immunological correlation between the Th cells subsets and Treg cells in the development of Myasthenia gravis (MG)

Materials and Methods
Changes in helper T cell subsets including Th1 and Th2, and regulatory T cells were investigated according to their cell surface markers and their signature cytokines using flow cytometry in a dog with focal MG compared to those of a healthy dog.

Results
The percentages of Treg cells were increased along with the increase of both Th1 and Th2 cell subsets in a dog with focal MG compared to those in a healthy dog.

Conclusion
Despite the increased percentage of Treg cells in a dog with MG in this study, the function of maintaining the self-tolerance of these Treg cells is considered to be impaired, resulting in the increased proliferation of Th1 and Th2 cells. This could be a possible evidence of increased auto-reactive T cells in the MG development.

This work was carried out with the support of “Cooperative Research Program for Agriculture Science” Technology Development (Project title: Functional feed material development and efficacy evaluation for detector dog’s ability improvement, Project No: PJ011989) Rural Development Administration, Republic of Korea.

Keywords: Focal Myasthenia Gravis, Helper T Cells, Regulatory T Cells, Th1, Th2

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Secondary Bacteremia Following Natural Parvovirus Infection in 35 Dogs

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Purpose
Canine parvovirus is a highly contagious virus and commonly causes severe gastroenteritis in puppies. The virus infects at the crypt epithelial cells then disrupts the gastrointestinal integrity. The objectives of this study were to identify the secondary bacteremia following parvovirus infection and the antibiotic susceptibility patterns in thirty-five infected parvovirus dogs.

Materials and Methods
The study period was during September 2016 to March 2017. Commercial enzyme-linked immunosorbent assay (ELISA) antigen test kits were used to confirm the disease. There were 35 dogs with positive parvovirus in this study. Routine treatments with fluid and antibiotic were performed. One ml of blood samples were collected from jugular vein with sterile technique then incubated in Brain heart infusion broth.

Results
Eleven of thirty-seven sample (11/37 or 29.73%) from 35 dogs had positive blood cultures. Gram-negative bacteria isolations were Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter species (27.27%, 18.18%, 18.18%, and 9.09% respectively). Three of eleven sample (3/11 or 27.27%) was found Staphylococcus coaglase gram-positive bacteria.

Conclusion
Positive bacteria isolations from blood culture were found although intravenously broad spectrum antibiotics were given. Gram-negative bacteria were commonly isolated and reported as they originated from the intestine of itself. Presenting of Staphylococcus coagulase in blood culture was suspected as contamination. Ciprofloxacin, cotrimoxazole, tetracycline and clindamycin shouldn't be select due to 100 percent resistant to Escherichia coli. Gentamicin showed strongly effect to cure Escherichia coli however must use with caution in young animal.

Keywords: Bacteremia, Canine, Parvovirus Infection

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Short and Long Term Pathologic Effects and Cellular Damage on Kidney of Experimental Radioiodine Treatment in Rats

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Purpose
The aim of this experimental study was investigated of short and long term kidney damage in rats with Radioiodine $^{131}$I (RAI) treatment.

Materials and Methods
Thirty rats were divided into three groups. Treatment groups were administered a single dose of 3 mci $^{131}$I by gastric gavage. One week (early term) and eight weeks (long term) after $^{131}$I administering animals were sacrificed and the kidneys were removed for histopathological, immunohistochemical and apoptotic examination. For the histopathological examination hematoksin-eosin and trichrome; for immunohistochemical examinations streptavidin-biotin-peroxidas was performed. For determination of RAI in the kidney NIS; identify of inflammatory cells CD19, CD20, CD68; for the cell proliferation PCNA and Ki67; for detection of apoptosis Caspas-8, Caspas-9 and TUNEL staining was applied.

Results
Histologically, focal interstitial nephritis, interstitial fibrosis, thickening of bowman capsule, tubule epithelial degeneration and necrosis were seen. In late term findings were more severe than short term, and also kidney calculi were seen in the tubules. Immunohistochemically; medullary tubules and renal papillary were more severe immunopositive for NIS than tubules in kortekeks, whereas glomeruli were negative. Similar immunopositivity were seen for PCNA and Ki67 antibodies. Caspas-8, Caspas-9 and TUNEL staining were markedly positive in medulla and medullary papilla. In addition, inflammatory cells were immunopositive with CD19, CD20 and CD68.

Conclusion
According to these results, patients treated with radioactive iodine suffer from thyroid cancer and hypothyroidism can occur damage in their kidney.

Keywords: Apoptosis, Kidney, Immunohistochemistry, Radioiodine, Toxication

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Radioprotective Effect of Montelukast Sodium Against Hepatic Radioiodine ($^{131}$I) Toxicity: A Histopathological Investigation in the Rat Model

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**Purpose**
This study aimed to evaluate the histopathological changes in rat livers at the third month following $^{131}$I treatment and the radioprotective effect of Montelukast sodium (ML) against $^{131}$I-related liver damage.

**Materials and Methods**
Thirty female Wistar Albino rats were randomly divided into three groups as control group (n=10), untreated rats; second (RAI) group (n=10), oral radioiodine (111 MBq) administrated rats, and third (ML) group (n=10), oral radioiodine and ML administrated rats. In the third group, ML administration was started 3 days before and ended 10 days after RAI administration. In the third month of radioiodine (RAI) administration, the animals were decapitated and the livers were removed for histopathological examination.

**Results**
In the comparison of the $^{131}$I and ML groups, hyperemia was determined respectively 80% to 40%, the presence of inflammatory cells 70% to 60% and capsule thickening 70% to 40%. Montelukast sodium was observed to have a protective effect especially on hyperemia and capsule thickening.

**Conclusion**
According to the study results, radioactive iodine ($^{131}$I) treatment seems to cause morphological damage to the rat liver, and Montelukast sodium effectively protects the liver against damage.

**Keywords**: Iodine-131, Liver, Histopathology, Montelukast Sodium, Rat

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**Organ-Specific Expression of Divalent Ion Channels in Canine Duodenum, Kidney, Spleen and Liver**

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**Purpose**
Cation channel include calcium channels, copper channels, iron channels. Each channel is physiologically significant and have been related to a lot of diseases. Previously, lots of studies researched about cation channels because of their physiological importance. But, the comparative study of transcriptional-translational levels of these molecules in canine organs has not been performed.

**Materials and Methods**
In this study, organ-specific expression of cation channels were examined; calcium channels (NCKX3, TRPV2), copper channels (CTR1, ATP7A), iron channel (IREG1) and ferroxidase for iron transporting (HEPH) in canine duodenum, kidney, spleen and liver.

**Results**
The NCKX3 protein expression was highest in the kidney, moderate in duodenum, and low in spleen and liver. In case of the TRPV2 protein, it was highly expressed in kidney, duodenum and liver. In contrast, it was low in spleen. The CTR1 protein levels were highest in the liver followed by duodenum, kidney and spleen. The ATP7A protein levels were highest in duodenum, but lowest in spleen. The IREG1 protein levels was highest in the liver followed by kidney, duodenum and spleen. In the case of HEPH protein, the levels were so high in liver, moderately high in duodenum and kidney, low in spleen. Results of the immunohistochemistry demonstrated that the cation channels were localized in intestinal villi, Red pulps and macrophages in spleen, hepatocytes, Kupffer cell, and tubules and glomerulus of the kidney.

**Conclusion**
These results suggest that cation channels are differently expressed among on organs, and they may be involved in organ-specific functions to maintain physiological homeostasis.

**Keywords**: Canis Lupus Familiaris, Canine Organ, Divalent Ion Channel

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Effect of in Ovo Injection of Plant Originated Aromatase Inhibitors on Sex Differentiation of Broiler Chicken

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Purpose
The present study conducted to survey the effect of in ovo injection of Nettle (Urtica dioica) extract, mushroom extract and fadrozole on sex differentiation of broiler chickens.

Materials and Methods
Three hundred fertile egg numbers from Ross 308 strain broiler breeders were divided into 5 groups with 4 replications. At the beginning of the fifth day of incubation in ovo injection operation was carried out. All hatched chickens than were reared in a completely randomized design for 42 days.

Results
The result indicated that in ovo injection of 0.1 ml of fadrozole, mushroom and nettle extracts led to 100, 66.67 and 37.5 percent sex reversal, respectively (P≤0.05). At total rearing period, daily feed intake and daily weight gain of fadrozole and mushroom extract were significantly higher than the control and nettle extract groups (P≤0.05). Compared to other treatments, in ovo injection of mushroom extract caused a significant increase in the average number of muscle fibers and the average of fiber diameter (P≤0.05). There were no significant differences in live weight, carcass weight and carcass cuts weight of chickens hatched from aromatase inhibitors treated eggs and those hatched from control eggs.

Conclusion
The findings of this study suggest that broiler sex reversal using aromatase inhibitors resulting to increases the growth rate of broilers. Also high anti-aromatase characteristic of mushroom extract can be a good alternative to chemical drugs such as fadrozole hydrochloride.

Keywords: Aromatase Inhibitors, Sex Differentiation, Broiler Chicken

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The Effectiveness of Goat Milk Bio-Peptide to Reduce Free Radicals on Lung Cancer in Rat

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Purpose
The objectives of the research to prove that goat milk bio-peptide has potentially to reduce lipid peroxidase on the lung cancer in rats indicate by serum malondialdehyde (MDA) and superoxide dismutase (SOD) levels.

Materials and Methods
A total of 12 male Rattus norvegicus averaging two months of age were used as an animals trial, and divided into four groups. Four treatments include; P0 group which classified as normal rats; P1 the group of lung cancer of rats without therapy; P2 the group of lung cancer of rat with goat milk bio-peptide type-2 therapy. P3 the group of lung cancer of rats goat milk bio-peptide type-3 therapy. Artificial lung cancer of rats prepared by induction of benzopyrene with the dose 200 mg/kg body weight every day during four days and incubated during 4 weeks. Only rats in group P2 and P3 treated by goat milk bio-peptide about 200 µml/h/d per oral as well as the type of goat milk bio-peptide treatment group for 1 week. At the end of the research, the blood samples of each rat in each group were collected and the blood serum analyzed to MDA and SOD levels by using TBA method and Northwest kit SOD, respectively.

Results
Results of the research showed the MDA level of lung cancer of rat decreased significantly (P<0.05) when treated by goat milk bio-peptides (F2 and F3), however, the level of SOD increased significantly (P<0.05) in these treatments group.

Conclusion
It was concluded that goat milk bio-peptides were effective as an antioxidant.

Keywords: Bio-Peptide, Goat Milk, Lung Cancer Rat, MDA, SOD

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Development of Pet Food Formulation Software for Dogs and Cats

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Purpose
Feed-formulation software using linear programming is commercially available and capable. However, most of the commercial feed-formulation software has two drawbacks: cost, and the calculations are something of a 'black box' with only the inputs and the final results visible. The objective of this study was to develop a simple and scientific pet food recipe program for companion animals.

Materials and Methods
A database of basic feed materials for companion animals was constructed using the Korean food and nutrient database, information on agricultural and fishery prices, and the National Institute of Animal Science database. In order to calculate the minimum price feed ratio, the Excel solver function was designed to be implemented on the web, and the NRC and AAFCO standards were applied to calculate nutrient profiles and feed rates according to dog and cat growth, weight and activity levels.

Results
The easy-to-feed formula for companion animals is based on selecting the type, breed, growth stage, gender, activity level of the companion animal, the amount of water and the total amount of feed, and then click on the Calculate feed rate button to generate the minimum cost custom feed recipe. In this process, the program user can precisely control the animal's nutritional requirements or the amount of feed materials used. This program can be found at www.nongsaro.go.kr/petfood.

Conclusion
This program will be a scientific and systematic tool for animal nutrition professionals to prescribe pet food suitable for companion animals and educate companion animal owners.

Keywords: Pet Food, Formulation, Software, Dog, Cat

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Gundersen Inlay Flap and Thermokeratoplasty for the Management of Corneal Endothelial Cell Degeneration in Dogs

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Purpose
The purpose of the study was to compare and evaluate the effectiveness of surgical therapies, including Gundersen inlay flap and thermokeratoplasty in the dogs diagnosed with corneal endothelial cell degeneration.

Materials and Methods
Seven eyes of five dogs (10.1± 0.9 years old) were presented with cloudy eyes, reduced vision and ocular pain. After regular ophthamlic examination, the patients were diagnosed with progressive corneal endothelial cell degeneration. All of the affected eyes were refractory to medical treatments. Each affected eye was treated with a superficial keratectomy followed by placement of (i) conjunctival hood flaps (2 eyes), (ii) pedicle flaps (2 eyes), (iii) "Letter box" flaps (concurrent conjunctival hood flaps on dorsal and ventral region of cornea) (2 eyes), and (iv) thermokeratoplasty (1 eye).

Results
Although all surgical treatments resulted in reduced ocular pain, epithelial bullae, cornea ulcer and corneal edema, and increased corneal transparency, "Letter box" flap showed better improvement in corneal transparency and vision of patient than others.

Conclusion
"Letter box" flaps might be a surgical alternative technique providing better corneal transparency to the refractory canine patients with corneal endothelial cell degeneration. Moreover, the conventional thermokeratoplasty could still be an effective technique enough to give a better quality of life to the patient with greater risk for anesthetic complications.

Keywords: Corneal Endothelial Cell Degeneration, Gundersen Inlay Flap, Thermokeratoplasty

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 Treatment of Acute Bullous Keratopathy with Third Eyelid Flap in a Cat

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\textbf{Purpose}
A 3-year-old castrated male Korean Shorthair cat was presented to the Animal Medical Center of the Jeonbuk National University with a history of sudden blepharospasm and formation of bullae on the cornea of the right eye (OD).

\textbf{Materials and Methods}
In ophthalmologic examination, menace response, dazzle reflex, and pupillary light reflex were present in both eyes (OU). Intraocular pressure of OD was 7mmHg. During slit-lamp examination, corneal edema and bullous lesions in the cornea of OD were found. After fluorescein staining, the tip of the bullae was stained. Based on these results, it was diagnosed as Acute Bullous Keratopathy (ABK). As a treatment, third eyelid flap was performed. In addition, topical Ofloxacin (Samil) q6h, topical 5\% Sodium Chloride (Bausch & Lomb Inc., Tampa, FL) q6h and oral cephalixin (22mg/kg; Ashish Life Science Pvt. Ltd.) q12h were prescribed.

\textbf{Results}
Third eyelid flap was removed after 21days. The corneal bullae lesion of OD was resolved and the corneal edema and vascularization were almost disappeared.

\textbf{Conclusion}
There are a few options for treatment of ABK including temporary tarsorrhaphy, 360-degree conjunctival grafting and third eyelid flap. However, there is no comparative data among the treatments because ABK is an unusual condition. In this case, third eyelid flap was applied and found that it could be a good option for Acute Bullous Keratopathy.

\textbf{Keywords:} Acute Bullous Keratopathy, Third Eyelid Flap, Cornea Bullae, Cat, Treatment

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**Corneal Intrastromal Abscess in a Shih-Tzu Dog**

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**Purpose**
To present a case of intrastromal abscess diagnosed by ultrasound biomicroscopy (UBM) and treated by intrastromal flushing with balanced salt solution (BSS) consisted of antibiotics and steroids.

**Materials and Methods**
An 8-year-old female Shih-Tzu dog was referred for the assessment of suspicious intracorneal material of yellow color in OD for 3 years. Neuro-ophthalmic examination, including menace response and pupillary light reflexes, was normal. Slit lamp biomicroscopy showed yellow lesion of the peripheral cornea at 8 to 4 o’clock accompanied by superficial corneal neovascularization and edema in OD. Corneal fluorescein staining was negative in OU. There are no other abnormalities except for incipient cataract OD and corneal opacity OS. UBM revealed hypoechoic masslike lesion surrounded by hyperechoic edematous cornea, which separated the stroma at a depth of three fourths of the cornea. Partial thickness corneal incision around the lesion was performed under topical anesthesia. After sampling for cytology and culture, intrastromal flushing was performed using combined cefazolin and dexamethasone diluted 10-fold with BSS.

**Results**
Necrotic lesion was identified on cytologic examination with no microorganisms or neoplastic lesions being detected. Neither aerobic nor anaerobic microorganisms were detected in the culture. Topical and systemic antibiotics and anti-inflammatory drugs were applied on the eye under bandage-lens. Two weeks later, UBM was performed again to confirm that there was no recurrence. Corneal neovascularization and edema gradually decreased until last examination.

**Conclusion**
Intrastromal flushing under topical anesthesia could be an easy, noninvasive treatment method for corneal intrastromal abscess.

**Keywords:** Cornea, Intrastromal Abscess, Intrastromal Flushing, Topical Anesthesia, Ultrasound Biomicroscopy

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The Effect of Short-Term Tocopherol Against Radioiodine (\(^{131}\)I) -Induced Early Lacrimal Gland Damage

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**Purpose**
Radioiodine (\(^{131}\)I) is a well-known radionuclide which is used in vivo both for diagnostic and therapeutic purposes, particularly for the treatment of hyperthyroidism in cats and dogs. The aim of this study was to evaluate whether there was a protective effect of short-term tocopherol on \(^{131}\)I induced adverse effect of lacrimal glands early damage in experimental animal models.

**Materials and Methods**
Twenty-four rats were randomly divided into two groups. The first group (\(^{131}\)I group) was administrated 3 mCi \(^{131}\)I by gastric gavage and 1 mL physiological saline intraperitoneally. The second group (\(^{131}\)I + Tocopherol) was administrated 3 mCi \(^{131}\)I by gastric gavage and 1 mL tocopherol intraperitoneally. After 24 h of the last dose being administered on the 7th day, the animals were decapitated. The Intraorbital (IG), extraorbital (EG) and harderian glands (HG) of the rats were removed for histopathological examination.

**Results**
Periductal and periacinar fibrosis in all lacrimal glands were observed to be statistically significantly less frequent in the \(^{131}\)I + Tocopherol group compared to the \(^{131}\)I group. The existence of the abnormal lobular pattern and peripheral basophilia and irregular nucleus shape in IG and EG, the poorly defined acidophilic cell outline and periductal infiltration in IG and in HG were observed to be statistically significantly less frequent in the \(^{131}\)I + Tocopherol group than in the \(^{131}\)I group.

**Conclusion**
According to study results, histopathological examinations revealed that tocopherol protects rat lacrimal glands against \(^{131}\)I -related early damage.

**Keywords:** Radioiodine, Tocopherol, Lacrimal Gland

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Outcome of Pars Plana Retinopexy with Perfluoro-N-Octane - Silicone Oil Exchange for Rhegmatogenous Retinal Detachment in Dogs: 9 Eyes

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Purpose
To describe the outcome of pars plana retinopexy with perfluoro-n-octane (PFO)-silicone oil exchange in dogs with rhegmatogenous retinal detachment in Seoul National University Veterinary Medical Teaching Hospital (SNU VMTH) from 2014-2017.

Materials and Methods
Medical records from eight dogs (9 eyes) underwent retinopexy in SNU VMTH from 2014-2017 were evaluated. Data were collected including signalment, duration from onset of blindness to surgical intervention, duration from surgery to regain vision, and postoperative complications.

Results
No eyes were visual before surgery. Of six eyes that regained functional vision, five remained visual until the last follow-up while one eye lost vision at 3 months postoperatively because of uveitic glaucoma. One of three eyes that failed to regain functional vision showed a weak positive menace response one day post-operation and maintained it for six days while the remaining two eyes never restored vision. Duration from onset of blindness to surgical intervention was 2-30 days (median 8 days); time for regaining vision was 1-14 days (median 6 days); follow-up time was 15-1090 days (median 44 days). Postoperative complications noted including corneal ulcer (7/9), uveitis (7/9), retinal degeneration (6/9), anterior chamber migration of silicone oil (6/9), corneal degeneration (4/9), retinal haemorrhage (4/9), re-detachment (4/9), glaucoma (3/9), subconjunctival leakage of silicone oil (2/9), vitreal haemorrhage (2/9), and chemosis (1/9).

Conclusion
Pars plana retinopexy with perfluoro-n-octane (PFO)-silicone oil exchange provided good outcome in dogs with retinal detachment in 66.67% cases described in this study.

Keywords: Retinopexy, Retinal Detachment, Silicone Oil, Blindness, Dog.

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Clinical Outcome and Synovial Fluids Analysis of TTA-Rapid in Dogs with Cranial Cruciate Ligament Rupture

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Purpose
The objective of the study was intended to apply the modified (TTA)-Rapid methods in dogs with cranial cruciate ligament rupture and report clinical outcome, complications and synovial fluids analysis.

Materials and Methods
The study materials was 17 dogs which were obtained with lameness in the hind limbs by primarily systemic and clinical examination. Radiographs of the affected joint obtained at weeks 0, 4, 8, 12 were examined. TTA-rapid surgery was followed by (Samoy et al., 2015). Lameness and pain were assessed by Hudson Visual Analog Scale and Canine Brief Pain Inventory. Synovial fluid gelatinase activity levels were determined by gelatin zymography (Coughlan et al.,1998).

Results
Arthroscopic examination and arthrotomy was shown no meniscus damage. On clinical examination at 3 months, 8 dogs (41.1%) had an excellent outcome, 6 dogs (35.2%) had a good outcome, and 3 dogs (17.6%) had a moderate outcome. Metalloproteinases activity were increased of 70 kDa enzyme in pre-operatif treatment synovial fluids. Enzyme activity was also seen at 204 kDa (MMP-9 pro-dimer) and 257 kDa (MMP-9 dimer). 100 kDa (MMP-9) band was totally inhibited within 60th day in some cases. The 70 kDa enzyme (MMP-2) was partially inhibited.

Conclusion
TTA-Rapid, relatively non-invasive soft tissue approach. Synovial fluids analysis for gelatinases were shown encourage results to decreased MMP 2 and MMP-9 activity after arthrocentesis in TTA-rapid group. Further studies must be needed to determine if the results remain the same in larger groups.

Keywords: TTA-Rapid, MMPs, CCL

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Cementless Pamuk’S Total Hip Replacement (PTKP) Implementation and the Results: The First Evaluation in Dogs

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Purpose
In this study, application and results of Pamuk's Total Hip Replacement (PTKP) system was evaluated the dogs with hip problems.

Materials and Methods
Twelve dogs with hip problems between 1-10 years of age from different breed, sex and weight average of 35.4 kg were used. Equipment, Ortho-Pet, Izmir, Turkey, are manufactured. It was approached with cranialateral incision of craniodorsal sides of the hip trochanter major level. Caput femoris excision was performed by osteotomy. Then the implants was placed for collum femoris. Implant head of the neck portion was steeled and the hip joint was in place. In controls, inspection, palpation and radiographic examination was performed for post-op 15 days and 1,3 and 6 months.

Results
Seven dogs (58.3%) in the postoperative prognosis is seen good or very good condition. Clinical examination of patients with a good prognosis in 15th and 30th days showed similarities between each other. Comparison of post-operative 90th days with 15th and 30th day prognosis show that the gait problems was eliminated and to considered to be very good condition. The extremities were not used very well and lameness of varying degrees in 5 dogs (41.6%). Radiographic examination of these dogs were showed loosening of the collum femoris apparatus and subluxation.

Conclusion
It was concluded that it is economic, development devices and encouraging of the first results. In addition, monitoring of longer-term outcomes PTKP’s more than 6 months is thought to provide important information.

Keywords: Dog, Hip Prosthesis, PTKP

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Nerve Rehabilitation on a Dog with Atlanto-Axial Instability

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Purpose
Atlanto-axial instability (AAI) was common neurological disorder in dogs. In the spinal cord injury of veterinary medicines, damage to the nervous system was considered permanent and irreversible. Recently, studies of neurological restore were progressed. From the damaged axon, a growing of new nerve branch or functional nerve restore through reconstruction of neural networks could be expected. The concept of plasticity has emerged as an important concept in medical treatment which was improving the grade of level and shorter the duration of post-operative care. The purpose of this article was to introduce the effect of nerve rehabilitation through AAI case in a dog.

Materials and Methods
A Maltese castrated male 2-year-old dog was visit "Royal Animal Medical Center". As chief complaint was tetraparesis. Through imaging diagnosis including MRI and CT the patient was diagnosed to AAI. After atlanto-axial fixation using Kirschner wire with polymethyl methacrylate (PMMA) the patient suffered neurological disorders, as ever, that improved than before surgery. Rehabilitation programs (e.g., body-weighted supported treadmill therapy and functional electrical stimulation) were performed for a month.

Results
Postoperatively a week active standing was possible. Ten days after surgery voluntary walking was started. A month after surgery the patient was walked naturally.

Conclusion
Nerve rehabilitation was needed on cervical neurological patients. It was shorter the duration of post-operative care.

Keywords: Nerve Rehabilitation, Dog, Atlanto-Axial Instability

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Results of Laparoscopic Supported Gastropexy in Dogs

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Purpose
Prophylactic gastropexy is an applied method for prevention in large breed dogs, especially gastric dilatation volvulus (GDV)’s surgery. In this study, ventral midline gastropexy and laparoscopic gastropexy which are effect of prophylaxis of GDV were compared for prognosis and surgical intervention.

Materials and Methods
The study material consisted of 12 dogs. Average weight of 20-30 kg dogs, respectively. The dogs were divided into two groups. The dogs in group 1 classic gastropexy method was applied. Laparoscopically assisted gastropexy was applied in dogs in group. Biochemical analysis, blood gas and hematocell have been investigated for examination in the preoperative and postoperative period by the 9th day. Ultrasonographic examinations was performed with convex probe 5-7.5 MHz. Radiographic examinations were performed direct and indirect positions.

Results
Post-operatively, dogs, daily controlled for 9 days after the operation. The results of both operative technique revealed fast and easy applicability. The average operating time for the classic gastropexy was 20 minutes. Laparoscopic group which time 41.5 minutes was determined to be average. There were no complications of both techniques of post-operative care. Postoperative gastric peristaltic contractions were counted on day 7 by Ultrasonography for both groups. Classic laparoscopic supported gastropexy and found an average of 4-5 contraction. Radiological examination of the stomach, location, size, shape, content, and rugal gastric wall were encountered with any pathological condition.

Conclusion
At the end of the study, both techniques, laparoscopic and conventional gastropexy can be applied for emergency surgery and prophylactic interventions.

Keywords: Dogs, Gastropexy, Laparoscopy

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Results of Interlocking Nail Stabilization in Dogs with Long Bone Fractures

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Purpose
The aim of the study to report the clinical and radiographic outcome after use of an interlocking nail (ILN) for stabilization of long bone fractures in dogs.

Materials and Methods
Twenty-six dogs were evaluated. There were ten femoral fractures, 12 tibial fractures and four humeral fractures. The equipment was manufactured by Orthovet (Orthovet, izmir, Turkey). Three ILN lengths with three different diameters (4, 6 and 8 mm) were used. Each ILN had a trocar tip on one end and four screw holes (two distal and two proximal). Ten fractures (four femoral, five tibial, one humeral) were associated with other orthopedic problems. Nine (39.1%) patients had aseptic nonunion and malunion fractures. A static fixation mode was used for nine fractures and a dynamic fixation mode was used in 17 (65.3%). The surgical time recorded was 45-52 minutes. Three dogs had a major complication requiring surgical intervention.

Results
At 6 months, the functional outcome was excellent in 15 (57.6%) animals, good in seven (26.9%), fair in three (11.5%), and poor in one (3.8).

Conclusion
In conclusion, the use of ILNs to repair diaphyseal fractures of the femur, tibia, and humerus in dogs resulted in a good or excellent functional outcome in most patients.

Keywords: Dogs, Interlocking Nail Stabilization, Long Bone Fractures

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Purpose
The aim of this study is to evaluate orthopedic problems of cats with diagnosis, treatment and complications between year 2015-2017 which were obtained to Department of Surgery, Small animal Clinics, Faculty of Veterinary Medicine.

Materials and Methods
Totaly, 275 orthopedic cases in cats were used as materials. After clinical examination of the patients, radiological examination completed by medio-lateral, cranio-caudal or latero-medial positions. Treatment options (intramedullary pin, plating, interlocking, cerclage wires, cancellous screw, external fixation, excisional arthroplasty, close reduction, cage rest) were selected specifically for the cases.

Results
Distribution of the problems, 93 (33.8 %) femur, 37 (13.5%) tibia, 34 (12.4%) coxa, 23 (8.4%) humerus, 21 (%7.6) vertebra, 16 (5.8%) hip joint, 13 (5%) antebraclium, 8 (2.9%) sacrum, 5 (1.8%) ilium, 5 (1.8%) ischium, 4 (1.5%) elbow joint, 4 (1.5%) metatarsal, 4 (1.5%) pubis, 4 (1.5%) radius, 2 (0.7%) metacarpal, 2 (0.7%) ulna have been diagnosed. 24 (8.7%) supported bandages, 36 (13.1%) excision arthroplasty, 81 (29.5%) intramedullary pins, 47 (17.1%) cage rests, 24 (8.7%) euthanasia, 12 (13.5%) rush pin and 5 (1.8%) cerclage methods were used as treatment option.

Conclusion
As a result, the older animal is likely to have a less competent immune system and may have compromised metabolic and endocrine function. In general, issues that should be addressed in the geriatric patient include reducing intraoperative and anesthesia time, enhancing bone and soft tissue healing, return to early function, control of postoperative pain, physical therapy, and proper nutrition.

Keywords: Cat, Orthopedic Problems, Treatment Options

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The Effect of Collagen-Alginate Composition on \textit{in Vitro} Characteristics of Microencapsulated Canine Adipose Tissue-Derived Mesenchymal Stem Cell

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\textbf{Purpose}
The purpose of this study was to find the effect of collagen-alginate composition on the size and shape of microbeads and proliferation and osteogenic properties of microencapsulated canine adipose-derived stem cells (ASCs) \textit{in vitro}.

\textbf{Materials and Methods}
Canine ASCs were microencapsulated in varied collagen-alginate composition by vibrational technologic encapsulator. The size and shape of microbeads were measured with light field microscope and the viability of microencapsulated canine ASCs was evaluated by live/dead viability/cytotoxicity kit. Proliferation and osteogenic properties of microencapsulated canine ASCs were evaluated by alamarBlue proliferation assay and alkaline phosphatase assay respectively.

\textbf{Results}
As the collagen ratio increasing in collagen-alginate composition, the size and size variation of microbeads tended to increase and the shape of microbeads was more irregular. Nonetheless, homogeneous microbeads were created with no significant difference in size and shape in the range of 0.75\% alginate-0.099\% collagen in 1.2\% alginate. There was no significant difference of viability in varied collagen-alginate composition. Both proliferation and osteogenic properties \textit{in vitro} increased with increasing collagen ratio.

\textbf{Conclusion}
Microencapsulation of canine ASCs with appropriate collagen-alginate composition increases cell proliferation and osteogenic properties \textit{in vitro} without significant affecting the shape and size of microbeads and cell viability.

\textbf{Keywords}: Microencapsulation, Collagen, Stem Cells

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Application of Alginate Microbeads as a Carrier of Bone Morphogenetic Protein-2

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Purpose
Recombinant human bone morphogenetic protein-2 (rhBMP-2) is used to enhance bone regeneration. However, the bone regeneration ability of BMP-2 depends on the delivery vehicle. In this study, we used alginate microbeads as a delivery vehicle for rhBMP-2, and confirmed that alginate microbeads are useful to deliver rhBMP-2 and alginate microbeads with rhBMP-2 promote bone regeneration.

Materials and Methods
Release ability of alginate beads was evaluated using BMP-2 ELISA between alginate microbeads with rhBMP-2 and collagen sponges with rhBMP-2 in 1, 2, 4, 7, 10, 14 and 21 days. Effect of bone regeneration in vitro was evaluated using ALP (alkaline phosphatase)-assay using canine adipose tissue-derived mesenchymal stem cells in 1 and 2 weeks. To confirm effect of bone regeneration in vivo, mice were injected with alginate microbeads containing rhBMP-2 into subcutaneous tissue. Eight weeks later after injection, Bone regeneration effect of rhBMP-2 was measured by micro-computed tomography and histological examination.

Results
Alginate microbeads rapidly secreted rhBMP-2 within 4 days and then secreted more constantly than collagen sponge did thereafter. The amount of released ALP is similar between alginate microbeads with and without rhBMP-2 In 1 week. However, in 2 weeks, released ALP was more detected in alginate microbeads with BMP-2 than those without BMP-2. Eight weeks later after injection of alginate microbeads containing rhBMP-2, we confirmed formation of bone in subcutaneous tissue in mice.

Conclusion
We conclude that alginate microbeads can act as an injectable delivery vehicle of rhBMP-2 to induce bone regeneration.

Keywords: Recombinant Human Bone Morphogenetic Protein-2, Alginate Microbead, Bone Regeneration

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Osteogenic Evaluation of Duck-Beak Bone Derived Bioceramic Microparticles

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Purpose
As an alternative material to the autogenous bone, duck-beak bone particles for bone substitute has been attracting great attention due to its biological properties. To deliver the most favorable outcome of the medical treatment, it is essential to study the effect of various processing methods of the duck-beak bone.

Materials and Methods
In this study, we compared the two deproteinizing agents for manufacturing duck-beak bone. Group 1 was treated by conventional chemical agent (ethylenediamine), and Group 2 was treated by hydrogen dioxide (H₂O₂). In vitro and in vivo experiments were conducted parallel to compare the cytocompatibility and osteogenic capability between two processing methods.

Results
For in vitro tests, human adipose-derived mesenchymal stem cells were planted onto each sample, and their attachment and growing were evaluated. For in vivo biocompatibility and osteogenic properties, the samples were applied on the critical sized calvarial bone defect of the rats. Group 2 showed significantly higher cell attachment, but Group1 showed slightly higher cell proliferation. In in vivo test, all groups have shown biocompatibility and increased level of osteogenic potential. However, Group 2 had significantly higher bone regeneration (p<0.05).

Conclusion
This experiment confirmed that H₂O₂ can be an optimal processing method for duck-beak bone particle.

Keywords: Duck-Beak, Osteogenic, Hydrogen Dioxide

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Distal Sesamoid Bursa in Cattle: Radiography, Fluid Taking, Injection Techniques and Fluid Analysis

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Purpose
A few techniques for injection into the distal sesamoid bursa (navicular bursa) or fluid taking have been described in cattle. In this study different techniques for approaching to DSB and analysis of synovial fluids were compared.

Materials and Methods
Five different techniques for entering to DSB were compared in 22 normal and 27 abnormal hooves of Holstein cows. The synovial fluids were collected from the DSB of all hooves. Physical characteristics of fluid, total nucleated cell counts, differential cell counts, protein and glucose concentrations and the activities of some enzymes were measured by standard methods.

Results
The placement of the needle through distal plantar approach parallel with the sole was the best for fluid taking or injection. The results of fluid analysis of bursa showed the parameters of DSB fluid are the same as other synovial fluids in cattle. The results revealed that the enzyme activity of the abnormal synovial fluid from the DSB significantly increased. Also, there were no significant differences between the mean concentrations of total protein in the normal and abnormal synovial fluid specimens.

Conclusion
Placement of the needle through distal plantar approach parallel with the sole was the best for fluid taking or injection. Differences of variable parameters in normal and abnormal hooves may be useful for the diagnosis of healthy and abnormal synovial fluid.

Keywords: Distal Sesamoid, Bursa, Cattle

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Operative Techniques of Percutaneous Endoscopic Limited-Lumbosacral Dorsal Laminectomy in Dogs

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Purpose
To describe the technical feasibility of percutaneous endoscopic limited-laminectomy, and to evaluate possibility of decompression and examination lumbosacral vertebrae spinal canals through endoscope in small dogs.

Materials and Methods
8 fresh canine cadavers were used for study. After injecting barium and agarose mixture (BA-gel) simulating intervertebral disc herniation, percutaneous endoscopic limited-laminectomy was performed using a dorsal approach between lumbar and sacrum vertebrae. The ligamental flava was removed by micro-punch and BA-gel was removed to decompress the spinal cord by elevator and rongeers after limited-laminectomy. Computed tomography (CT) scans were obtained pre-operatively and post-operatively to evaluate surgical outcomes. Intra-operative complications through endoscopic video, incision length and procedure time were recorded.

Results
All procedures were completed with clear observation of the spinal cord and floor of the spinal canal. The mean total operating time was 30.00 ±12.01 minutes. Lengths of incision were under 1 cm in all dogs. The average amount of removed BA-gel was 40.00 ±21.91 mm³, and the average size of bony defect was 29.50 ±13.61 mm². In two dogs, iatrogenic nerve root injuries were caused by micro-rongeur as intra-operative complication. Evaluated by CT scans, BA-gel was removed enough to decompress spinal cord.

Conclusion
Percutaneous endoscopic lumbosacral limited laminectomy is feasible to decompress spinal cord by removing BA-gel and provides a good view of the spinal canal, and could be an alternative surgical option for treatment of lumbosacral disk disease in dogs.

Keywords: Percutaneous Endoscopic Limited Laminectomy, Minimal Invasive Spine Surgery, Intervertebral Disc Disea

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Hepatic Lobe Torsion in a Golden Retriever Puppy

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Purpose
Liver lobe torsion is quite rare and often occurs in middle aged dogs, this case happened in a puppy. This case report was conducted as an addition of this disease detail to veterinary literature.

Materials and Methods
Study subject was a 3 month-old puppy, female Golden Retriever, being sent to Animal Emergency Center for the condition of sudden defecation then became lethargic and started dyspnea, pale mucous membrane, abdominal pain and vomit. Abdomen was distended with palpable fluid wave.

Laboratory test result showed the condition of acidemia. Higher PCV/TP of abdominal fluid compared to peripheral blood indicated active haemorrhage in abdominal cavity.

Ultrasound and abdocentesis found fluid-filled bloody fluid, decreased blood flow in liver lobe. Radiography found an abdominal mass, loss of abdominal detail due to peritoneal effusion.

Blood Transfusion and emergency exploratory laparotomy performed. Liver lobe torsion noted. Before being resected, the torsed lobe was not untwisted in order to avoid releasing bacteria, toxins and thromboli into the circulation. The nontorsed liver lobes were examined for adequate blood supply in case of infarction of the adjacent lobes after removal of the affected liver.

Results
The patient was well recovered after reconstruction surgery. There were not many complications, just heamaturia and tarry diarrhea after transfusion.

Conclusion
The clinical signs of liver lobe torsion were non-typical. Imaging diagnosis is a recommended method for liver lobe torsion. Emergency surgery is necessary and if there is any evidence of necrosis in the torsed lobe, that lobe should be excised.

Keywords: Hepatic Lobe Torsion, Diagnosis, Hepatic Lobectomy, Laparotomy, Puppy

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Hemilaminectomy Technique, Solution for Intervertebral Disc Disease

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Purpose
This case study confirmed the efficiency of hemilaminectomy technique to deal with intervertebral disc disease.

Materials and Methods
Studied subject was Eddie King, Jack Russell Terrier, male neutered, he was 6 years. He had been showing reduced activity levels for 48 hours and he developed hind limb ataxia. He was found to have conscious proprioception deficits and weak motor function in both hind legs. Neuroexamination shown non ambulatory paraparesis on the gait but pain was presented on thoracolumbar spinal. On pelvic limbs, withdrawal and patella reflexes were increased.
CT and myelogram shown disc extrusion was present on spinal cord from 12th to 13th of lumbar disc space. Disc material presented as minalized material on left side of the spinal cord, on section of dorsalventral view, it accounted one third of the volume of subarachnoid space, and compressed on spinal cord. Hemilaminectomy provides good decompression and easy access to the floor of the vertebral canal for removal of disc material. A window in the vertebral lamina was created to reveal the spinal cord and take out all of disc material on ventral, dorsal and dorsolateral of the spinal cord.

Results
A slow progression shown on the neurological examination. Five days after surgery, he could stand and walk by himself.

Conclusion
Hemilaminectomy provides good decompression and easy access to the floor of the vertebral canal for removal of disc material, by create a window in the vertebral lamina in order to solve the intervertebral disc disease.

Keywords: Intervertebral Disc Disease, Hemilaminectomy, Myelopathy, Chondrodystrophoid Dogs, Diagnosis

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Removal of Oral and Cutaneous Tumors by Diode Laser in 4 Dogs

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Purpose
Treatment of tumors include surgical resection, chemotherapy, radiation therapy and so on. The purpose of this report is to introduce efficiency of laser for tumor resection.

Materials and Methods
Tumors were removed using laser and procedure was performed under local anesthesia (3/4) and inhalant anesthesia (1/4) in 4 cases. Samples from fine needle aspiration or biopsy were used for pathological examination. Patients' records included breeds, age, gender, regions, pathological examination and recurrence ratio.

Results
The regions of tumor in 4 cases were oral cavity and skin. Types of tumor were acanthomatous ameloblastoma, melanoma, perianal gland adenoma and soft tissue sarcoma respectively. All patients showed no recurrence except one patient. This patient was amputated right thoracic limb because of tumor. After one month, cutaneous mass was detected around amputated region and it was removed by laser under local anesthesia. Although recurrence was not shown on that region, the patient was euthanized because of metastasis of tumor.

Conclusion
Laser can generally be applied under local anesthesia because of relatively less pain compared with resection using blade. With its advantage, procedures were done under local anesthesia except one patient. Another advantage of laser is that it causes less bleeding and this character is magnified when it is used for oral mass resection because oral cavity is prone to bleeding. Prognosis of patients was good when laser was applied for oral mass resection. In consideration of these facts, laser can be an effective method for tumor resection.

Keywords: Laser, Tumor, Dogs, Oral, Cutaneous

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Tibial Plateau Leveling Osteotomy Method in Dog

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Purpose
This case study confirmed the efficiency and apply the Tibial Plateau Leveling Osteotomy (TPLO) method in cruciate ligament rupture cases.

Materials and Methods
Studied subject was Monty Skinner, Labrador, male neutered, he was 7 years. He was hopping and pain in right hind limb. There was no abnormalities from neuroexamination. Distance observation of the standing and walking posture in combination with the palpation and radiograph result gave the suspect of cruciate ligament rupture. Arthroscope was used for confirmation.
Surgery was conducted accompanied with the epidural anesthesia. The surgery started with cutting the tibia bone by special curved saw blade with suitable size depended on the Tibial Plateau Angle measurement from radiograph. The tibial plateau was rotated to a determined angle then was stabilized by TPLO plate and screws to level the slope. After closure, X-ray was re-used to confirm the surgery result. Posture during x-ray affects the angle in TPLO cases that plays an important role in treatment succeed.

Results
The next morning, he could bear his weight on all legs, started eating and ready to go home but he needed strict rest in two weeks.

Conclusion
Cruciate ligament rupture causes the uncomfortable condition for dogs. TPLO is an effective solution but it requires special facilities and technique especially identify the Tibial Plateau Angle.

Keywords: Tibial Plateau Leveling Osteotomy, TPLO, Cruciate Ligament Rupture, Tibial Plateau Angle, Knee Surgery

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Xenotransplantation of the Porcine Trachea in the Dog

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Purpose
This study evaluated the feasibility of the xenotransplantation of the trachea in the pig to the trachea in the dog to help solved the partial problem of the recipient trachea.

Materials and Methods
Three healthy beagle dogs weighing between 8-9 kg were used as recipients and two pigs weighing 20 kg were used as donors. 2 cm x 2 cm quadrate tracheal section was resected from each recipient, and tracheal xenotransplantation were performed. All animal were carefully monitored during 90 days of the observation for anatomic complication or infection. The recipients were sacrificed at 90 days after operation the grafts were assessed.

Results
All of the dogs survived during experiment, and all of their wounds heals well. On bronchoscopy, airways were open and no stenosis or malacic changes were seen in the dogs. At the 28 days after xenotransplantation, the white xenograft was exhibited in the surface airway epithelium. At the 60 days after xenotransplantation, the xenograft was covered by mucous membranes. At the 90 days after xenotransplantation, the airway epithelium was normally regenerated. The levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ) were significantly increased at the 7 days after xenotransplantation. The results of the polymerase chain reaction showed that tissue component from pigs could be identified in the xenograft of the beagle.

Conclusion
The porcine tracheal section was successfully xenotransplanted into the trachea of the recipient.

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Keywords: Trachea, Transplantation, Pig, Dog

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3D-Printing Bone Model for Surgical Planning of Total Hip Replacement for Treatment of Hip Dysplasia After Unsuccessful Triple Pelvic Osteotomy in a Dog

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Purpose
To describe technical efficacy of using 3D-printing bone model for surgical planning of THR for treatment of persistence coxo-femoral joint incongruity after TPO.

Materials and Methods
A 2-year old, 26kg, castrated male, chow chow was presented for assessment of weight-bearing lameness of the left hind limb. The patient had a history of TPO on left side to correct hip dysplasia a year prior to clinical sign presentation. On physical examination, pain and crepitis were noted on the left hip joint during extension and medial patellar luxation was identified on the same side. Radiological examination revealed coxo-femoral joint subluxation, degenerative bone changes on the left hip joint and pelvic angles were changed after TPO. Increased possibility of postoperative hip luxation because of changed pelvic long axis and angles after TPO. Preoperative computed tomography had applied for 3-dimensional printing to establishing accurate surgical plan. Measurement of the changed angles of the pelvis after TPO and Rehearsal surgery was done by using 3D-printing bone model. Revision acetabular cup was fixed by cortical bone screw at the acetabulum in premeasured angles and surgery was done.

Results
Cup Implant angle of lateral opening was 50° and the angle of retroversion was 15°. The function of the affected limb had been getting better without complications and concurrent medial patellar luxation was resolved.

Conclusion
Using 3D-printing bone model is a reliable method of THR for treatment of unsuccessful TPO.

Keywords: 3D-printing Bone Model, Total Hip Replacement(THR), Triple Pelvic Osteotomy(TPO), Incongruity

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**Comparative Study of Self-Retaining Distractor and External Manipulation for Hip Joint Arthroscopy in Small-Breed Dogs**

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**Purpose**
The purpose is to describe technical efficacy and to evaluate potential iatrogenic neurovascular, articular damages of femur head and acetabulum when self-retaining distractor and external manipulation technique are used to perform hip-joint arthroscopy.

**Materials and Methods**
Forty hip-joints(20 canine cadavers) were used and randomly sorted external manipulation group and self-retaining distractor group (n=20 each). In the distractor group, two K-wires were inserted followed by jig placement at the level of lesser trochanter and cranial acetabular rim. The arthroscopy examination was done in all hip joints and technical feasibility and visualization of joint distraction technique were evaluated. Then, the hip-joint disarticulation was performed to calculate the degree of iatrogenic articular and sciatic nerve damages by using India ink-staining and digital image.

**Results**
Mean±SD of joint distraction distance was 8.88±3.54mm in the self-retaining distraction group and 2.37±0.82mm in the external manipulation group. The acetabular cartilage was damaged (P = 0.004) significantly in external manipulation group. However, articular damage of femur head (P = 0.940) was similar.

**Conclusion**
It’s a good modality to use joint distractor device to perform hip arthroscopy because it’s better for surgeons to do the procedure without assistants. It also offers better window and reduces iatrogenic cartilage-damage than manual traction technique.

**Keywords**: Arthroscopy, Distraction, Visualization

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Evaluating of Pre-Surgical Treatment Options with Prednisone and Diphenhydramine on Mastcell Degranulation

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Purpose
Evaluating the effect of pre-surgery treatment with diphenhydramine (Benadryl®) and prednisone (Deltasone®) on reducing the risk of mast cell degranulation in canine cutaneous mastocytoma.

Materials and Methods
Six patients with cutaneous mastocytoma were referred for surgery after diagnosis and studied on mast cell degranulation during surgery, in veterinary hospital of university of Tabriz. Patients were divided into 2 groups without considering the grade of tumors. group 1 treated with Benadryl (1mg/Kg), and group 2 with deltasone (1mg/Kg) during induction for controlling the risk of degranulation during surgery. (drugs ordered from Raha pharmaceutical co., Sofe city, Isfahan, Iran)

Clinical signs of degranulation include redness, shrinking and swelling on mass areas and signs of anaphylactic shock like respiratory distress and reduced blood pressure were evaluated during surgery.

Results
None of cases showed any anaphylactic shock during surgery. only one patient showed signs of degranulation and made the surgery team use a dose of (0.5 mg/Kg) prednisone for controlling the inflammation. This patient had 9 well differentiated masses also had the largest number of masses between all cases.

Conclusion
By comparing the effect of two drugs, as there were two other patients in both groups which had fewer poorly differentiated masses but no signs of degranulation were seen on them during surgery, we can conclude that diphenhydramine is not effective for patients with large number of masses and differentiation of cells probably is not as risky as the number of masses is. If the masses are large in number and also in size, using a corticosteroid would be a better option for pre-surgical treatment.

Keywords: Mastocytoma, Prednisone, Diphenhydramine, Surgery, Anaphylaxis

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Effects of the Cetyl Myristoleate in Osteoarthritis Dog

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Purpose
Cetyl myristoleate (CM), an ester of a fatty acid, has been known to have effect in reducing arthritic pain and improving mobility of joint. We investigate the effect of CM on improvement of joint function and quality of life in osteoarthritic dogs.

Materials and Methods
Thirteen osteoarthritic dogs were used for the treatment trial. CM (80 mg/dog) administered every day during 10 weeks. Clinician and owner's scores were sum of each criteria score and were evaluated at baseline and 2, 4, 6, 8, and 10 weeks respectively. Clinician score criteria included lameness, weight bearing, and pain. Owner's score criteria included quality of life, activity, joint stiffness, and lameness. Owner's satisfaction rate were also examined. The changes of blood chemistry levels were evaluated at baseline and 10 weeks respectively. Statistical analysis was performed using SPSS version 23.0

Results
The clinician scores before and after 10 weeks CM trial were 2.9 ± 0.8 and 2.1 ± 1.5 respectively (p < 0.05). The owner's scores gradually decreased and were 6.3 ± 2.0 at 10 weeks which was a significant decrease in comparison to the baseline (p < 0.05). Owner's satisfaction rate with CM trial was 84%. There were no significant differences in blood chemistry and body condition during the 10 weeks.

Conclusion
Owner's satisfaction rates were high and CM might prevent worsening of clinical symptom and improve quality of patient life.

Keywords: Cetyl Myristoleate, Fatty Acid, Osteoarthritis

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Generation of Recombinant VLRB Antibody Against Viral Nervous Necrosis Virus (VNNV) in Hagfish (Eptatretus Burgeri)

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Purpose
Variable lymphocyte receptors (VLRs) are unique antigen receptors in jawless vertebrates (agnathans) such as lamprey and hagfish. VLRB that consists of leucine rich repeats (LRRs) modules. Here we describe the production of recombinant VLRB antibodies specific for viral nervous necrosis virus (VNNV), which is a serious viral disease in marine aquaculture industry.

Materials and Methods
-Hagfish immunization
50㎍ of VNNV mixed with Freund’s complete adjuvant was injected to hagfish
-VLRB cDNA library
Leukocyte was collected from blood of immunized hagfish. The synthesized cDNA was cloned into the pKepta vector.
-VLRB library screening and dot blot
96-well plates and PVDF membrane coated with VHSV or VNNV were blocked with skim milk followed by VLRB transfectant supernatant. VLRB antibodies detected with anti VLRB mAb (11G5) followed by HRP conjugated goat anti-mouse IgG.
-Mutation of LRRCT domain
Amplification was performed from Igk to CP of VLR76 and LRRCT domain was amplified by new cDNA. These were amplified together.

Results
From VLRB transfectants, we found a recombinant VLRB antibodies, VLR76, which recognized VNN virus at the highest levels in ELISA. As a result of mutation of LRRCT domain, we selected a clone, VLR18, which recognized VNNV with higher level in ELISA. In dot blot, VLR76 and VLR18 could detect VNN virus under non-reducing condition.

Conclusion
This study revealed that we found the VNNV-specific recombinant antibodies, VLR76 and VLR18. Also, this VLRB antibodies might be useful for antibody therapies and treatment against viral diseases.

Keywords: Hagfish, Variable Lymphocyte Receptor, Viral Nervous Necrosis

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Expression of Secretable CD4-2 Molecule of Olive Flounder (*Paralichthys Olivaceus*) Using Mammalian Expression System

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**Purpose**
Although many studies about CD4 have been proceeded in teleost, it is mainly at the genenic level and little is know about its function. In this study, we design a method of producing secretable, recombinant CD4-2 molecule of olive flounder, which is helpful to make monoclonal antibody, for further indentifying functional role of CD4 related cells.

**Materials and Methods**
Leukocyte was collected from blood sample of olive flounder from a commercial source. Two insert fragments of CD4-2 were amplified from cDNA synthesized from total RNA of the leukocyte. The amplified CD4-2s were cloned into the KINGeo vector derived from pTracer-EFA mammalian expression vector and the plasmid was transfected into HEK-293F cell using Lipofectamine2000. At 48 hrs post-transfection, pellet and supernatant were collected and western blot was conducted by using V5-specific mAb.

**Results**
Based on TMHMM server, which is predicting transmembrane domain (TMD), we designed two forms of CD4-2, full (906 bp) and TMD truncated form (450 bp). At 48 hrs post-transfection, pellet and supernatant from transfectants were exploited for detecting expression of recombinant proteins using V5-specific mAb. Target bands, full sequence for 40 kDa and truncated sequence for 20 kDa, were well shown at pellet. However, only truncated form was observed at supernatant, which is by due to deletion of membrane-spanning sequences.

**Conclusion**
In this study, we produce secretable recombinant CD4-2 molecule of olive flounder, will be helpful to further monoclonal antibody production.

**Keywords**: CD4-2, Olive Flounder, Recombinant Protein, Mammalian Expression System, Cloning

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**Determination of Oxytetracycline Resistance of Yersinia Ruckeri Isolated from Rainbow Trout in Turkey**

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**Purpose**

*Yersinia ruckeri*, also known Enteric redmouth disease (ERM) is a serious septicemic bacterial disease of salmonid fish species. It has a significant economic losses in the rainbow trout industry that causes highly antimicrobial using, especially tetracycline groups. We aimed to determine of oxytetracycline susceptibility for *Yersinia ruckeri* strains isolated from rainbow trout farms in Turkey between 2011-2013.

**Materials and Methods**

Isolated bacteria were identified by conventional biochemical, rapid identification kits (API 20E) and molecular methods (PCR). Minimal inhibitory concentration (MIC) assessment, using the broth micro dilution method according to proposed standards. MIC values were determined against oxytetracycline of 10 *Yersinia ruckeri* strains and NCTC 12266 (reference strain). MIC results were evaluated according to the European Committee on Antibiotic Sensitivity Testing (EUCAST).

**Results**

Each isolate were identified by API 20E as *Yersinia ruckeri*. Confirmation was done with PCR at 575 bp. MIC values of *Y. ruckeri* to Oxytetracycline from 0.512 to 2 µg / ml was found. When evaluated according to EUCAST data, 10 of 11 isolates were sensitive and 1 of them were moderately sensitive.

**Conclusion**

10 isolates isolated from the ERM outbreaks in our country, it has been determined that the resistance has not yet developed but the susceptibility has decreased. The unconscious use of oxytetracycline is the inevitable result that the resultant sensitivity will decrease and the resistance will develop. If vaccination and biosecurity measures are taken in fish farms, the use of antimicrobial will be reduced.

**Keywords**: Yersinia Ruckeri, Antimicrobial Resistance, Oxytetracycline, MIC, Rainbow Trout

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Histological Description of a Post Estrous Adult Female Reproductive Tract in a South American Fur Seal of the Peruvian Subpopulation (Arctocephalus Australis)

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Purpose
Peruvian subpopulation of South American fur seals (Arctocephalus australis) are categorized as vulnerable under IUCN and as in danger of extinction in Perú. Currently, one of the most important colonies of this subpopulation is being studied within the natural protected area Punta San Juan (PSJ), but no formal histological description of adult female reproductive tract had been made for this subpopulation so far.

Materials and Methods
It was possible to obtain a fresh post estrous adult female reproductive tract of an individual that died from direct interaction with fisheries in January 2016. The whole reproductive tract was fixed in formalin and preserved in 70° alcohol. Afterwards, tissues were sliced and submitted for routine histological processing.

Results
Ovaries had peripheral cortex zone with highly vascularized central medula. Ovarian cortex had irregular dense connective tissue and simple cubic epithelium that invaginated into subcapsular crypts, also growing ovarian follicles and plenty of interstitial cells. Oviduct had a mucous membrane of cylindrical epithelium with many folds towards the lumen; along with basal lamina of loose connective tissue and muscular layers. The uterine horn presents glands with few uterine secretions. In the body of uterus, the mucous membrane had simple cylindrical epithelium and evidenced glands without secretion. The vagina had an aglandular composed cubic epithelium, with basal lamina of irregular dense connective tissue and a muscular layer.

Conclusion
A fresh adult female reproductive tract was described histologically and may help understand female anatomical features during different times of the breeding/annual cycle.

Keywords: Histology, Female Reproductive Tract, South American Fur Seal

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Detection of the First Sulfanamid Resistance Genes Positivity in Aquatic Myroides Spp. Isolates in Turkey

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**Purpose**
The genus Myroides, belongs to the family flavobacteriaceae and its species have been found in various terrestrial and aquatic environments, and also as causative agents in various infections. In addition, it has been recognized as an human pathogen because of its ability to develop resistance to antimicrobials and an opportunistic pathogen in humans. The aim of this study was to investigate the presence of sulfonamide resistance genes among aquatic Myroides spp. in Turkey.

**Materials and Methods**
Isolated bacteria were identified by conventional biochemical and 16S ribosomal RNA gene sequencing. Minimal inhibitory concentration (MIC) assessment, using the broth micro dilution method according to proposed standards. MIC values were determined against sulfonamide, tetracycline, florfenicol, oxolinic acid and erythromycin of 5 Myroides spp. strains. The detection of the sulfonamide genes in the genomic DNA was examined via PCR with gene specific primers.

**Results**
Each isolate were identified by biochemical test and 16S rRNA sequencing as Myroides spp.. MIC values of them to sulfonamide from 64 to 256 µg / ml was found. 3 of 5 isolates Sul1 and Sul2 resistance genes was detected.

**Conclusion**
In 5 isolates isolated from rainbow trout and aquatic environment in Turkey, it has been determined that the sulfonamide resistance has developed. Containment of the environment with antibiotics or other contaminants causes the spread of antibiotic resistance genes. For this reason, it is inevitable that different resistance genes in water-borne microorganisms are transferred to other bacteria and detected in clinical isolates.

**Keywords:** Myroides Spp, Antimicrobial Resistance, Sulfonamide, Antimicrobial Resistance Genes, MIC

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Atypical Strains of *Enterobacter Cloacae* and *Enterobacter Asburiae* Isolated from Rainbow Trouts

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**Purpose**

Some *Enterobacter* species showed oxidase positive and negative characteristics and they were identified by specific primer that generated for Aeromonas species while they belongs Enterobacteriaceae family. We aimed at identification of some atypical Enterobacteriaceae species in this study.

**Materials and Methods**

Isolates were collected from the Aegean, Central Anatolia, and Mediterranean Sea regions in 2013-2014. All seven *Enterobacter* strains were isolated from clinical, subclinical, and moribund fish cases originating from different herds, fish weights, months, and regions. The biochemical characteristics of all isolates were determined with conventional microbial tests. The identification was performed in the 16S rRNA sequence with 27F and 1387R primers and further identifications were performed using the housekeeping gene with gyrB 3F and 14R primers.

**Results**

A total of seven *Enterobacter* isolates were studied that were recovered from the livers and spleens of rainbow trout weighing 30-3,000 g. All strains were Gram negative, catalase positive, O/F fermentative, vibriostat (O/129) resistant and oxidase positive. All *Enterobacter* isolates showed oxidase positive characteristics and belonged on Enterobacteriaceae family, however isolates were genotypically identified as *E. cloacae* and *E. asburiae* in the sequence similarities with the gyrB gene region.

**Conclusion**

In the literature, the *Enterobacter* family known as oxidase negative and bigger family of *Enterobacteriaceae*. But we detected all *Enterobacter* strains as oxidase positive strains by biochemically and determined *Enterobacter cloacae* in the genotypic studies. This study showed that atypical strains could be confused with other non-Enterobacter agents.

**Keywords**: Enterobacter Spp., *E. Cloacae*, *E. Asburiae*, Rainbow Trout, GyrB Sequence

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Efficient and Rapid RNA Extraction by a Field-Deployable Automatic System for Molecular Detection of Avian Influenza Virus from Avian Tissue and Swab

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Objective
Real-time RT-PCR (rRT-PCR) is used regularly for the diagnosis of diseases caused by RNA viruses. Nucleic acid (NA) extraction is generally required to provide RNA of good quality and quantity for rRT-PCR. Here, the performance of a field-deployable automatic NA extraction system (taco™, GeneReach) for RNA from commonly used avian samples was evaluated.

Materials and Methods
Avian influenza A virus (AIAV)-positive oropharyngeal swab (n = 26) and AIAV-spiked tissue (n = 8; brain, lung and spleen) were subjected to taco™ (taco™ DNA/RNA Extraction Kit) and RNeasy Mini Kit (Qiagen) simultaneously. Supernatant aliquots of tissue homogenate were spiked with serial dilutions of AIAV H5N1 strain (16A59). Reproducibility was evaluated by triplicate extractions with 6 tissues and 6 swabs of various AIAV titers. AIAV RNA from the samples were quantified by a published qRT-PCR.

Results
Ct value was compared between RNAs from the two extraction methods. Regression coefficients were very close to 1.0 with both tissue (0.997) and swab (0.956). The repeats of taco™ extraction had Cts of CV% < 2.5% for all samples, except for two swabs (3.70, 7.77%).

Conclusion
The taco™ (taco™ DNA/RNA Extraction Kit) system had great efficiency and reproducibility for two most common avian sample types for diagnosis and surveillance of RNA viruses. This automatic system could extract DNA and RNA simultaneously at a relatively low cost, providing solutions to the inconsistency problem due to human errors, and high reagent cost.

Keywords: Taco, RNA Extraction, Avian Tissue and Swab

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A Field-Deployable Insulated Isothermal PCR-Based System for Rapid and Sensitive Detection of Avian Influenza A Virus at Points of Need

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Purpose

Influenza A viruses (IAV) have a wide host range, including birds, pigs, and human. Rapid on-site identification of IAV could help disease management and control. The field-deployable PCR system, POCKIT™ COMBO (GeneReach), including an automatic NA device and an insulated isothermal PCR (iiPCR) device, allows timely pathogen detection. The performance of an avian IAV RT-iiPCR targeting the M gene was evaluated.

Materials and Methods

Analytical sensitivity of the RT-iiPCR was compared with a qRT-PCR using H5N1 isolate (16A59) or H7N9 isolate. The inclusivity panel included H3, H4, H5, H6, H9 and H10 subtypes. NA from oropharyngeal swabs and tissue homogenates (brain, lung and spleen) extracted by taco™ were tested to evaluate the RT-iiPCR by comparison with the qRT-PCR. Interrater agreement was calculated by the kappa test.

Results

The RT-iiPCR had LoD95% of 8 GEs. Detection endpoints (fold dilution) of qRT-PCR and RT-PCR were at $10^5$ and $10^6$ for H5N1, and $10^5$ and $10^7$ for H7N9, respectively. The reaction detected the different IAV subtypes. Contingency analysis shows that 38 were positive and 9 were negative in both assays, while three were qRT-PCR negative/RT-iiPCR positive. The two methods had 94% agreement ($\kappa = 0.82$).

Conclusion

The RT-iiPCR had performance comparable to the reference qRT-PCR. Therefore, this method on the field-deployable POCKIT™ COMBO system can be useful for timely IAV detection in poultry to facilitate avian disease management and control, as well as minimize risks to public health.

Keywords: Avian Influenza A Virus, Insulated Isothermal PCR, Point-of-Need Detection

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Gross and Histopathological Lesions, and Immunohistochemical Analysis in Layer and Korean Native Chicken with Natural Highly Pathogenic Avian Influenza Virus(H5N6) Infection

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Purpose
The pathological lesions and localization of HPAIV in natural highly pathogenic avian influenza virus(H5N6) infection in chicken were rarely reported. Therefore we describe those data for HPAI case, 2016.

Materials and Methods
Necropsy was performed on one case of layer and two cases of Korean native chicken, December, 2016. Parenchymal tissues were collected for histopathological and immunohistochemical analysis, and virus isolation.

Results
Grossly, all breeds (layer and Korean Native chicken) showed lesions in various parenchymal tissues. Reddening of tracheal mucosa, and pulmonary reddening and edema were observed. And necrosis in spleen, liver, kidney, heart and pancreas were observed. Histopathologically, moderate congestion and thrombosis were seen in lung. Moderate to severe congestion, necrosis with or without chronic inflammation in spleen, liver, kidney, pancreas, brain and heart. Immunohistochemically, avian influenza virus antigens were seen in blood vessel walls and necrotic parenchymal cells of many organs. At virus isolation, HPAIV(H5N6) was isolated.

Conclusion
We confirmed that the H5N6 virus circulated in entire body of chicken and caused fatal damages.

Keywords: Highly Pathogenic Avian Influenza Virus(H5N6), Layer, Korean Native Chicken

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Epidemics of Highly Pathogenic Avian Influenza (HPAI) in the Republic of Korea

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Purpose
The present study describes the characteristics of highly pathogenic avian influenza in the Republic of Korea from the first epidemic in 2000 up to 2017.

Materials and Methods
The epidemiological investigation and analysis reports for the epidemics from 2003 to 2016 were reviewed. Release of information on the web page of Animal and plant Quarantine Agency were examined.

Results
From the first epidemic in 2003/2004 with type H5N1, virus inflow from overseas was shown the highest possibility of association with migratory birds. The first H5N8 virus in January 2014 was also thought to have flowed in through winter migratory birds at the end of 2013. A similar route was inferred for the epidemic from November 2016 with the first detection of H5N6 type. The diffusion into poultry farms in the country occurred through various factors of mechanical transmission such as vehicles, livestock owner (employees), etc. in addition to wild birds similarly to previous outbreak cases.

Conclusions
Biosecurity rules must never to be violated to keep free poultry farms in Korea from the recursive outbreaks of highly pathogenic avian influenza.

Keywords: Highly Pathogenic Avian Influenza, Epidemiology, Republic of Korea
Big Data Based Risk Assessment on Highly Pathogenic Avian Influenza for Poultry Farms

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Purpose
The explosive increase and interest in big data is a general trend. Big data analytics means the process of all complex operation using mathematical optimization to uncover useful information. This study presents big data analytics during the 2016/2017 highly pathogenic avian influenza (HPAI) epidemic in Korea.

Materials and Methods
Farm-level HPAI risk was assessed using a ready-made big data based analytic model, on-boarded in the Animal and Plant Quarantine Agency (APQA)’s decision making support system. Training data set included farm information, movement pattern of livestock related vehicles between farms and their effective contacts.

Results
From November 2016 to April 2017, the APQA’s big data analytic model provided risk of HPAI outbreak for 4,973 poultry farms (with duplicates), which were connected with HPAI outbreak farms through movement of livestock related vehicles. The farms were allocated into Severe (10.98%), Warning (0.86%), Alert (1.79%), and Attention (86.37%) risk level. Major farm types at severe risk level were broiler (61.26%), layer hen (17.26%), and broiler duck (11.58%) in decreasing order. These farms were mostly associated with feed-lorry (38.94%), animal-carry (21.64%) and egg-carry (13.50%) vehicles.

Conclusion
The APQA’s big data analytic model used to warn and prepare tailored control strategy for high-risk farm or zone during the 2016/2017 HPAI epidemic. This study has a significance of the first pre-emptive offer of farm-level risk. People in the animal disease management domain appreciated this big data based analytic model for its support for decision-making during the 2016/2017 HPAI epidemic.

Keywords: Highly Pathogenic Avian Influenza, Epidemiology, Republic of Korea

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Avian Influenza Surveillance in Captured Wild Bird in South Korea Between 2008 and 2017

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Purpose
National surveillance of avian influenza virus (AIV) in South Korea has been annually conducted for early detection of highly pathogenic avian influenza (HPAI). In this study, we report on the result of a nationwide surveillance study for AIV in captured wild birds between 2008 and 2017.

Materials and Methods
During the AIV surveillance programs between 2008 and Feb. 2017, 16,469 wild birds were captured and samples of swab (oropharyngeal and cloacal) and serum collected. The virus isolation was performed by egg inoculation and subtyped by sequencing. Seropositive cases detected by hemagglutination inhibition assay using H5, H7 antigen.

Results
13,299 captured birds were belong to family Anatidae (14 Species), 3,170 captured birds were belong to other family (101 species). A total of 144 AIV were isolated only from family Anatidae in winter (annually positive rate; 0.23 % ~ 1.31 %). Among 144 isolates, 35 HPAI viruses of H5 subtype were isolated. one H5N1 (clade 2.3.2.1), 15 H5N8 (clade 2.3.4.4), 17 H5N6 (clade 2.3.4.4), 2 H5N8 (clade 2.3.4.4) were isolated in 2010, 2014/15, 2016, 2017, respectively when poultry outbreak occurred. The LPAIV with various HA subtypes were belongs to including H1-H7 and H9-H11. H5, H7 HI Positive serums were collected from family Anatidae in winter.

Conclusion
Surveillance in captured wild birds had detected early detection of HPAI in waterfowls in winter, when every poultry outbreak. Enhanced surveillance in wild birds should be implemented to control AIV introduced by wild birds and eradicate HPAI.

Keywords: Avian Influenza Virus, Surveillance, Captured Wild bird

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Serological and Antigenic Surveillance of Avian Influenza Viruses for Mammalian Transmission in Korea

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Purpose
Mammalian transmission of avian influenza A viruses (AIVs) have been steadily occurring over the last 100 years. In Korea, new subtypes of the highly pathogenic influenza viruses (HPAIs) have been occurred during 2014-2016 and caused severe damages on poultry industries. Therefore, we aimed at serological and antigenic surveillance of AIVs in wild mice and humans with high risks of AIVs infections.

Materials and Methods
We captured 191 of wild mice (Striped field mouse, House mouse, Eurasian harvest mouse, and lesser shrew) around poultry farms and migratory bird habitat twice in the spring of 2013 and 2014 at Gyeongki province. We collected 420 of human sera from live bird market traders, poultry processing workers, and quarantine officers during 2013-2015. Serological testing was performed by using NP-based ELISA and HI tests for H5, H7 and H9. The antigen detection was examined by inoculating samples to 10-day-old SPF embryonated chicken eggs (ECEs).

Results
Any antibodies or antigens of influenza A virus were not detected by ELISA (0/138) and ECEs inoculation method (0/191) in wild mice, respectively. The sero-positive rate of high-risk humans was 97.6% by ELISA, but anti-H5, H7 and H9 antibodies were not detected.

Conclusion
According to our results, the risk of mammalian transmission of AIVs is low in Korea. However, the continuing active surveillance of AIVs in wild mammals and humans with high risk may be important for preparedness and prevention of next pandemic outbreaks.

Keywords: Avian Influenza Virus, Mammalian Transmission, Serological and Antigenic Surveillance

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The Improvement of H9N2 Vaccine Strain by Using Non-Pathogenic NS Gene

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Purpose
The H9N2 low pathogenic avian influenza virus strain A/chicken/01310/2000 (E20) (01310 E20) has been used as vaccine strain. This virus was passaged 20th time in Embryo chicken eggs (ECEs), showed significantly higher viral titer than that of 01310 E2, but tended to acquire higher mortality in ECEs. In this study, we improved 01310 E20 vaccine strain by using non-pathogenic NS gene.

Materials and Methods
We generated recombinant 01310 viruses by using 7 genes (PB1, PB2, PA, HA, NP, NA, and M) of 01310 E20 virus and NS gene of A/chicken/Korea/KBNP-0028/ 2001(H9N2) by reverse genetics. Mutations in 0028 NS gene were made by site-directed mutagenesis. Recombinant viruses were inoculated in ten 10-d-o SPF ECEs and measured the mean times to death (MDT).

Results
The °01310-NS(0028) significantly increased mean times to death (MDT) than that of recombinant 01310 (r01310). Interestingly, change in MDT was affected by the previously discovered pathogenicity related factors, PDZ binding domain (PL) motif EPEV, G139N, and S151T mutations in NS gene.

Conclusion
We proved that G139N, S151T, and EPEV in NS gene contribute higher mortality in ECEs, and established recombinant H9N2 vaccine strain acquiring low mortality of ECEs.

Keywords: Nonstructural(NS) Gene, H9N2, Vaccine, Pathogenicity, Mean Death Time(MDT)

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Development of More Accurate and Sensitive Influenza A Virus Detection Technique

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Purpose
Several outbreaks of HPAI (Highly pathogenic avian influenza) have occurred recently in Korea, causing huge economic damages to the poultry industries. In this situation, the need of the most accurate and rapid diagnostic technique to detect influenza A virus (IAV) has been emphasized. Thus, we developed a new diagnostic technique with more sensitivity and specificity.

Materials and Methods
A set of novel primers was designed which have higher conformity above 98% with the corresponding sequence of influenza A virus in nature. The efficiency of the primers was evaluated by qRT-PCR assay. In addition, we developed an effective method to enrich influenza’s viral RNP from the fecal samples using magnetic bead based antibody technique. We evaluated the effectiveness of the method by qRT-PCR using the novel primers that we designed in this study.

Results
In the primer efficiency evaluation study, the novel primers presented lower limit of detection (LOD) than those of currently used primers. The specificity was confirmed by melting curve analysis. By the RNP enrichment method, we enriched viral RNP from the fecal samples by 60 times compared to the common RNA extraction method. In addition, we optimized the protocol of the method to shorten the experiment time.

Conclusion
We developed an improved IAV diagnostic technique with higher sensitivity and specificity using improved primer set, RNP enrichment method, and melting curve analysis. This study will contribute to the early diagnosis of HPAI, which is important to reduce the magnificent damage of the poultry industries.

Keywords: Avian Influenza Virus, QRT-PCR, Viral RNP Enrichment, Diagnosis, Primer

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Avian Influenza

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Evaluation of the Zoonotic Potential of Multiple Subgroups of Clade 2.3.4.4. Influenza A (H5N8) Virus

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Purpose
H5N8 Highly pathogenic avian influenza viruses (HPAIVs) that emerged early 2014 in poultry in East Asia spread to worldwide. Phylogenetic analysis revealed that two distinct genetic groups of the H5N8 HPAIVs were identified in the South Korea, defined as group A and B. Between two groups, the dominant viruses belonged to group A further evolved into four distinct subgroups: C1 (South Korea), C2 (South Korea/Japan), C3 (North America/Japan), and C4 (Europe/Japan/South Korea). Here, we evaluated five genetically distinct subgroups (A, B, C1, C2, and C4) of the H5N8 HPAIVs for zoonotic potential.

Materials and Methods
Receptor binding specificity of the HA protein for α2,3- or α2,6-linked sialic acid (SA) was determined by a solid-phase binding assay. The pH of fusion-induced hemolysis was also determined. The replication kinetics of the viruses were assessed in the human bronchial epithelial cell line Calu-3 at 33°C or 37°C.

Results
All the representative H5N8 HPAIVs preferentially bound to α2,3-SAs, with minimal binding to α2,6-SAs. The midpoint pH for membrane disruption in hemolysis assays was 5.51 to 5.62 for the H5N8 HPAIVs except A/domestic mallard duck/Korea/H1924/2014(C4) virus. In Calu-3 cells, replication of all H5N8 viruses except A/mallard duck/Korea/H2102/2015 (C2) was delayed and less efficient at 33°C than at 37°C.

Conclusion
Although the HPAI H5N8 viruses have not yet acquired the entire features required for mammalian host adaptation, their ability to rapidly spread worldwide and continuously evolve highlight continued monitoring and underscores the need for continued risk assessment in mammals.

Keywords: HPAI, H5N8, Mammalian Adaptation

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Public Release of Wild Birds Movement with GPS Device on Web for Prevention of HPAI

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Purpose
Korea experienced five major epidemics of highly pathogenic avian influenza (HPAI) with variable virus types: H5N1 (years 2003–2011), H5N8 (2014–2017) and H5N6 (2016–2017). Migratory birds are suspected to be major factor of introducing HPAI virus into Korea. This study presents a project aiming at preventing HPAI by informing the public about the route of migratory birds using visualization of migratory birds with GPS.

Materials and Methods
A web-site examining real-time and archive positions of wild birds were constructed: mbird.or.kr. This site was based on signals from geographical positing system (GPS) devices attached through Animal and Plant Quarantine Agency (APQA)’s program for preventing HPAI. Signals of wild birds’s position is captured through GPS combined with wide band cede division multiple access (WCDMA) mobile phone network with global roaming.

Results
We launched the website(mbird) available to the public. The public can see the movement route in movie format. The website contains migratory routes of wild birds with GPS by year, type, time and region. This public website presents migratory routes of birds attached GPS by the APQA research time and its associates not only in Korea but also in China and Mongolia.

Conclusion
Tracking device is useful to monitor movement of wild and domestic animals. It will provide valuable information on evidence disease transmission. Accumulation of data will confirm the role of animal movement as career of pathogen.

Keywords: GPS, Wild Birds, Web, HPAI, Migration

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Fatal H5N6 Influenza Virus Infection in Cats in South Korea, 2016

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Purpose
The human infection by H5N6 virus was firstly reported in China in 2014. Here, we report H5N6 virus isolation from cats during H5N6 HPAI outbreaks among wild birds and poultry farms in South Korea in 2016.

Materials and Methods
On December 26, 2016, carcasses of 1 domestic male cat and 1 juvenile stray cat were submitted the regional veterinary office. The virus isolation was performed by SPF egg inoculation. Phylogenetic and genetic analyses were conducted for molecular characterization.

Results
The H5N6 viruses isolated from cats belonged to group C of H5 clade 2.3.4.4. Korean H5N6 viruses from poultry and wild birds were divided into five distinct geno-groups (C-1 to C-5). The feline isolates showed high similarity with C-4 geno-group of H5N6 viruses, which were isolated from poultry farms located in same area (Pocheon, Gyeonggi province).

Conclusion
This study is the first report HPAI H5N6 infection in cat in South Korea, although fatal infection by H5N6 in domestic cats has been reported in China. Our results demonstrated that domestic cats are also at risk for H5N6 infection. Hence, it should be taken measures to minimize the risk of transmission with companion animals, like cat and dog, and controlled by preventing any contact with affected poultry or wild birds.

Keywords: H5N6, Cat, South Korea

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Occurrence of TEM, SHV and CTX-M Genes in Diarrhea Chickens and Antibiotic Resistance

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Purpose
To survey the frequency of TEM, SHV and CTX-M genotypes in extended-spectrum beta-lactamase producing Escherichia coli (ESBL-producing E. coli) isolated from diarrhea chickens collected from different regions in Tra Vinh province of Vietnam.

Materials and Methods
A total of 100 fecal samples of diarrhea chickens were collected. The phenotypic identification of ESBL was confirmed by combination disc methods. In vitro, susceptibility to ESBL-producing E. coli isolates than 14 antimicrobial agents was performed by Kirby-Bauer disk diffusion method. The frequency TEM, SHV and CTX-M ESBL-producing E. coli was assessed by Polymerase Chain Reaction method.

Results
The frequency of ESBL-producing E. coli was 47%. In vitro, susceptibility to ESBL-producing E. coli showed that the majority of isolates were highly susceptible to amikacin (92%), fosfomycin (86%), doxycycline (79%) and colistin (71%). The rates of resistance to other antibiotics varied from 37% to 100%. Through 40 tested isolates, the prevalence of TEM, SHV and CTX-M genes was determined to be 77.5%, 67.5% and 77.5% respectively. All isolates showed the presence of the antibiotic resistance genes including 87.5% CTX-M gene, 75% TEM gene, and 70% SHV gene.

Conclusion
Due to the increase of E. coli with multiple ESBL genes, continuous surveillance in order to use appropriate antibiotics and the control of infections in chickens is necessary.

Keywords: Escherichia Coli, Extended-Spectrum Beta-Lactamase, Diarrhea Chickens

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Establishment and Evaluation of Colibacillosis Models

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Purpose
Avian pathogenic E. coli (APEC) causes embryonic death, acute and chronic mortality of young and adult Aves. To date, various colibacillosis models with various E. coli strains were reported, but we established models to simulate all kinds of ways of APEC infection.

Materials and Methods
For evaluation of embryonic death model, SPF embryonated chicken eggs (ECEs) were dipped in saline with 10⁷ cfu/ml of a serotype O78 E.coli strain (E104) for 15min and antibiotics or saline were injected into allantoic cavity. Hatching rate between antibiotics-treated and non-treated groups were compared. For 1-day-old chick mortality models E104 (10⁸ cfu/100ul) was inoculated via subcutaneous route (SC) after feeding or per os before feeding. In case of SC inoculation model, chicks were treated with antibiotics or saline and fasted for three days. Respiratory infection model was established by pre-infection of an IBV (KM91, 10⁴ EID₅₀/chickens) before intratracheal inoculation of 10⁸ cfu/100ul of E104 to 4-week-old SPF chickens.

Results
For embryonic death model, the mortality of antibiotics-treated group (3%) was significantly different from that of non-treated group (90%) (P<0.05). The SC inoculation model showed 0 and 100% mortalities in antibiotics-treated and non-treated groups. Per os inoculation model resulted in 40 and 10% mortalities in challenged and non-challenged groups. Respiratory infection model showed 0 and 50% mortalities in IBV- and IBV+APEC-inoculated groups, respectively.

Conclusion
In this study, we successfully established avian colibacillosis models which covering all kinds of infection patterns of APEC. These models may be useful for evaluation of antibiotics and vaccines against avian colibacillosis.

Keywords: Avian Pathogenic E.coli, Embryonic Death Model, 1-Day-Old Chick Mortality Models, Respiratory Infection

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Vaccination of Chickens with PoulShot Cocci\textsuperscript{®} Induces Protective Immunity against Coccidiosis

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Purpose
Avian coccidiosis is one of the most important diseases affecting the poultry industry worldwide. The coccidia species in the chicken belongs to the genus Eimeria. Feed additive antibiotics are used to prevent and treat coccidiosis but there is increasing concern about antibiotic resistance. In line with this, we developed a live anticoccidial vaccine, PoulShot Cocci\textsuperscript{®}, to control and prevent Eimeria infections.

Materials and Methods
Thirty SPF and commercial (field) chicks aged 7 days old were used as test animals. The chicks were randomly divided into three groups of 10 chicks each. The chicks in one group (vaccine) were vaccinated orally with 1 dose of PoulShot Cocci\textsuperscript{®}. The other groups were used as positive and negative controls and inoculated orally with PBS. At 28 days of age, vaccinated and positive control animals were challenged orally with Eimeria tenella. Chicks were observed daily up to 7 days post challenge. Mortality rate and lesions indicative of Eimeria tenella during necropsy were recorded.

Results
Control chicks started to die at 4 days post challenge (DPC). At 7 DPC, 60~80\% of control chicks were dead. On the other hand, 100\% of vaccinated chicks survived. Also, the average lesion scores were 3.6±0.9, 3.5±0.8 for control chicks and 1.8±1.3 and 1.2±0.7 for vaccinated chicks. Lesions were not observed in negative control chicks.

Conclusion
The results revealed that vaccination with PoulShot Cocci\textsuperscript{®} induces protective immunity against coccidiosis. Based on these evidences, PoulShot Cocci\textsuperscript{®} can be used as an alternative anticoccidial drugs.

Keywords: PoulShot Cocci\textsuperscript{®}, Live Anticoccidial Vaccine, Avian Coccidiosis

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Isolation and Characterization of Probiotic 
*Bacillus Subtilis* VL28 on Chicken Farms in Vietnam

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**Purpose**
The aim of this study was to isolate, identify and examine potential probiotic activities of *Bacillus subtilis* strains from soil and feces on chicken farms in Mekong Delta, Vietnam.

**Materials and Methods**
Bacillus spp. strains were isolated according to Eman (2013). The isolates were identified by biochemical tests, API CH50B kits and 16s RNA sequencing techniques. 
Probiotic activities were assessed based on extracellular enzyme secretion (Harley et al., 2001), temperature stability at 50°C (Barbosa et al., 2005), acid and bile salts tolerance (Corcoran et al., 2005, and Dunne, 2001), aggregation ability (Kos et al., 2003, AlGburi et al., 2016), and antagonistic ability by agar diffusion method (Moore et al., 2013).

**Results**
From 296 Bacillus spp. isolates, twenty-one *Bacillus subtilis* strains were identified. Of these, *B. subtilis* VL28 showed the greatest probiotic potential and its gene has been registered on the Genbank with accession number KY346980. *B. subtilis* VL28 was capable of producing extracellular enzymes (amylase, protease, and lipase). It can growth at 50°C and was stable at pH 2 and 0.05% bile concentration. It had the ability to auto-aggregate (82%), and co-aggregate with pathogens (16.1% and 36.4% in co-aggregation of *E.coli* and *Salmonella*, respectively), and showed antagonistic activity against *E.coli*, *Salmonella* spp., *Staphylococcus* spp., and *Streptococcus* spp. with inhibition zones about 14 - 30mm in diameter.

**Conclusion**
Based on these results, it is suggested that the newly found *B.subtilis* VL28 is a potential probiotic strain that can be applied in poultry production.

**Keywords:** Bacillus Subtilis, Chicken, Probiotic

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Serologic Investigation for West Nile Virus Infection in Commercial Domestic Chickens (**Gallus Gallus Domesticus**)

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**Purpose**
West Nile Virus (WNV) is a virus of the family *Flaviviridae*. The virus mainly infects birds, but is known to infect humans, horses, dogs, cats, bats, chipmunks, skunks, squirrels and domestic rabbits as well. The virus is transmitted through mosquito vectors, which bite and infect birds. In this study, West Nile Virus antibody presence (WNV) in white leghorn chickens located in various commercial domestic chicken establishments in Konya was studied serologically.

**Materials and Methods**
Blood sampling from 380 white leghorn chickens within the age range of 20-40 weeks was carried out. Blood serum samples were studied by West Nile Competition ELISA kit (ID-VET, Montpellier, France).

**Results**
All samples were found seronegative.

**Conclusion**
We have the assumption that this could be due to protective precautions-applications by establishments and advantageous natural geographical structure of the area where these establishments are located.

**Keywords**: West Nile Virus, Domestic Chicken, Serology

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AM-0093

Efficacy of an Effervescent Tablet Presentation of the Newcastle Disease VG/GA Strain Vaccine AVINEW NeO® Against a Virulent ND Challenge

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Purpose
Newcastle disease (ND) control requires good management practices, high biosecurity and efficacious vaccination programs. Technology improvements and the simplification of the vaccination process impact positively its efficacy. The substitution of the freeze dried presentation by an effervescent tablet provides with convenience, security and an ecofriendly option. The aim of this work was to attest the efficacy of an effervescent tablet presentation of the Newcastle disease VG/GA strain vaccine (AVINEW NeO®) against a virulent ND challenge and its bioequivalence with the freeze dried form.

Materials and Methods
The viral titers provided by freeze dried and effervescent vaccines were evaluated. A total of 180 broilers were divided in 5 groups: groups 1= AVINEW NeO® applied at 1 and 10 days, Group 2= AVINEW NeO® applied at 1 and 10 days plus day 1 inactivated ND. In groups 3 and 4 AVINEW NeO® was substituted by Freeze dried AVINEW®. Group 5 remained as unvaccinated/challenged control. At 26 days birds were challenged with 10^6,0 EID50 of a genotype XII Peruvian ND virus. Clinical signs and mortality were assessed.

Results
No significant differences between the titers of the reconstituted AVINEW NeO® and AVINEW® were observed (10^7.2 and 10^7.3 respectively). All groups provided at least 85% protection against a very stringent heterologous challenge. The protection was higher in the groups including the killed vaccine (above 94%).

Conclusion
These results confirm the bioequivalence of the two vaccine presentation and suggest together with the convenience for the vaccination process that the use of effervescent live viral vaccines in a step forward in poultry vaccination.

Keywords: Effervescent Tablet, Newcastle Disease, Vaccination

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Berberine Attenuates *Riemerella Anatipestifer* Infection by Suppressing Th17 Responses Through NF-κB Signaling Pathway

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Purpose
Berberine is used as an herbal medicine in ancient China and an anti-inflammatory agent for various inflammatory diseases. In this study, we elucidated the ameliorative effect of berberine against *Riemerella anatipestifer* infection.

Materials and Methods
The effects of berberine on survival rate, bacterial burden, Th17-related cytokine expressions and NF-κB signaling pathway in *R. anatipestifer* stimulated duck splenocytes and infected tissues were investigated. Duck splenocytes were stimulated with killed *R. anatipestifer* and treated with berberine. Two-week-old ducks were intramuscularly infected with *R. anatipestifer* and orally treated with berberine for 10 days. Survival rate and bacterial load from each spleen and liver obtained at day 4 were monitored. Tissue and cell samples were subjected to histopathological, qRT-PCR and Western blot analyses.

Results
Berberine treatment in experiment *R. anatipestifer* infection in ducks increased survival rate and reduced bacterial burden in spleen and liver of infected and berberine-treated ducks compared to infected and untreated ducks. The mRNA expression levels of IL-17A, IL-6, IL-1β and TGF-β in duck splenocytes stimulated with killed *R. anatipestifer* and infected tissues were significantly downregulated after berberine treatment. Interestingly, berberine downregulated transcription and signal molecules like MyD88 kinase, TAK1, STAT3 and NF-κB1, thus, suppression of NF-κB signal pathway activity.

Conclusion
The protective effects of berberine against *R. anatipestifer* infection in ducks, *in vitro* and *in vivo*, were associated with suppression of Th17-related cytokines and NF-κB signaling pathway inhibition, leading to amelioration of *R. anatipestifer* infection. This research was supported by IPET (MAFRA-716002-7).

Keywords: Riemerella Anatipestifer, IL-17A Downregulation, Berberine

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In Vitro Screening of Isolated Lactobacilli from North of Iran Native Chickens to Control Salmonella Enteritidies, Salmonella Typhimurium and Escherichia Coli

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Purpose
The aim of this study was screening of lactobacilli isolated from north of Iran’s native chickens for in vitro probiotic potential to control food born pathogenes.

Materials and Methods
Two hundred and seventy gram positive, catalase negative, rods happed bacterial strains were isolated from different part of backyard chicken’s intestine. Three Lactobacillus johnsonii and nine Lactobacillus reuteri strain were chosen according to their in vitro survivability in chicken gut through bile and acid resistance tests and 16s RNA sequencing. Their ability to control pathogens was evaluated using spot-the lawn technique. The bio-safety tests including beta haemolysis and antibiotic susceptibility test using ampicillin, clindamycin, gentamicin, streptomycin, tetracycline, erythromycin, kanamycin and chloramphenicol carried out.

Results
The selected bacteria survived at pH=3 for three hours and their 0.3% bile resistance was varied between 56 to 71% for 8h. The Isolated Lactobacillus reuteri had better ability to in vitro inhibition of Salmonella enteritidies and Salmonellatyphimurium than Escherichia coli. Twelve lactobacillus strains were beta haemolysis negative. Two of the three Lactobacillus johnsonii strain showed resistance to chloramphenicol and one of them showed resistance to clindamycin + tetracycline. Among nine Lactobacillus reuteri strain three strains showed antibiotic resistance to tetracycline, chloramphenicol and tetracycline + erythromycin + clindamycin respectively.

Conclusion
The results demonstrated that Lactobacillus reuteri isolated from native chickens intestine were able to survive under gastrointestinal tract condition and were able to inhibit food borne pathogens in vitro.

Keywords: Lactobacilli, Native Chickens, Probiotic Potential

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Evaluating Efficiency of Vaccine LASOTA with Newcastle Disease Viruses Isolated from Outbreaks in the Province in the South of Vietnam

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Purpose
The study was conducted in purpose of defining the virulence of Newcastle disease virus (NDV) strains and evaluating the efficiency of vaccine Lasota by challenging to chickens in the province in the south of Vietnam.

Materials and Methods
With 5 samples (chicken brain; named A1, A2, A3, B1 and B2) collected from the outbreaks in two districts of this province, isolated to determine NDV by HA, HI test, RT-PCR and challenged to 6-week-old chickens after vaccinating by vaccine Lasota.

Results
The tests show that all viruses were high virulent NDV. The NDV strain in the sample A3 was the highest virulence with mean death time (MDT) of 52.8 hours, and LD<sub>50</sub> of 10<sup>-7.5</sup>. The protection rate of chickens after vaccination was 100% with challenging by the strain A3.

Conclusion
The result shows that the antigen structure between the infected NDV and the NDV in vaccine Lasota was homologous. Therefore, vaccine Lasota can protect the local chickens from the virulent Newcastle disease virus strains.

Keywords: Newcastle Disease Virus (NDV), Vaccine Lasota, Chicken in the South of Vietnam

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The Efficacy of a Mixture of *Azadirachta Indica* Extract and Wood Vinegar Against the Feather Mite *Megninia* Spp Naturally Infecting Laying Hens

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**Purpose**
The research objective was to investigate the acaricidal potential of the neem (*Azadirachta indica*) extract and wood vinegar mixture at different concentrations for its use as botanical acaricide to control the feather mite infestation in chicken

**Materials and Methods**
The experiment was conducted in 6 groups according to the concentrations of the *Azadirachta indica* extract (AIE) and wood vinegar (WV) mixture: group1 2% WV, group2 4%WV, group3 2% AIE, group4 2%AIE and 2%WV mixture, group5 2%AIE and 4%WV mixture and group6 untreated control. The treatments were applied by dipping the laying hens. The results were recorded at day 0, 1, 2, 3 and 7 by observing the number of mites on hen body in three sites: neck, under wing and back. Each treatment was studied in triplicated

**Results**
The examinations of on laying hens after treatment on day1 showed considerable reduction of living mites in all treatment groups compared to controls. The 2%AIE and 2%WV mixture showed the highest efficacy by letting hens free of mites from day1 till day 7. Both 2%AIE and 2%WV mixture and 2%AIE and 4%WV mixture showed the greater results than those of 2% AIE and 2%WV treatment

**Conclusion**
These results show a very high acaricidal potential of the *Azadirachta indica* extract and wood vinegar mixture, if the mites come in direct contact with the mixture.

**Keywords**: Azadirachta Indica Extract, Wood Vinegar, Megninia Spp

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Biochemical and Histopathological Study of Aflatoxicosis on Ross 308 Broiler Chick

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Purpose
This study was designed to establish a dietary dosage and exposed time among AFB₁ level in diet on broiler performance.

Materials and Methods
One-days Ross 308 broiler feed contained diet 0, 0.5, 1.0 and 2.0 mg AFB₁/kg in feed for 21 days. The body weight gain, serum biochemistry and histopathological lesions of liver were evaluated on 7, 14 and 21 days post feeding (dpf), respectively.

Results
The body weight on AFB₁ - treated broilers was lower than that in the control diet. On 14 and 21 dpf of 2.0 mg AFB₁ broilers was significantly lower than that of another group (p < 0.01, p < 0.001), respectively. The relative liver weights were significant increased in a dose-dependent manner and the relative spleen weights were significantly increased of 2.0 mg AFB₁/kg - treated on 21 dpf (p < 0.001).

Biochemical analyses showed that the total protein and albumin were significantly decreased on 7 and 14 dpf of 2.0mg AFB₁ treated compared with another treatments (p <0.05). AST levels were significantly increased dose dependently (p <0.05).

Histopathological analyses showed that liver tissues on AFB₁ - treated birds showed significant lesions such as hemorrhage, hepatocyte necrosis, inflammatory cells infiltration and fatty degeneration. Hepatocyte necrosis or infiltrated inflammatory cell was more severe to dose- and exposed length - dependently and the liver fibrosis was increased dose dependently (p <0.05).

Conclusion
The results could aid in understanding the underlying basis for parameters for evaluate to aflatoxicosis.

Keywords: Aflatoxin B1, Broiler Performance, Dose Dependently Eeffect

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Effects of Saccharomyces Cerevisiae on Tibial Dyschondroplasia in Broiler Chickens

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Purpose
The aim of this study was to determine the effect of Saccharomyces cerevisiae (yeast) on tibial dyschondroplasia in broiler chickens.

Materials and Methods
The experiment was carried out on broiler chicks (n=300) for 42 days. The experiment groups were: Control, Yeast1 (added 0.1% yeast), Yeast2 (added 0.2% yeast) and Yeast4 (added 0.4% yeast). Feed and water were provided ad libitum. At the end of the experiment the left tibia of broiler chickens was used for bone weight, length, ash, mineral content and tibial dyschondroplasia evaluation.

Results
Chickens fed with different doses of yeast supplement have significantly higher body weight when compared to control group. 0.4% yeast supplementation to the diet of broiler chickens significantly increased bone ash and bone calcium content (p<0.05). While 0.2% yeast supplementation to the diet significantly increased bone phosphorus content, 0.1% and 0.4% yeast supplementation significantly decreased bone phosphorus content. Adding 0.2% yeast supplement significantly increased bone length, while adding 0.4% of yeast supplement increased the bone weight significantly. However, there was no significant effect of yeast supplement on carcass weight (p>0.05). Also, there was no significant effect on tibial dyschondroplasia rates between groups (p>0.05) but increasing doses of yeast supplement decreased the severity of the tibial dyschondroplasia.

Conclusion
Yeast supplement to diet of broiler chicken has positive effects on broiler performance. Our data show that there is no significant effect on tibial dyschondroplasia rates between groups (p>0.05) but increasing doses of yeast supplement decreased the severity of the tibial dyschondroplasia.

Keywords: Saccharomyces Cerevisiae, Tibial Dyschondroplasia, Broiler

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Molecular Epidemiological Analysis of *Mycoplasma Gallisepticum* Isolates from South Korea by Gene Targeting Sequencing (GTS)

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**Purpose**

*Mycoplasma gallisepticum* (*M. gallisepticum*) causes chronic respiratory disease in chickens and economic losses of decreased meat and egg production. For control, live vaccines are used in commercial layers and breeders. Molecular methods including RAPD have been used for the differentiation between vaccine strains and field isolates. Targeted sequencing is known to be a highly reproducible typing method for strain differentiation. In this study, we used the GTS(gene targeted sequencing) analysis using multiple gene sequences for epidemiological study of the Korean field isolates.

**Materials and Methods**

Sixteen *M. gallisepticum* isolates from chicken flocks during 2005 to 2016 were used. Genomic DNA extraction and PCR amplification were performed as described previously (Ferguson et al., 2005). GTS analysis using sequences of gapA, MGA_0319, mgc2 and pvpA was conducted.

**Results**

Six dendrograms were constructed from sequence alignments. Korean isolates were divided into 3 to 4 sequence types by analysis of individual genes and 5 sequence types by analyses of mgc2/pvpA or gapA/MGA_0319/mgc2/pvpA combined genes. Discrimination using MGA_0319 and pvpA sequencing was poor. Two isolates, CSG17 and 13AD117 were related to the vaccine strains of ts-11 and F strains, respectively.

**Conclusion**

The Korean isolates could be differentiated by the GTS analyses of individual genes (gapA and mgc2) and combined genes (mgc2/pvpA and gapA/MGA_0319/mgc2/pvpA). GTS analysis using multiple gene sequences showed better discriminatory power. This research shows that GTS can be used for strain typing for epidemiological study of *M. gallisepticum* in South Korea.

**Keywords**: Mycoplasma Gallisepticum, Sequencing, GTS(Gene Targeted Sequencing) Analysis, Dendrogram, Typing

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Molecular Epidemiological Investigation of Infectious Bronchitis Virus Isolated from Vietnam

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Purpose
Infectious bronchitis virus (IBV) exists in most of countries around the world and has numerous serotypes and variants because of its unstable genetic property. Despite the economic impact of IBV on poultry industry, to date there have been no information on the epidemiology of IBV in Vietnam. Therefore, in the present study, we reported virus isolation and characterization of IBV from Vietnam.

Materials and Methods
Virus isolation and identification were performed for tissue samples from dead chickens from Vietnam between 2014 and 2015. The full length S and N genes of IBV isolates were amplified by RT-PCR. Each of PCR products was sequenced and analyzed by multiple alignment and phylogenetic analysis.

Results
Three field isolates of IBV were successfully isolated from dead chickens in Ha Noi, Thai Nguyen, and Hai Phong provinces. In phylogenetic analysis, three IBV isolates were classified into three distinct genotypes, including Q1-like, QX-like, and TC0702-like genotypes at the level of S1 gene. Each of Vietnamese IBVs was closely related to Chinese IBVs of corresponding genotypes at the levels of N and S2 genes, as well as S1 gene. Amino acid alignment revealed that Vietnamese IBVs showed no significant genetic change such as deletion, insertion and recombination.

Conclusion
This is the first report of IBVs circulated among chickens in Vietnam. Our results indicate that at least three genotypes of IBV, which might be introduced from neighboring countries, especially China, are co-circulating among chickens in the Northern Vietnam.

Keywords: Infectious Bronchitis Virus, Vietnam, Genotype, Phylogenetic Analysis

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Molecular Characterization of Salmonellae Collected from Chickens in Some Selected Poultry Farms of Bangladesh

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Purpose
Salmonella infections are the major constraints of poultry industries in Bangladesh. It causes great economic loss every year. It has also zoonotic importance. The present investigation was designed with the following objectives:
Isolation and identification of salmonellae;
Molecular characterization;

Materials and Methods
A total 557 bacteriological samples were collected aseptically by using cotton swab in tetrathionate broth from cloacae of live chickens, liver of dead chickens and, and farm premises.
Isolation and Identification of salmonellae:
All the samples were subcultured on different media of Salmonella. The Gram’s staining and motility test were done.
Molecular characterization:
For PCR analysis of salmonellae, 139(F) and 141(R) oligonucleotide primers (Genei, Banglore, India) targeting the Salmonella invA gene were used.

Results
Isolation and identification of salmonellae:
On the basis of colony characters, 63 (38 from cloacal swab of alive birds, 28 from liver swab of dead birds, and 7 from farm premises) were identified as Salmonella spp.
Among 63 isolates, 21 (33.33%) were Salmonella Pullorum, 15 (23.80%) were Salmonella Gallinarum and 27 (42.85%) were other Salmonella spp. based on the motility and biochemical tests.
Detection of salmonellae by PCR:
All isolates were confirmed by PCR tests. In PCR salmonellae organisms were shown 284 pb band. No band was shown in control.

Conclusion
Poultry industry faces problem for salmonellae infections in our country. The salmonellae isolates may be used for phylogenetic analysis, pathogenesis study and vaccine production that will help diagnosis, prevention and control of economically important Salmonella infections in Bangladesh.

Keywords: Chickens, Salmonellae, Characterization

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Development of LAMP for Detecting Immunosuppressive Viruses in Chicken

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**Purpose**
Chicken infectious anemia virus, marek’s disease virus, reticuloendotheliosis virus and infectious bursal disease virus are important immunosuppressive viruses in chicken. We developed LAMP-CIAV:MDV:REV:IBDV to detect immunosuppressive viruses more rapidly, conveniently and accurately in poultry farms.

**Materials and Methods**
The five LAMP primers were designed based on the published sequences in CIAV, MDV, REV and IBDV using Primer Explorer V4 software, respectively. In order to determine the sensitivity of the viruses, the LAMP assay was carried out using different amounts of DNA from the plasmid V2-CIAV, V2-MDV, V2-REV and V2-IBDV containing target genes. The specificity of the LAMP assay for IBDV detection was carried out using RT-LAMP kit(Mmonitor), and the LAMP assay for specificity test of CIAV, MDV, and REV was carried out using genomic DNA from CIAV, MDV, REV and other avian diseases viruses. The specificity of the LAMP assay for the detection of the viruses was consistent with conventional PCR.

**Results**
The optimal condition to confirm the sensitivity for CIAV, MDV, REV and IBDV detection was 63°C for 40min with a detection limit of 50 copies(CIAV), 20copies(MDV),16copies(REV) and 250 copies(IBDV), respectively. And the optimal temperature of specificity test for IBDV detection using RT-LAMP kit was 58°C for 40min. The LAMP products were successfully amplified and detected by DNA electrophoresis and by naked eye.

**Conclusion**
We checked the specificity and sensitivity of the developed LAMP- CIAV:MDV:REV:IBDV method and confirmed that the method was more efficient than previously reported detecting methods such as PCR.

**Keywords**: LAMP, Immuno-Suppressive Virus, Chicken

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Development of LAMP for Detecting Bacterial Pathogens Causing Arthritis in Broiler

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Purpose
Escherichia coli, Enterococcus sp. and Staphylococcus sp. are the major bacterial pathogens causing arthritis in broiler. We developed LAMP-E. coli:Entero:Staphylo to detect more rapidly, conveniently and accurately.

Materials and Methods
The strains used in this study were Enterococcus species (E. faecium, E. faecalis, E. hirae, E. durans, E. avium, E. gallinarum, E. cecorum and E. columbae), Staphylococcus species (S. aureus and S. cohnii) and E. coli. The LAMP primers were designed based on the published common sequences in Enterococcus species, Staphylococcus species and E. coli, respectively. The LAMP assay for specificity test was carried out in a total of 25 ul of reaction mixture containing genomic DNA of the Enterococcus species, Staphylococcus species, E. coli and other several avian pathogens. In order to determine the sensitivity of Enterococcus species, Staphylococcus species and E. coli, the genomic DNA was serially diluted 5-fold. The reaction result was monitored visually.

Results
The optimal temperature for the LAMP assay using the screened primers was 63°C for 40min with a detection limit of 400fg~50pg/ul(Enterococcus species), 400fg~2pg/ul (Staphylococcus species) and 800fg/ul(E. coli). And the specificity of the Enterococcus species, Staphylococcus species and E. coli was 100%, respectively.

Conclusion
We checked the specificity and sensitivity of the developed LAMP-E. coli:Entero:Staphylo method and confirmed that the method was more efficient than conventional detecting methods such as identification using chemicals and PCR methods.

Keywords: LAMP, Arthritis, Broiler

*Corresponding Author: Seongjoon JOH (johsj0901@korea.kr)
Characterization of *Clostridium Perfringens* Isolates and Reproduction of Necrotic Enteritis in Broiler Chickens

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**Purpose**
Necrotic enteritis (NE) is caused by *C. perfringens* (CP) and emerging problematic disease in broiler industry after banning feed antibiotics. Experimental reproduction of NE is essential for understanding pathogenesis of CP and development of disease control measures. Recently NetB toxin is known to important for reproduction of NE. Therefore, we aimed at characterization of toxin genes of CP isolates, and reproduction of NE by challenging an isolate possessing netB gene.

**Materials and Methods**
The presence of α, β, ε, ι, β2, pfoA, netB, and enterotoxin genes were assessed by PCR for 13 isolates. A netB-positive CP isolate was orally administered to broilers by 3 times at 14-16 day-old with 10⁸cfu/ml, and fishmeal was added to feed. Body weights were measured once a week, and necropsy was performed at 21 day-old to observe intestinal lesions.

**Results**
Alpha toxin and pfoA genes were present but β and ε toxin genes were absent in all isolates. β2 toxin gene was detected in nine strains, and ι toxin, netB, or enterotoxin genes was detected in only one isolate. Mean body weight of broiler chickens challenged with a netB-positive isolate showed significant differences from that of control group at 21 day-old (P<0.05). Also, fibrin deposition or ulcer in the small intestine was observed in the challenge group.

**Conclusion**
NE was successfully reproduced by challenging netB-positive CP and suppling fishmeal-added feed. This result may be useful for understanding pathogenesis of CP and development of disease control measures.

**Keywords**: Necrotic Enteritis, Clostridium Perfringens, NetB Gene

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Analysis of Antibody Positive Rate by Vaccination of FMD According to Age in Cattle and Pigs

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Purpose
We report the positive rate of antibody after foot-and-mouth disease (FMD) vaccination in cattle and pigs. The positive rate of antibody against FMD was analyzed at age.

Materials and Methods
The FMD vaccine injected at 4~7 months intervals in cattle and pigs raised at the National Institute of Animal Science. Hanwoo was vaccinated with two serotypes (O Manisa+O 3039+A22 Iraq) FMD vaccine. Pig and dairy cattle were vaccinated with single serotype (O Manisa+O 3039) vaccine. The positive rate of FMD antibody was analyzed in blood by ELISA.

Results
In the case of cattle, positive rate of FMD antibody was high in all ages. The positive rates in Hanwoo and dairy cattle were 99.5% and 97.7%, respectively. In pigs, the antibody positive rate was 96.9%. However, antibody positive rate of FMD vaccine was relatively low at 3 to 5 months of age, especially 57.6% at 3 months of age.

Conclusion
Both cattle and pigs showed high rates of antibody against FMD vaccination over 95%. In cattle, high antibody positive rates of FMD were found at all ages, whereas pigs showed significantly lower antibody rates at 3 months of age.

Keywords: Foot-and-Mouth Disease, Vaccination, Antibody Positive Rate

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Efficacy Study of Three High Potency Oil-Adjuvant Vaccines Against a Heterologous Strain, FMDV O Jincheon Strain (O/SKR/2014), in Pigs

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Purpose
For the application of new vaccine strains in pigs, the vaccine evaluation is fundamentally important. Therefore, we investigated the efficacy of three high potency (>6 PD₅₀) oil-adjuvant commercial vaccines, one vaccine (containing O1/Campos strain) from Argentina and two vaccines (containing O/Primorsky/14 and O/Taiwan/97 strain, respectively) from Russia, against heterologous FMDV, O/Jincheon strain in pigs.

Materials and Methods
30 pigs were divided into 3 groups, vaccinated with O1/Campos, O/Primorsky/14 and O/Taiwan/97 vaccines, respectively. Among each vaccinated group, 5 pigs were challenged 14 days after vaccination, and other 5 pigs were challenged 28 days after vaccination. Additionally, 5 pigs were used as unvaccinated control. As donors, 4 pigs were inoculated with O/Jincheon strain and directly contacted with all groups of pigs for 24 hours. The animals were bled and observed for 14 days.

Results
All non-vaccinated animals showed obvious FMD clinical signs after challenging. However, vaccinated groups showed no clinical or only one-spot mild sign. None of the vaccinated pigs had detectable antibodies to FMDV non-structural protein (NSP). Most vaccinated pigs were sero-positive to the FMDV SP O ELISA since 7 days after vaccination. From 7 DPV to 0 DPC, the mean titers for neutralizing antibodies were between 1.2~2.1 log10 for O1/Campos, between 2.1~2.5 log10 for O/Primorsky/14, and 1.4~2.0 log10 for O/Taiwan/97.

Conclusion
This study showed that three high potency oil-adjuvant vaccines can provide proper protection against challenge of O/Jincheon strain by showing the significant decrease in clinical signs in pigs.

Keywords: FMDV, Vaccine, Pig, Virus Neutralization Test

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Laboratory Mouse Model to Evaluate Efficacy of Food-and-Mouth Disease Vaccine

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Purpose
The use of mouse models to evaluate foot-and-mouth-disease (FMD) vaccine candidates can save cost and time compared with experiments on target animals. The aim of this study is to develop FMD challenge viruses that are lethal to adult mice and to demonstrate the FMD vaccine evaluation using mouse model

Materials and Methods
Six-serotypes (O, A, Asia1, C3, SAT1 and SAT3) of FMD viruses, non-pathogenic in C57BL/6 mice, were inoculated IP in 8-week-old C57BL/6 mice. Sera were collected at 3 DPI and filtered through 0.2 um syringe filters to inoculate to ZZ-R cells for two passages. These steps of passages were repeated five times. To demonstrate the potential of mouse-challenge models, mice were vaccinated IM with two commercial trivalent FMDV vaccines containing A/Malaysia/97 and A22/Iraq antigen, respectively. Three weeks after the vaccination, mice were challenged IP with 100 LD₅₀ of mouse-adapted lethal FMD virus, namely A/Malaysia/97-M5Z10 strain. The body weight and survival rates of mice were monitored for 9 days.

Results
Eight mouse-adapted FMDV challenge strains (O/Jincheon, O/Vietnam, A/Malaysia/97, A/Vietnam, Asia1/MOG/5, C3/Resende, SAT1/BOT1, SAT3/ZIM4/81) were established. After challenging with A/Malaysia/97-M5Z10, mice vaccinated with A/Malaysia/97 antigen did not lose body weight and demonstrated a 90-100% survival rate for all groups (1/20 to 1/1,000 dose), while mice vaccinated with low dose (1/500 and 1/1,000 dose) of A22/Iraq antigen showed continuous weight loss and low survival rate (25%).

Conclusion
These results show the potential of mouse-adapted FMD challenge viruses for an effective efficacy test for FMD vaccines in mice

Keywords: Food-and-Mouse-Disease, Vaccination, Mouse-Adaptation

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Purpose
Since the current foot-and-mouth disease (FMD) vaccine is an inactivated vaccine and to differentiate infected animals from vaccinated ones, it is essential to remove nonstructural protein (NSP) during vaccine production process. According to the OIE standards, the vaccine purity test is currently examined using final vaccine product for cattle. This study was performed to develop a novel in vitro assay alternative to the current in vivo test.

Materials and Methods
The gene for the NSP 3AB was expressed in E. coli as fusion proteins using a p-BAD102/D-Topo vector. Hybridomas producing monoclonal antibody (MAb) to FMDV 3B were generated. The lateral-flow assay (LFA) was constructed with the selected MAb that was conjugated with gold particles and was immobilized as a test line on a cellulose membrane. NSP including 3B epitope present in a sample binds to the gold particles and results in a colored band.

Results
The optical condition of LFA was established with the MAb and recombinant NSPs. Through reactivity with the serially diluted recombinant NSPs, the analytical sensitivity of LFA was revealed to be less than 1 ng. This performance was comparable to the commercial PrioCHECK FMD IPC-3ABC kit that is known as the only in vitro kit for the detection of NSP in FMD vaccine.

Conclusion
Considering that the lowest amount of 3ABC that can elicit antibodies in vaccinated cattle was reported to be around 10.2 ng, the LFA developed in this study could be usefully employed for in-process quality control of FMD vaccine in the future.

Keywords: Foot-and-Mouth Disease, Vaccine, Purity, Nonstructural Protein

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Foot and Mouth Disease

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Co-Injection of a Vaccine and Anti-Viral Agents Can Provide Fast-Acting Protection from Foot-and-Mouth Disease in Pigs

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Purpose
We have developed two recombinant adenoviruses that simultaneously express porcine interferon-α and interferon-γ (Ad-porcine IFN-αγ) and multiple siRNAs (Ad-3siRNA) for rapid protection against foot-and-mouth disease virus (FMDV) until vaccine-induced protective immunity occurs. In the present study, we show that the use of Ad combination (Ad-porcine IFN-αγ and Ad-3siRNA) with a conventional foot-and-mouth disease (FMD) inactivated vaccine exerts rapid and consistent protective effects against FMDV.

Materials and Methods
Human embryo kidney (HEK) 293 cells, porcine kidney (LF-BK) cells, and FMDV O/Andong/SKR/2010 strain were used in this study. FMDV challenge was performed after application of vaccine and (or) Ad combination after 1, 2, or 7 days after administration in pig. And then clinical observation, sample collection, and analysis (viral copy numbers, antibody and cytokine) were conducted. The t-tests and the log rank test were performed for statistical analysis.

Results
We found that most of the pigs (five out of six) that received vaccine + Ad combination and were challenged with FMDV at 1 or 2 dpv were clinically protected from FMDV. In addition, most of the pigs that received vaccine + Ad combination and all pigs inoculated with the vaccine only were clinically protected from an FMDV challenge at 7 dpv.

Conclusion
We can say that the combination of the FMD inactivated vaccine and effective antiviral agents may offer both fast-acting and continuous protection against FMDV. In further studies, we plan to design co-administration of Ad combination and novel vaccines.

Keywords: Foot-and-Mouth Disease, Vaccine, Antiviral, Combination

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**Purpose**
Foot-and-mouth disease (FMD) is the most contagious disease of cloven-hoofed animals, leading to the severe economic loss in the country. The current FMD vaccine is an inactivated vaccine that has several disadvantages including the risk of virus escape during vaccine production. To overcome these drawbacks, we tried to develop a novel recombinant FMD vaccine using chimeric construct of vesicular stomatitis virus (VSV) glycoprotein fused to FMD virus VP1 GH loop epitope.

**Materials and Methods**
Sequence corresponding to VP1 amino acid GH loop of FMD virus was inserted into VSV glycoprotein whole protein. This construct was used to get the recombinant vaccine candidate. The candidate protein was mixed with ISA206 adjuvant, and injected into six piglets twice, one month apart. As a control group, commercial inactivated vaccine was also injected to piglets. The antibody titer was measured by FMDV type O ELISA.

**Results**
The recombinant vaccine protein was successfully expressed to be around 64kDa containing glycoprotein, FMD virus VP1 epitope and six histidine residues by SDS-PAGE. This expression was also confirmed by Western blot using monoclonal antibodies against FMD virus and vesicular stomatitis virus. The recombinant protein vaccine exhibited higher antibody titer than the commercial vaccine, though the difference not being significant.

**Conclusion**
The recombinant protein vaccine candidate showed comparable immunogenicity to the commercial vaccine. Although further study using more animals is needed, this preliminary test results shed light on the development of a novel FMD vaccine which can replace the traditional inactivated vaccine in the near future.

**Keywords:** Foot-and-Mouth Disease, Vaccine, Vesicular Stomatitis Virus, Glycoprotein

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Separation of Non-Structural Proteins and Foot-and-Mouth Disease Virus Using Chromatography

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Purpose
Foot-and-mouth disease (FMD) virus negatively affects animal husbandry development of the world. To differentiate the infected animals from vaccinated ones in vaccinated regions, non-structural protein (NSP) should be eliminated during the process of FMD vaccine production. Herein, several types of columns were tested for separating NSP and intact FMD virus particle.

Materials and Methods
FMDV strain O/JINCHEON was proliferated in the BHK-21 cell line by suspension culture method. In this study, we used five chromatographic columns (cation exchange, anion exchange, affinity, hydrophobicity, and size-exclusion) for FMD virus purification and NSP removal. FMD virus particle was quantified by sucrose density gradient analysis for each fraction of the columns and the amount of NSP was measured by lateral-flow assay developed with monoclonal antibody binding to 3B epitope.

Results
All but one column failed to separate between NSP and virus particle. While at low concentration of buffer condition, both virus particle and NSP were found in flow-through fractions, as the buffer concentration increased, NSP was still in flow-through fractions and the virus particle was detected in eluted fractions. This method obviates the additional treatment such as buffer exchange, so that it did not interfere with next process of FMD vaccine production.

Conclusion
FMD virus purification and NSP removal from crude FMD virus supernatant using one-step chromatography would guarantee the purity of FMD vaccine. Furthermore, this high throughput technology should be useful to simplify the whole manufacturing process of FMD vaccine in a large scale.

Keywords: Foot-and-Mouth Disease Virus (FMDV), Chromatography, Purification

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Unnecessity of Delaying Foot-and-Mouth Disease Vaccination for Maternally-Derived Antibody Decline in Pigs

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Purpose
In South Korea, all kinds of cloven-hoofed domestic animals have been recommended to be vaccinated to Foot-and-mouth disease (FMD) with an interval of 4 weeks at ages between 8 and 12 weeks after birth. However, growing pigs are vaccinated only once in those ages because of the costs involved, vaccine shortage, and injection-induced histopathological changes. Therefore, this study was performed to determine the optimal time of single vaccination for pigs with the currently used FMD vaccine consisting of O1 Manisa and O 3039 virus strains.

Materials and Methods
Group I, II, III, and IV pigs were vaccinated at the ages of 8 weeks, 10 weeks, 12 weeks, and 14 weeks, respectively. All pigs in the four groups were regularly bled after vaccination until slaughter age (24 weeks). To check antibody to FMD virus, the blood samples were tested using commercial type O enzyme-linked immunosorbent assay.

Results
Pigs vaccinated at 8 weeks (group I) showed higher seroprevalence than the other groups at the ages of 16, 20, and 24 weeks (p< 0.05), although the maternally derived antibody level at the vaccination point of group I was the highest among the four groups.

Conclusion
Though double vaccination is necessary to completely protect finishing pigs from FMD virus infection with the current FMD bivalent vaccine, the age of 8 weeks can be considered as the optimal time for piglet vaccination if the booster injection is unavailable because of several realistic reasons.

Keywords: Foot-and-Mouth Disease, Pigs, Vaccine

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Comparison of Clinical Symptoms and NSP(Nonstructural Protein) Antibody Formation in Experimentally Infected Pigs with Foot-and-Mouth Disease Virus (FMDV)

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Purpose
When an animal is infected with FMDV, it is generally known that NSP antibodies are detected within 10 days. However, the NSP antibody may not be detected in an animal whose clinical symptoms are reduced due to vaccination. Therefore, we compared clinical symptoms and NSP antibody production in experimentally infected pigs with FMDV.

Materials and Methods
The pigs were divided into four groups: 7 days after vaccination(A), 14 days after vaccination(B), 21 days after vaccination(C) and unvaccinated(D). Two pigs of group D were inoculated in the heel bulb with O/GJ/2016 and used as donors. The clinical symptoms were observed for 35 days. The probang samples and sera were used for viral antigen and NSP antibody detection, respectively.

Results
All animals in group D showed generally high clinical scores and NSP antibodies were detected. Two animals with the highest clinical scores in group A were NSP antibody positive. In group B, no NSP antibody was detected in all animals. However, the antigen was detected in the probang samples of this group. In group C, NSP antibodies were detected in only one pig with a relatively high clinical score.

Conclusion
We confirmed that there may be a close relationship between clinical symptoms and NSP antibody formation. NSP antibodies were sometimes negative even in the presence of antigen. Therefore, in order to find out FMDV infected animals, it’s very helpful to combine antigen tests as well as antibody tests.

Keywords: Foot-and-Mouth Disease, Nonstructural Protein, Viral Antigen

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Early Protection Against Foot-and-Mouth Disease in Mice and Pigs Treated with Ribavirin and FMD Vaccine

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Purpose
Co-injection of antiviral agent with FMD (foot-and-mouth disease) commercial vaccine could immediately protect mice and pigs from FMD. We investigated replication levels of FMDV, clinical score in pigs and survival rate in mice after FMDV challenge.

Materials and Methods
C57BL/6 mice were injected with Ribavirin which was known to inhibit replication of RNA virus including FMD. Ribavirin was administrated by oral route with feed and FMD vaccine was injected by intramuscular(IM) route. The agent was co-injected with adjuvants or vaccine by IM in mice. Mice were challenged with 50 median 50% lethal dose(50LD₅₀) of FMDV Asia1/Shamir on day 0, and on day 3 or 10. Survival rates of the mice were monitored for 15 days. Pigs were injected the agents with adjuvants or vaccine. Virus titer for pig challenge was 10⁶ tissue culture infectious dose₅₀(TCID₅₀) of Asia1/Shamir FMDV and the virus was inoculated into foot-pad. The pigs were monitored for 7 days.

Results
Ribavirin treated mice with adjuvants or vaccine showed higher survival rate than control-group. The mice administrated Ribavirin with adjuvants or vaccines even though mice were challenged twice by FMDV. Pigs had reduced copy number of viral RNA in blood and no clinical-score when they were injected with Ribavirin with adjuvant and vaccine. We suggest that the Ribavirin is an excellent antiviral-agent to control FMD.

Conclusion
Co-injection of Ribavirin and FMD vaccine could be efficient control agents for early protection of pigs against FMD. We suggest Ribavirin is an anti-FMDV agent to protect pigs from FMDV, if escape lethal dosage.

Keywords: Foot-and-Mouth Disease, FMD, Ribavirin

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Development of Two Assays to Quantify Foot-and-Mouth Disease Virus Intact Particle

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Purpose
Foot-and-mouth disease (FMD) virus capsid proteins self-assemble into a pentamer (12S) and then a whole virus particle (146S). Since immunogenicity of the 146S is superior to the 12S particle, it is important to obtain high yields of 146S during FMD vaccine production process. In this regard, it should be useful to develop an assay to quantify 146S amount, differentiating degraded form of 12S pentamer.

Materials and Methods
FMD virus whole particle (146S) was obtained by BEI inactivation, PEG concentration, and sucrose density gradient ultracentrifugation. Heat treatment of the 146S yielded into the 12S particle completely. Monoclonal antibody for the detection of 146S and 12S were produced in mice by immunizing inactivated FMD virus. The assays to detect the 146S and 12S were established by double antibody sandwich (DAS) enzyme-linked immunosorbent assay.

Results
While the 146S DAS ELISA exhibited the same pattern of optical density parallel to that of the sucrose density gradient ultracentrifugation fraction, the 12S DAS ELISA showed reversed pattern of result in comparison to the ultracentrifugation. Besides, the 12S DAS ELISA worked for FMD virus type O, A, Asia1, and C. It could also quantify 146S amount indirectly through before and after heat treatment of the 146S.

Conclusion
The DAS ELISA is easy to perform compared to the sucrose gradient ultracentrifuge to quantify FMD virus 146S. This will allow us to monitor whether the FMD virus is degraded or not during FMD vaccine production and to screen 146S stabilizer in a high-throughput way.

Keywords: Foot-and-Mouth Disease, Pigs, Vaccine

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**Quantification of 146S Particles of Foot-and-Mouth Disease Virus by UV Spectrophotometer Photometry of Sucrose Density Gradients**

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**Purpose**
For foot-and-mouth disease (FMD) vaccine production, the immunogenicity is strongly associated with the quantity of 146S virus particles. So in this study we tried to reproduce the technology used in the industry to measure the quantities of 146S particles of FMDV and applied it to newly developed vaccine strain.

**Materials and Methods**
We obtained viral antigens of the Korean FMDV isolates (A-Pocheon 2010, O-Jincheon 2014) produced through BHK21 suspension cells in the laboratory and A22 Iraq antigen received from a vaccine company for the quantification of 146S particles. The inactivated and concentrated viral antigen was centrifuged in sucrose density gradients (SDG) and subject to the continuous monitoring of the flow through spectrophotometer. In the absorbance profiles of the SDG, the 146S specific peaks were identified and the area of peak (APQ) was calculated to estimate the quantity of the 146S particles.

**Results**
For the A22 Iraq antigen, the linearity of the APQ over a range of diluted viral antigen was shown, and there was correlation between the APQ and the estimated quantity of the 146S particles corresponding to the 146S specific peak fraction. With these results as a basis, the quantity of 146S particles of A-Pocheon 2010 and O-Jincheon 2014 could be accurately estimated from their APQs. The integrity of the 146S particles contained in those 146S specific fractions was confirmed by electron microscope.

**Conclusion**
In this study, we could show that automatic fractionation system linked to the photometric system generating continuous UV absorbance profile is a robust technology to quantify 146S particles in the SDG of FMDV accurately.

**Keywords:** Foot-and-Mouth Disease, Quantification, 146S Particle

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Comparison of Protective Spectrum of Serotype O Korean Isolates of Foot-and-Mouth Disease Virus as Vaccine Candidates in Guinea Pig

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Purpose
From the continual and sporadic outbreaks of foot-and-mouth disease (FMD) by the Mya-98 lineages within O SEA topotype in the Republic of Korea from 2010 to 2016, the development of local vaccine strains more relevant to Korean situation can to be justified. The new vaccine strain needs to have broad spectrum as high as much, so we selected vaccine candidate strains from the 2014 outbreaks and compared their immunogenicity and width of protective spectrum in guinea pig.

Materials and Methods
Three vaccine candidate virus isolates were chosen from the clinical samples collected in the field from 2014-15; O/SKR/Jincheon/2014, O/SKR/Cheonan/2015, O/SKR/Anseong/2015. For each of them, we made monovalent experimental vaccine with the inactivated purified 146S virus particles mixed with Montanide ISA201VG (Seppic, France) for vaccination of guinea pigs. Serum neutralizing antibodies were measured in the sera bled at 21 days post vaccination against a panel of challenge viruses of ME-SA or SEA topotypes to compare the protective spectrum of them.

Results
The sera of the guinea pig immunized with the isolate, O/SKR/Jincheon/2014, showed broader protective spectrum from virus neutralizing titers against homologous or intratypic heterologous FMDV viruses.

Conclusion
Closely related FMDV viruses could elicit different protective immune spectrum in guinea pig and this test result would be used and evaluated for vaccine strain selection and development in the following study.

Keywords: Foot-and-Mouth Disease, Vaccine, Serotype O

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Epidemiology of Foot-and-Mouth Disease (FMD) in the Republic of Korea

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Purpose
This study describes the experience of dealing with foot-and-mouth (FMD) disease outbreaks in the Republic of Korea

Materials and Methods
Epidemiological characteristics of the FMD outbreaks were examined through review of epidemiological investigation sheet for each outbreak farms.

Results
Korea has experienced nine epidemics of FMD in the 21st century. Outbreaks were recorded in March-April 2000, May-June 2002, January 2010, April-May 2010, November 2010-April 2011, July-August 2014, December 2014-April 2015, January-March 2016, and February 2017. For each epidemic, new inflow of virus from overseas and transmission through fomite (i.e. vehicles, people, materials) were thought major factors of the FMD outbreaks in Korea.

Conclusion
In the epidemic of FMD, early detection was the most important factor of control measures of outbreak. Early detection and minimize the number of outbreak farms can only be achieved through the collaboration of farmers, local veterinarians, related industries and animal health services. This fact underlines the importance of public education about FMD.

Keywords: FMD, Epidemiology, Korea

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Epidemiological Analysis of Foot-and-Mouth Disease Virus (FMDV) Antibody Positive Farms in the Republic of Korea During 2016

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Purpose
The aim of this study is to analyze FMD NSP antibody positive farms in South Korea during 2016 spatio-temporally.

Materials and Methods
Animal and Plant Quarantine Agency (APQA)'s disease reporting data were used for the study. Each farm data were collected from epidemiological inquiry with farmers by the veterinary officers of APQA or local veterinary authorities. FMD NSP antibody was determined in sera collected from farms or slaughterhouse using ELISA during 1 Jan to 6 Nov. 30km control zoning centered on positive farms was established and high risk area was confirmed by accumulation rates for recent 4 months.

Results
186 FMD NSP antibody detected farms including 175 pig farms and 11 cattle farms during the investigation period were analyzed spatio-temporally. A remarkable peak in March due to the whole farm-examination in Chungcheongnam Province with FMD outbreaks was shown. When a risk area was checked by month, Hongseong had the highest risk, followed by Cheonan, Asan and Cheongyang. For recent 4 months, Hongseong and Yongin centered with the most positive farms in Chungcheongnam and Gyeonggi Provinces, respectively, posed high risk for the FMD recurrence.

Conclusion
As FMD NSP antibody is continuously detected among pig farms located in Chungcheongnam and Gyeonggi Provinces, risk for FMD recurrence does still exist. Specific control measures of the NSP antibody positive farms without FMDV antigen and high risk areas to identify and eradicate virus circulation should be made in the near future.

Keywords: Food-and-Mouth Disease Virus (FMDV), Antibody, Epidemiology, Republic of Korea

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Post-Vaccination Monitoring for the Structural Protein After Vaccination in Cattle Following Foot and Mouth Disease Outbreak

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Purpose
Foot and Mouth Disease (FMD) occurred in 3 provinces (Chungbuk, Chonbuk and Gyeonggi) from Feb. to Mar. in 2017. FMD O vaccines were injected to all the Korean native and dairy cattle throughout the country. After 3 weeks, the monitoring was carried out to assess the immunity of the population targeted for protection by vaccination after FMD outbreak.

Materials and Methods
A total of 450 farms (Korean native cattle farm 300, dairy cattle farm 150) were selected in order to estimate the SP antibody seropositivity in the farm. The sera were collected from 5 cattle according to sex and age distribution in the farm tested. The SP antibody testing was done using SP ELISA (PrioCheck, Netherland). The t-test was applied to compare the differences of means between groups.

Results
The seropositive rate of SP antibody was 98.5% in cattle. The positive rate of Korean native cattle and dairy cattle was 98.3% and 98.9%, respectively. There was no difference between ages (< 2 year: 96.5%, >2 year: 99.4%) and sex (female: 98.7%, male: 97.2%). Two farms [1 Korean native cattle (20%) and 1 dairy cattle (40%)] showed below the positive rate of 80%.

Conclusion
It was likely that SP antibody was produced over average 95% in cattle irrespective of breed, sex and age. Therefore, vaccine must be injected to prevent the outbreak and spread of FMD.

Keywords: Foot and Mouth Disease, Monitoring, Structural Protein

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Genetic Characterization of the Foot-and-Mouth Disease Viruses Serotype O and A in Korea, 2017

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Purpose
In February 2017, two different serotypes O and A outbreaks of foot-and-mouth disease (FMD) occurred simultaneously in Korea. As for Serotype O, the outbreak continued for a period of 9 days and affected 8 cattle farms in two separate regions. The outbreak of serotype A was terminated with only one occurrence in a dairy cattle farm. This reports the genetic characterization of Korean isolates to establish the possible epidemiological relationship during FMD epizootic of 2017 in Korea.

Materials and Methods
Viral RNA were extracted from clinical samples including vesicular fluid. The VP1 regions were amplified and PCR products were directly sequenced, according to the manufacturer’s protocol. Phylogenetic trees were based on the VP1 gene (636bp) using the neighbor-joining method in Mega6.

Results
Based on the phylogenetic trees for VP1 gene, all FMDVs from eight serotype O outbreaks belonged to the ME-SA/Ind-2001d genotype and closely clustered with recent Russian, 2016 and South East Asian FMDVs. And one serotype A virus grouped into Asia/SEA-97 genotype, closely clustered with South East Asian A viruses in 2016 (99.8%), but showed relatively low relationship with the last Korean A virus in 2010 (91.41%).

Conclusion
Based on the phylogenetic analysis of VP1 gene, both of the Korean FMDVs serotype O and A in 2017 might be introduced newly from neighbored FMD circulating countries via unknown source. Six consecutive serotype O viruses within the same region were closely related to the first serotype O outbreak.

Keywords: Foot-and-Mouth Disease Virus, Korea, 2017, Phylogenetic Analysis

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Comparative Phylogenetic Analysis of Foot-and-Mouth Disease Viruses Between Vietnam and Korean Isolates

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Purpose
Since foot and mouth disease virus (FMDV) both for serotype O and A had simultaneously introduced into South Korea in 2017, genetic analysis on FMDV isolates circulating in neighboring countries could give valuable information. Therefore, this study examined the genetic correlation between Korean FMDVs, and Vietnamese circulating both serotype A and O simultaneously.

Materials and Methods
We isolated 22 FMDVs from field outbreak samples from Vietnam, NVCD, between October of 2014 and March of 2015. We used Korean isolates since 2014 for comparison. The VP1 region was amplified and PCR products were directly sequenced, according to the manufacturer’s protocol. Phylogenetic tree based on the VP1 gene(636bp) using the neighbor-joining method in Mega6.

Results
Out of 22 Vietnamese FMDVs, fifteen were belonged to serotype O and the other seven to serotype A. All serotype O viruses were clustered into the SEA/Mya-98 genotype. Among them, three isolates from pig grouped closely into the Korean isolates in 2014 and 2016(96.09~96.76%). No Vietnamese isolates were grouped into recent Korean isolates in 2017. As for serotype A, all seven isolates were clustered into the Asia/SEA-97 genotype. Six isolates were closely grouped into the latest Korean isolates of 2017(99.21~99.37).

Conclusion
In this study, we provided recent phylogenetic information regarding genetic relationship between Vietnamese and Korean FMDVs, based on complete VP1 gene. But further accumulated genetic data are required to understand the comprehensive epidemiological situation in Asia region for effective FMD control.

Keywords: Foot-and-Mouth Disease Viruses, VietNam, Korea, Phylogenetic Analysis

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Analysis of Immune Response to Recombinant 3AB Antigen of FMDV in Animal Models and Pig

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Purpose
In this study, an animal model using recombinant non-structural protein has been applied to induce antibody of FMDV. Therefore guinea pigs and pigs vaccinated with experimental vaccines containing recombinant 3AB has been applied to study the persistence of antibodies against NSP of FMDV.

Materials and Methods
To test the minimum amount of a recombinant FMDV NSP (3AB) to induce detectable humoral responses in guinea pig, experimental oil vaccines were prepared containing 30, 60, 120 and 250 ng/ml of a recombinant 3AB and were 3 times vaccinated every two week. Five out of ten pigs were vaccinated 2 times containing 250 ng/ml recombinant 3AB and five were infected by 10⁵ TCID₅₀ FMDV (O/SKR/Gimje/2016) without vaccination. Sera were obtained every two weeks for 49 days in vaccinated group and for 35 days in challenged one. The samples were tested by NSP ELISA kit (Median 3AB, Bionote 3ABC).

Results
Antibodies against NSP were detected at 120 and 250 ng/ml showing 27% and 93% positive reactions respectively at 28 DPV, however, low concentration groups (30 and 60 ng/ml) were not detectable in guinea pigs. Positive rate of antibodies against NSP was 75% at 21 DPV and increased up to 100% at 48 DPV in vaccinated pigs. Also, positive rate of antibodies against NSP was 100% in experimentally infected pigs.

Conclusion
Dose-response experiments indicated that 120 ng/ml is the lowest protein amount detected at 28 DPV in guinea pig. These results demonstrated a recombinant FMDV NSP(3AB) can substitute for a live virus of FMD to induce antibodies against FMDV NSP.

Keywords: Foot-and-Mouth Disease Virus, Non-Structural Protein Antibody, NSP Antibody Persistence

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Analysis of Duration of Induced Antibodies Against Recombinant 3AB Protein of FMDV in Pigs

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\textbf{Purpose}
In order to perform the effective management of infected animals which were detected NSP-sero positive during sero-surveillance of FMD, it is extremely important to predict the duration of antibodies of nonstructural proteins against FMDV. This study was performed to determine how long the antibodies to 3AB recombinant antigen lasted in growing pigs and sows. We also studied the persistence of maternally derived antibodies to the 3AB antigen in piglets born from vaccinated sows with 3AB recombinant antigen.

\textbf{Materials and Methods}
Groups of 4 growing pigs and 5 sows were vaccinated 2 times at 2 week intervals with experimental vaccines (water-in-oil single emulsions 50%-50%), containing 250 ng/ml recombinant 3AB in the aqueous phase. Sequential sera were collected from 4 growing pigs every 2 weeks for 6 months. Also, a total of 5 sow sera, colostrum and sera of their 24 offsprings were collected every 4 weeks for 5 months. The samples were tested by NSP ELISA kits (Median 3AB, Bionote 3ABC).

\textbf{Results}
All 4 growing pigs and 5 sows induced antibodies against non-structural protein at 21 DPV and were continually detected for 6 months. It was confirmed that the maternal derived antibodies against the 3ABC antigen, transferred through the colostrum, were detected in all piglets.

\textbf{Conclusion}
This data demonstrated the kinetics of Abs against 3ABC in experimentally immunized pigs.

\textbf{Keywords}: Foot and Mouth Disease, Non Structural Proteins, Antibody Duration, Recombinant 3AB, Pigs

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Foot and Mouth Disease

Tissue Distribution of Foot-and-Mouth Disease (FMD) Viral Antigen in Experimentally Infected Pigs

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Purpose
In the case of vaccinated animals, it can be difficult to find animals infected with FMD virus due to a mild clinical symptom. In order to eradicate FMD, it is important to identify infected animals. Therefore, the aim of this study is to identify the optimal tissue for FMD viral antigen detection in vaccinated animals.

Materials and Methods
The pigs were divided into four groups: 7 days after vaccination (V7,n=7), 14 days after vaccination (V14,n=7), 21 days after vaccination (V21,n=7) and unvaccinated (UV,n=6). Two pigs of UV group were inoculated intradermally in the heel bulb with O/GJ/2016 as donors for a pig-to-pig time-limited contact exposure. All pigs were autopsied 28 days or 35 days after contact infection. The tissues were screened for FMD viral antigen using qRT-PCR and IHC.

Results
In UV group, viral antigens were detected in most of the target tissues, with the most frequent detection (75%) in the tonsils and popliteal lymph nodes. In addition, the popliteal lymph node was the site where the antigen was detected most frequently in the vaccinated groups (V7=57%, V14=57%, V21=43%).

Conclusion
This study suggests that FMD viral antigens can persist in popliteal lymph node for up to 35 days after infection. Therefore, in cases of asymptomatic FMD infected animals in NSP (nonstructural protein) antibody-positive farms, it is considered possible to use popliteal lymph nodes to monitor the antigens at the slaughterhouse.

Keywords: Foot-and-Mouth Disease, Real-Time PCR, Popliteal LN

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**Efficacy of SuiShot® Flu-3, a Trivalent Swine Influenza Vaccine, Against H1N1, H1N2, and H3N2**

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**Purpose**
Swine influenza A virus causes significant economic loss in swine industry by co-infection with other respiratory pathogens. Moreover, the fast and complex evolution and multispecies infection of this pathogen has increased public health concern. Therefore, the need of a vaccine against SIV has been proposed. In this study, we evaluated the efficacy of SuiShot® Flu-3 (CAVAC), an inactivated trivalent SIV vaccine against H1N1, H1N2, and H3N2.

**Materials and Methods**
In this study, 36 4-week-old pigs were separately housed as follows: 18 pigs in group 1 (vaccination and challenge group), 12 pigs in group 2 (challenge group), and 6 pigs in group 3 (negative group). The pigs in group 1 were vaccinated twice with 2 weeks interval, and then $10^{4.0}$ EID₅₀/ml of H1N1, H1N2, and H3N2 viruses were challenged in groups 1 and 2 at 8 weeks of ages (one strain per 6 pigs in group 1 and one strain per 4 pigs in group 2). HI titer against SIV was evaluated after vaccination. Clinical signs, virus shedding through nasal route, and pneumonia score were evaluated after challenge.

**Results**
One week after 1st vaccination, antibody titer against each strain was detected by HI test. The vaccinated group showed significant reduction of clinical signs, virus shedding, and pneumonia score in comparison with non-vaccinated group after challenge.

**Conclusion**
SuiShot® Flu-3 showed satisfactory efficacy against homologous challenge of H1N1, H1N2, and H3N2. This report will widen the knowledge of vaccination regarding swine influenza A virus.

**Keywords:** Swine Influenza, Vaccine, Influenza A Virus

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Serotypic Prevalence and Antibiotic Susceptibility Test Analysis of Actinobacillus Pleuropneumoniae Isolated in Korea

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Purpose
Actinobacillus pleuropneumoniae (APP), a causative agent of porcine pleuropneumonia, is distributed worldwide. A total of 15 serotypes are recognized and distribution of prevalent serotypes varies within countries. Because of the variety of serotypes, knowledge on prevalent serotypes is very important for the prevention of this disease. In this study, we discussed the serotypic prevalence and antibiotic resistance of APP isolates in Korea.

Materials and Methods
From 2012 to 2016, a total of 212 APP strains were isolated from pig farms in Korea. Serotypes of the isolates were identified by PCR method. Also, disk diffusion method for antimicrobial susceptibility testing was performed using nine antibiotics for 127 isolates out of 212.

Results
The majority of isolates were serotypes 1 (47.6%) and 5 (27.8%). Notably, the proportion of serotype 1 is gradually increasing. One hundred and twenty four (97.6%) isolates were resistant to at least one antibiotic and two isolates were resistant to all antibiotics. Resistance was most frequently detected in Trimethoprim/Sulfamethoxazole(SXT) whereas susceptibility was most frequently detected in Amoxicillin/Clavulanic acid (AMC).

Conclusion
In this study, it was found that the most prevalent serotypes of APP in Korea are 1 and 5. APP field isolates were generally highly resistant to antibiotics but the most effective antibiotic for APP is Amoxicillin/Clavulanic acid (AMC).
Considering the ban of antibiotics in feed for pigs, the use of vaccines containing prevalent serotypes in farms or regions is recommended for the prevention and control of porcine pleuropneumoniae.

Keywords: Actinobacillus Pleuropneumoniae, Serotype, Antibiotic Susceptibility Test

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Isolation and Annual Prevalence of *Actinobacillus Pleuropneumoniae* Serotype 1 in Korean Pigs

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**Purpose**
Porcine pleuropneumonia is caused by *Actinobacillus (A) pleuropneumoniae* and results in significant economic losses in global swine industry. Among 15 different serotypes of *A. pleuropneumoniae*, serotype 2 and 5 are known as major prevalent serotypes in Korea. However, serotype 1 was increasingly recognized in Korean domestic pigs since the late 2000s. Therefore, this study was conducted to investigate the annual prevalence and clinical features of serotype 1 isolates in Korea.

**Materials and Methods**
A total of 292 *A. pleuropneumoniae* positive lungs from 2010 to 2016 were used in this study. All lung samples were inoculated on blood agar plate with NAD or chocolate agar plate and incubated for 48h at 37°C with 5% CO₂. Serotype of the isolates was identified by PCR methods. Some lung samples were fixed in 10% buffered formalin for histopathologic examination.

**Results**
Among the 292 isolates, serotype 1 was the most frequent serotype (175 isolates, 59.9%) in this study. The percentage of cases caused by serotype 1 was increased from 2010 to 2014 and detected in 87.8% to 94.1% between 2014 and 2015. For the microbiologic and histopathologic examinations, serotype 1-positive cases were also observed in suckling and early weaned pigs between 2011 and 2013.

**Conclusion**
Through serotyping of *A. pleuropneumoniae*, it is observed that serotype 1 is most frequent serotype in Korea. Interestingly, some isolates were able to infect and develop clinical diseases in young piglets. The results of this study suggest that *A. pleuropneumoniae* serotype 1 seems to be an emerging serotype in Korea.

**Keywords:** Actinobacillus Pleuropneumoniae, Serotype 1, Pig

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Safety and Serological Response of Edema Disease Vaccine in Piglets.

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Purpose
The objective of the present study was to evaluate the effect and safety of ED toxoid vaccine.

Materials and Methods
Forty, healthy, seronegative piglets were selected at 10~14days of age and randomly allocated to 3 treatments(n=10/different adjuvant, G1: CVC1, G2: CVC2, G3: CVC3 vaccine). Ten additional pigs were used as control piglets(G4). Piglets were vaccinated intramuscularly at age of 14, 28 days. Blood samples were collected at post vaccinated day0(T0), day14(T2), day21(T3), day28(T4). Every day during the nursery period, observed whether the piglets showed any clinical sign of edema disease(ED). Indirect ELISA and S/N test were performed to evaluate antibody response. The results were statistically processed with SPSS.

Results
During the experiment until challenge, neither adverse reactions to the vaccine nor the occurrence of ED were observed. Mortality dropped from 90% in the control group to 35%(G1), 30%(G2), 80%(G3) in the vaccinated group. Mean of clinical sign score were 2.125, 0.856, 3.075, 2.8 at G1, G2, G3 and control group, respectively. Average of ELISA and S/N antibody titer in vaccinated nursery piglets at T4 increased significantly compare to control piglets.

Conclusion
In this study the effect of vaccination of ED was evaluated by mortality, clinical score, ELISA and S/N test. In ELISA and S/N test, vaccinated group had significant higher titer than control. In the vaccine G1 and G2 a significant reduction in mortality rate were observed. The clinical score post challenge results suggested that CVC2(G2) is effective ED prevention vaccine in the piglets.

Keywords: Edema Disease, Vaccine, Porcine

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Antimicrobial Susceptibility of *Streptococcus Dysgalactiae* Subsp. *Equisimilis* Isolates from Pigs

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**Purpose**
The *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) infections give rise to arthritis, endocarditis, or meningitis in pigs. The purpose of this study is determining the antimicrobial susceptibility based on minimum inhibitory concentration (MIC) of SDSE isolates from pigs with lameness, respiratory symptoms, or sudden death, which should inform the proper application of effective antimicrobial agents.

**Materials and Methods**
A total of eight swine SDSE isolates were obtained from individual farms, which were submitted for postmortem diagnosis. The suspected β-hemolytic colonies were identified as SDSE by 16s rRNA sequencing. To confirm the Lancefield serological group, the Strep LA kit was used. MICs of SDSE were determined using Sensititre system with antimicrobial testing plates (BOPO6F). Antimicrobial resistance genes were detected using previously published primers for macrolides [erm(A), erm(B), and *mef*(A/E)] and tetracyclines [tet(M), tet(O), tet(K), and tet(L)].

**Results**
All of eight isolates were categorized into Lancefield group C. The MIC values were various especially for enrofloxacin, clindamycin, and macrolides. The tested β-lactams showed the lowest MIC values (ampicillin and ceftiofur, ≤0.25 µg/ml; penicillin, ≤0.12 µg/ml), while the highest values were presented for the tetracyclines (chlortetracycline and oxytetracycline, >8 µg/ml), neomycin (>32 µg/ml), and danofloxacin (>1 µg/ml). Of the eight isolates, 2 and 7 isolates carried more than one resistance gene to macrolides and tetracyclines, respectively.

**Conclusion**
The susceptibility test results of β-lactams were expected, and this is still widely used in the treatment of SDSE infections in pigs. Nevertheless, continuous monitoring is essential to obtain updated trends of antimicrobial resistance in swine SDSE isolates for veterinary medicine.

**Keywords:** Antimicrobial Susceptibility, *Streptococcus Dysgalactiae* Subsp. *Equisimilis*, Pig, Antimicrobial Resista

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Virulence-Associated Genes and Antimicrobial Susceptibility of Pasteurella Multocida from Clinically Diseased and Slaughtered Pigs in Korea.

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Purpose
Pasteurella multocida is an important pathogen of pneumonia in pigs. The purposes of this study are to detect virulence-associated genes and suggest effective antimicrobials of P. multocida isolates in Korea.

Materials and Methods
A total of 233 P. multocida isolates were recovered from clinically diseased (n=191) and slaughtered (n=42) between 2008 and 2016. PCR was performed to determine capsular type and detect virulence-associated genes; toxA, oma87, psl, ompH, pfhA, ptfA, sodA, sodC, tonB, tbpA, hgbA, hgbB, nanB, and nanH. Minimum inhibitory concentrations of P. multocida were determined using Sensititre system with antimicrobial testing plates (BOPO6F).

Results
Among 233 isolates, 72% of them belonged to the capsular A, while type D and F were determined 25% and 3%, respectively. The toxA was detected in 4% of isolates. The hgbB and pfhA were detected in 78% and 25% of isolates, respectively. None of the isolates had tbpA, while the other genes were detected in all isolates. Among 18 antimicrobials tested, tylosin was the most resistant agent (97%) to P. multocida. Tiamulin and Tetracyclines were resistant to 61% and 58% of the isolates, respectively. In addition, ampicillin (7%), penicillin (8%), and ceftiofur (0%) were not resistant to the most isolates.

Conclusion
The most prevalent capsular type is A and toxA, hgbB and pfhA are useful virulence genes to characterize of P. multocida isolated from Korea. Moreover, β-lactams are recommended in the treatment of infections caused by P. multocida in Korea.

Keywords: Pasteurella Multocida, Capsular Typing, Virulence-Associated Genes, Minimum Inhibitory Concentrations, β

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Development of a Co-Agglutination Test for the Serology of *Mycoplasma Hyopneumoniae* in Farm Pigs

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**Purpose**
Our aim is to develop a simple and fast test for this purpose.

**Materials and Methods**
The microorganism was propagated in Friis medium (reference strain SJ) and grown massively in order to have enough biomass for the trial. Afterward it was and the antigen was reacted to latex microparticles and the proper union between bacteria and latex particles was confirmed by electron microscopy. For determination of sensitivity and specificity, positive sera obtained from previous experiments were used. Finally, the results were compared with an ELISA test.

**Results**
The microorganism was propagated in Friis medium (reference strain SJ) and grown massively in order to have enough biomass for the trial. Afterward it was and the antigen was reacted to latex microparticles and the proper union between bacteria and latex particles was confirmed by electron microscopy. For determination of sensitivity and specificity, positive sera obtained from previous experiments were used. Finally, the results were compared with an ELISA test.

**Conclusion**
The diagnostic of *Mycoplasma hyopneumoniae* from a simple coagglutination test can be a rapid and inexpensive that allows us to identify this microorganism in the farm thus helping us define the agents causing respiratory problems.

**Keywords:** Mycoplasma, Hyopneumoniae, Coagglutination, Test, Pigs

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S42 Mutation of Swine Influenza Virus (H1N1) NS1 Protein Influence Protein’s Function in Vitro and in Vivo

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Purpose
To test the role of NS1 42 and 92 amino acids of a novel triple reassortant virus A/swine/Shanghai/3/2014(H1N1) in escaping host immune response.

Materials and Methods
First, mutants NS1S42P, NS1D92E and NS1S42P/D92E of A/swine/Shanghai/3/2014(H1N1) were constructed and expressed in 293T cells in which cytokines production were detected by real-time PCR. Furthermore, three mutant viruses were rescued by reverse genetics technology and then identified their characteristics and roles in cellular antiviral response.

Results
The recombinant proteins were successfully expressed in 293T cell and showed no affection to the proteins’ localization in A549 cells. Viruses of SIV-NS1S42P, SIV-NS1D92E, SIV-NS1S42P/D92E showed similar growth kinetics with the parental virus except SIV-NS1S42P growing slower. Furthermore, S42P mutation can enhance mRNA transcript level of IFN-β (P<0.01) and IFN-α (P<0.05) while had no effect on TNF-α transcription (P>0.05) in either recombinant plasmids or rescued virus.

Conclusion
S42 is the key amino acid, which can influence NS1 proteins’ localization and cells antiviral immunization. It played important role in reducing cytokines production and help virus escaping the cells immune response. This work was supported by Basic research project (NO. 2014-3-3), Youth Talents Growth plan (NO. 2014-1-35) from Shanghai Ministry of Agriculture, and Shanghai Minhang High-end Talent Program (2012). All authors of this research declare no conflicts of interest.

Keywords: Swine Influenza Virus, NS1 Protein, Cellular localization, Antiviral Immune Response, IFN-α/β/γ, TNF-α

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Genomic Sequence Analysis Reveals New Bacteriophage Genome, JH2

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Purpose
Genomic Sequence Analysis Reveals New Bacteriophage Genome.

Materials and Methods
The DNA of phage JH2 was sent to Shanghai Biorefer Technology for sequencing. This company conducts direct sequencing on genomic DNA.

Results
An E. coli phage JH2 was isolated from fecal samples taken from a pig farm in Jiangsu, China. Phage JH2 could infect 85% (n=32) O8 ETEC strains, when 81 alike-strains from Vietnam and China were tested. The genome size could be as large as JH2 had 77187 bps, a linear, double-stranded and CG=38.89%. It shared height similarity of 94.9% to Staphylococcus phage SA1 of GU169904 with 147303 bps, which is about double the size. JH2 carried 22 tRNA and no gene for known toxins. Under the electronic microscope the phage showed icosaheiral heads, necks, contractile and long tails, and it belonged to the Myoviridae family. The phage demonstrated high stability to the physical and chemical factors, thermal sensitive 60°C and acid and alkaline resistance (pH5-9). The genome of JH2 contained 131 putative ORFs. One hundred and twenty-six ORFs (out of 131) were predicted and 121 ORFs had putative functions. From database, 22 tRNAs were predicted in JH2 genome. Staphylococcus phage SA1 contains an almost complete phage genome of JH2 phage.

Conclusion
The complete genome sequence of JH2 was analyzed. Comparing the complete genome sequences of JH2 and Staphylococcus phage SA1 showed 94.9% similarity

Keywords: JH2 Bacteriophage, E. Coli, O8

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Evaluation of a Universal Influenza Virus Vaccine Candidate in Pig Model

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Purpose
Influenza vaccines are specific for respective vaccine strains, but are less effective against antigenically drifted strains. The strategy for a universal influenza vaccine was developed based on the humoral immune response towards the antigenic conserved hemagglutinin stalk region. VAERD discussed as a side effect of universal vaccination. The purpose of this study was to investigate the level of protection conferred by universal vaccines and correlate between universal vaccine and VAERD.

Materials and Methods
Universal influenza vaccine candidates were tested in a pig model with maternal derived antibodies against pandemic H1N1. Three week-old piglets were immunized three times at four-week intervals either with (i) universal (chimeric hemagglutinin-based) vaccines with either adjuvant or without adjuvant; (ii) an inactivated pH1N1 vaccine; (iii) an inactivated H1N2 vaccine (VAERD positive control) or (iv) mock- vaccinated. Pigs were intratracheally challenged with pH1N1 virus four weeks after the final boost and euthanized 5 days post challenge. The detection of virus shedding, histological examination and ELISPOT of IFN-γ were done for evaluation of vaccine efficacy.

Results
The results revealed a positive effect of universal vaccine on most analyzed parameters when compared to the non-vaccinated, the pH1N1 influenza vaccinated or H1N2 vaccinated groups and VAERD was not observed in the universal vaccine group.

Conclusion
Our results indicate that a stalk-based universal vaccine has protective effects in pigs even in the presence of maternal antibodies and it does not induce VAERD. This indicated a universal vaccine strategy might be used to broadly protect human and animals from influenza infections.

Keywords: Influenza Virus, Universal Vaccine, Hemagglutinin Stalk

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In Silico Analysis of Putative Drug and Vaccine Target of the Metabolic Pathways of Actinobacillus Pleuropneumoniae Using Subtractive/Comparative Genomics Approach

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Purpose
Actinobacillus pleuropneumoniae, a Gram negative bacteria residing in the respiratory tracts of pigs, causes a significant loss in pig industry worldwide. Currently, emergence of drug resistance in A. pleuropneumoniae is increasing. Hence, identifying proteins/genes which could be targets for drug and vaccine development has a paramount importance.

Materials and Methods
The Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, the essential genes databases (DEG), DrugBank databases and Swiss-prot databases were referred to identify the non-homologous essential genes and prioritization of proteins for their druggability.

Results
Among the metabolic pathways 20 of them were unique and contained 273 non-homologous proteins, of which 122 were essential to the pathogen. The cytoplasmic and transmembrane proteins were 95 and 11, showing suitability for drug and vaccine targets, respectively. Among the proteins, 25 have a hit from the DrugBank database and three have similarity with the metabolic proteins of Mycoplasma hyopneumoniae, which can serve as a common therapeutic target for porcine respiratory disease complex.

Conclusion
The glyoxylate and dicarboxylate pathways can be a target for antimicrobial therapy. Furthermore, tetraacyldisaccharide 4’-kinase and 3-deoxy-D-manno-octulosonic-acid transferase could serve as a vaccine target against A. pleuropneumoniae.

Keywords: Actinobacillus Pleuropneumoniae, Dendropanax Morbifera Leveille, Metabolic Pathways

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Biofilm Formation and Determination of Minimum Biofilm Eradication Concentration In *Mycoplasma Hyopneumoniae*

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**Purpose**
The study was designed to evaluate biofilm formation in *Mycoplasma hyopneumoniae* and to determine minimum biofilm eradication concentration of antibiotics.

**Materials and Methods**
Determination of minimum inhibitory concentrations and minimum biofilm eradication concentrations were made in Friis broth medium for the four field isolates and two standard strains of *M. hyopneumoniae*. Biofilm forming ability was determined by crystal violet staining and architecture of the biofilm was evaluated using Confocal scanning laser microscopy. The viability of the cells was further investigated using BacLight stain.

**Results**
Crystal violet staining on coverslips demonstrated an apparent line of biofilm growth in three of the field isolates. The confocal imaging of BacLight stained biofilms revealed that the majority of the cells were viable after 336 hr of incubation. Polysaccharide production was not affected in biofilm populations of *M. hyopneumoniae*, except at much higher concentration. Biofilm formation did not affect the minimum inhibitory concentrations of any of the antibiotics tested. Most importantly, all the drugs were unable to eradicate the biofilm even at 10 time’s higher dilutions of their corresponding MICs.

**Conclusion**
*M. hyopneumoniae* can form biofilm which allows it to survive in the presence of higher concentrations of antibiotics. However, additional studies are needed on how *M. hyopneumoniae* initiates biofilm formation and adhere to surfaces.

**Keywords**: Biofilm, Eradication, Mycoplasma Hyopneumoniae

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Surveillance for African Swine Fever Virus in Republic of Korea, 2016

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Purpose
African swine fever (ASF) is a serious hemorrhagic viral disease of pigs of all breeds and ages caused by African swine fever virus (ASFV). Due to its strong contagiousness and high mortality rates, it is classified as notifiable disease by the World Organisation for Animal Health (OIE). Although ASF has never occurred in the Republic of Korea (ROK), there is a potential for ASFV introduction into ROK through various routes. In this study, we report on the result of serological and virological surveillance of ASFV conducted in 2016 in the ROK.

Materials and Methods
For ASF antigen test, a total of 186 samples were collected in 2016: 108 tissue samples from 21 sick pigs showing swine fever-like symptoms and 78 smuggled pork and pork products confiscated from airport and port. Virological test was conducted using real time PCR in accordance with OIE manual. Also, a total of 3,642 sera were collected from domestic and wild pigs and tested for antibodies against ASFV in 2016: 2,528 from domestic pigs and 1,113 from wild pigs, respectively. Serological test was conducted using two commercial ELISA kit according to the manufacturer’s instructions.

Results
All samples tested in this study were confirmed as negative for ASFV antigen and antibodies.

Conclusion
The result of this study shows that there is no evidence for ASFV circulation in domestic and wild pigs in the ROK. To maintain ASF-free status, persistent surveillance among populations at risk and monitoring of risk factors are required.

Keywords: African Swine Fever, Surveillance, Republic of Korea

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Purpose
Our objective was to test the gliceril monooleate gel in cubic phase as a toxin protector and carrier of Apx toxins and evaluate its potential as an immunogen.

Materials and Methods
The serotypes 1, 3, 5 and 7 of reference strains of Actinobacillus pleuropneumoniae were grown to obtain Apx toxins in the supernatant. The supernatants were subjected to tangencial ultrafiltration to enrich them; The toxin concentration was assessed by protein titration by the Bradford and PAGE-SDS electrophoresis of the four supernatants. The capacity of the gel to sequester the toxins and include them in the cubic phase were also evaluated by polarised light microscopy. The toxin release was evaluated by in vitro dissolution tests of the gel. The antigenic effect of the product was evaluated by oral administration into mice, where the changes in the populations of T and B lymphocytes were evaluated by flow cytometry and the antibodies against the Apx toxins was measured with an ELISA test.

Results
The monoolein gel was capable of trapping 400 mg/ml without affecting the formation of the cubic phase of the gel. Approximately 60% of the trapped protein was released from the gel in four hours.

Conclusion
The flow cytometry test developed a change in the lymphocyte populations with CD4 and CD19 markers, which is consistent with a possible stimulation of the immune system, as well as the presence of detectable antibodies with the ELISA test.

Keywords: Actinobacillus, Pleuropneumoniae, Toxins, ELISA, TEST

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Characterization of Pig Inflammasomes; NLR Family Caspase Recruitment Domain Containing 4 and Absent in Melanoma 2

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Purpose
Pigs are an important livestock and serve as a large animal model due to physiological and anatomical similarities with humans. Thus, components of the porcine immune system such as inflammasomes need to be characterized for disease control, vaccination, and translational research purposes. Previously, we and others elucidated porcine nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family Pyrin domain containing 3 (NLRP3) inflammasome activation. However, until now, porcine NLR family caspase recruitment domain (CARD)-containing 4 (NLRC4) and absent in melanoma 2 (AIM2) inflammasomes have been not well studied.

Materials and Methods
Porcine peripheral blood was freshly drawn from jugular veins of domestic pigs, and mononuclear cells (PMBCs) were isolated by Lymphocyte Separation Medium. After LPS priming, PBMCs were subjected to Salmonella typhimurium or Listeria monocytogenes to activate NLRC4 or AIM2 inflammasome.

Results
In this study, we treated well defined NLRC4 and AIM2 inflammasome triggers to porcine peripheral blood mononuclear cells (PBMCs) and murine bone-marrow derived macrophages (BMDMs) and observed interleukin (IL)-1β maturation as a readout of inflammasome activation. NLRC4 and AIM2 triggers led to IL-1β secretion in both porcine PBMCs and mice macrophages. Porcine Salmonella- or dsDNA-mediated IL-1β secretion was blocked by high KCl solution and diphenyliodonium, an inhibitor of potassium efflux and reactive oxygen species production. However, murine IL-1β secretion resulting from Salmonella- or dsDNA-mediated inflammasome activation was not regulated by high KCl solution.

Conclusion
Taken together, we suggest that porcine NLRC4 and AIM2 inflammasomes recognize the same triggers as in mice, although their cellular signaling mechanisms slightly differ from those of murine inflammasomes.

Keywords: Pig, Interleukin-1β, Macrophages

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Development of PEDV Inactivated Vaccine Using Bio-Molecule Expression Technique

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Purpose
Due to the variant of PED virus which is appeared since year 2013, Not only Korea but also various countries such as China and USA has been suffered. In order to solve this problem, KOMIPHARM has developed a new concept of PED inactivated vaccine (PED-Fc vaccine) by applying bio-molecule expression technique to china-derived PED variant virus

Materials and Methods
In order to develop a PED-Fc vaccine, a retroviral vector system is used to create a new cell line of Vero-Fc which is expressing Swine's IgG Fc molecules, and PED-Fc virus is developed using this line.

Results
After vaccination to pregnant sows, perform the challenge test with pathogenic PEDv to suckling piglet which is fed with colostrum of the sow. And, Compared with piglets born in non-vaccinated sows, the piglets born in vaccinated sows show that excellent efficacy on cumulative mortality, diarrhea severity and body weight gain.

Conclusion
By applying the bio-molecule expression technique to current PED vaccines, the efficacy of the vaccine can be successfully improved.

Keywords: Vaccine, Fc molecule, PED Virus

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Construction of Regulated Delayed Attenuated *Salmonella* Choleraesuis Vector with Enhanced Heterologous Antigen

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**Purpose**
Development of a safe and effective attenuated *S. Choleraesuis* vaccine vector

**Materials and Methods**
The regulated delayed attenuated systems (RDAS) regulated by mannose and arabinose were introduced into the wild type *S. Choleraesuis* strain C78 with the mutant of regulated delayed antigen synthesis \(\Delta\text{relA}:\text{araC}\) \(\text{p}_{\text{BAD}}\text{lacI}\) \(\text{TT}\) and \(\Delta\text{asdA}\) to constitute a balanced-lethal plasmid system, resulting in the RDAS *S. Choleraesuis* strain rSC0011. The 6-phosphogluconate dehydrogenase (6-PGD) of *S. suis* serotype 2 (SS2) were cloned and inserted into the prokaryotic expression vector pYA3493, and resulting in plasmid pS-6PGD. The safety and immunogenicity of the rSC0011 (pS-6PGD) were evaluated *in vitro* and *in vivo*.

**Results**
Compared with the wild type parent strain C78-3 and attenuated vaccine strain C500, the survival curves of rSC0011 were similar to those of strains C78-3 and C500 at the early stage of infection, but lower than those of C78-3 and higher than those of C500 at the later stage in both porcine alveolar macrophages and peripheral porcine monocytes. The LD\(_{50}\) of rSC0011 orally in mice was close to that of C500 and 10,000-fold higher than that of C78-3, suggesting that the RDAS rSC0011 achieved similar attenuation as C500 did. However, the RDAS strain rSC0011 was superior to C500 in colonization of Peyer’s patches. Adult mice orally immunized with strain rSC0011 (pS-6PGD) developed strong immune responses against 6-PGD and *Salmonella* antigens, and conferred high protection against challenge with SS2 by i.p.

**Conclusion**
The vaccine strain provided excellent protection against SS2 challenge.

**Keywords:** Salmonella Choleraesuis, Vaccine Vector, Regulated Delayed Attenuation System, Regulated Delayed Antige

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Histogenesis of Ghezel Sheep Kidney

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Purpose
Study of histogenesis of kidney of Ghezel sheep embryo

Materials and Methods
Embryo samples were collected from Tabriz slaughter house. Samples were taken to the laboratory, then samples were divided into four groups in terms of time by measurement of CRL (Crown-Rump Length) in embryos. Groups were divided in 0-40, 40-80, 80-120 and 120-150 days and were numbered to 1, 2, 3 and 4, respectively. Kidneys of the embryos were removed and were fixed in 10% formalin. Parafinized blocks were prepared by routine histological techniques and 7 μm sections were stained by H&E method.

Results
Results were showed that renal vesicles were developing and from mid second period developed relatively and were changing to glomeroles and nephrons. Also renal glomerules with a simple squamous epithelium in parietal layer were observed in medulla in first period. Macula densa was observed in hemal pole of glomeroles in end of third period. Distribution of renal vesicles was decreasing in end of third period and they were very low in mid of fourth period. Developed renal glomeroles were observed in end of fourth period. Renal lobes were forming in beginning third period and first sings of medullary rays were observed in this period. Invagination of medullary rays into cortex was observed in beginning fourth period that it dedicated formation renal lobules. Renal lobules developed completely in end of fourth period. In the first period, a few collecting ducts that surrounded by high level mesenchymal tissue were observed in medulla. Then medullary collecting ducts were observed in groups 25 or more in the second period. In the fourth period, collecting ducts were observed in cortex and end parts epithelial tissue of collecting ducts were changing to transitional epithelium. This was sign to formation of pelvice.

Conclusion
Generally, most changes of ghezel sheep kidney histogenesis are happening in 40-80 days after fertilization

Keywords: Ghezel Sheep, Histogenesis, Kidney, Embryo

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Identification of Microbial Pathogens and Microbiota of Bovine Mastitis Raw Milk

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Purpose
Bovine mastitis has been one of the most important diseases in the dairy industry and caused by Staphylococcus aureus, Streptococcus agalactiae, and E. coli etc. In case of subclinical mastitis coagulase negative Staphylococci (CNS), Gram negative rods and S. aureus were identified at high frequency in order. To understand the recent microbial pathogens and microbiota of bovine mastitis in Korea we performed the present study.

Materials and Methods
We isolated and identified microbial pathogens from raw milk specimens from cows suffering from mastitis (42 dairy farms) by using VITEK compact and characterized microbiota of raw milk by using 16S rDNA pyrosequencing.

Results
Staphylococcus spp. (90.5%) were most frequent and followed by Enterococcus spp. 40.5%, Streptococcus spp. (31.0%), Kocuria spp. 26.2%, and Lactococcus spp. 21.4%. According to the frequency of individual species E. faecalis was most frequent (28.6%) followed by S. aureus (23.8%), Staphylococcus chromogenes (21.4%), Kocuria rosea (21.4%), Streptococcus uberis (16.7%), Staphylococcus hemolyticus (14.3%), Lactococcus garvæae (11.9%), Aerococcus viridans (11.9%), L. lactis (9.5%), E. coli (7.1%) and Raoultella planticoli (7.1%). However, the microbiota of raw milk characterized by 16S rDNA pyrosequencing showed unexpectedly different compositions of bacterial species from the culture-based bacterial identification results above.

Conclusion
Thus, further studies on the microbial population changes win raw milk when cows are suffering mastitis may provide basic information to solve mastitis problem in dairy industry.

Keywords: Bovine Mastitis, Raw Milk, 16S rDNA Pyrosequencing

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Delayed Insemination Optimizes Conception in Dairy Cows with Natural Estrus

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Purpose
The objective of this study was to compare conception risk in cows inseminated at different times after estrus detection via pedometers when breeding only once per day.

Materials and Methods
This was a retrospective study at 2 dairy farms in the US, milking 3x and with freestalls. Cows were fitted with pedometers (AfiAct) and coded for high activity at each of milking sessions separately (H1, H2 and H3). The proportion of cows that conceived when inseminated 1, 2 or 3 sessions (approx. 8, 16 and 24 hrs) after the last high activity code was compared using a Z-Test (α=5%).

Results
A total of 1,964 inseminations were included. Significantly (P≤0.004) more cows conceived when inseminated 2 or 3 sessions after the last high activity code (33.9% and 31.2%, respectively) compared to cows inseminated in the session immediately following the high activity detection (21.1%). Due to the timing at which cows showed high activity (late afternoon and early evening) and the daily insemination time (morning), most of the pregnant cows (51.4%) had conceived when inseminated 2 session after high activity was detected.

Conclusion
Cows vary in the time at which they start natural estrus. With an average life-span of viable sperm of about 12 hours, and 2/3 of the cows showing high activity for only 1 session (8h), inseminating once per day is likely limiting reproductive performance in many dairy farms. A second time for insemination in the day should be considered to improve reproductive performance.

Keywords: Conception Risk, Insemination Timing, Pedometer

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Pathological Changes on Rumen of Bali Cattle That Infected by Paramphistomiasis

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Purpose
The study was conducted to observe the changes of anatomical and histopathological pathology of bali cattle’s rumen that infected by worms Paramphistomum sp.

Materials and Methods
Rumen samples were obtained from Pesanggaran Slaughterhouse, Bali. A total of 32 samples of rumen organ of bali cattle that infected by paramphistomiasis were used as samples in this study. Macroscopic observation made by observing changes in organ samples of rumen, while the microscopic observation made by making a histology preparations of rumen samples and stained by Hematoxylin Eosin. Histopathological lesions observed by binocular microscope with 100x and 400x magnification.

Results
Based on an inspection of anatomical pathology changes in the rumen of bali cattle largely seen erosion and ulceration on the mucosa of rumen. However, the averages changes of histopathological pathology showed epithel desquamation, ulcerative necrosis, necrosis in keratin, oedema, congestion, inflammatory cell infiltration of eosinophils, and fat degradation.

Conclusion
Pathological changes in both macroscopic and microscopic on the rumen of bali cattle that infected by paramphistomiasis mostly occurs on the mucosa rumen of bali cattle.

Keywords: Paramphistomiasis, Pathology, Rumen, Bali Cattle

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Results of Right Paralumbar Fossa Colostomy in Calves with Atresia Coli

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Purpose
Atresia coli is characterized by the complete closure of different segment of the colon. Atresia of colon in calves usually occurs at the level of the spiral colon. The etiopathogenesis of this congenital abnormality is not completely understood. The Several hypotheses have been put forward including hereditary and non-hereditary factors. It is seen in more holstein breed. The purpose of this study was to evaluate the clinical and surgical (right paralumbar fossa colostomy) results in calves with atresia coli.

Materials and Methods
This study was conducted on 1 to 6 days old (24 holstein, 5 simmental) on 29 calves, admitted between years 2015-2017 to the Department of Surgery, Faculty of Veterinary Medicine, University of Selcuk, Konya, with typical symptoms of atresia coli. Absences of faeces, progressive depression and abdominal distension were the most common clinical signs with calves in atresia coli. In these cases the only alternative is surgical treatment.

Results
Right paralumbar fossa colostomy was made in 11 calves with atresia coli. The rest of 18 calves with atresia coli has not accepted the surgical treatment. The reason for not accepting the surgical approach of postoperative prognosis. In the post operative period, the calves lived for between 9-270 days. The average survival time was 73 days.

Conclusion
It is concluded that early prognosis was found to be crucial for the success of surgical treatment with calves in atresia coli.

Keywords: Calves, Atresia Coli, Surgery

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Clinical Experience of Interlocking Nail Stabilization of Long Bone Fractures in Calves

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Purpose
The aim of the study to report the clinical and radiographic outcome after use of an interlocking nail for stabilization of long bone fractures in calves.

Materials and Methods
A total of 21 different breed, age, weight and sex calves with the front and hind limb fractures were obtained to the Selcuk University, Faculty of Veterinary Medicine, Department of Surgery. Proximal or distal diaphyseal transverse, oblique fractures of the humerus, radius, femur and tibia were evaluated. 3rd generation of interlocking pin was used in this study. 6, 8, 9 and 10 mm in diameter was used for interlocking pin. Interlocking pin was placed for 15 calves.

Results
The distribution of cases are 7 femur (46.6%), 4 tibia (26.6), 2 humerus (13.3%) and 2 radius (13.3%), respectively. Six out of the 15 patients (40%) were good condition without any problems in the post-operative period. In 8 patients (60%) were ex in the post-operative period. They have shown various causes (infection, opening of suturing area, etc.). The success of 40% in results is particularly linked to the post-operative care conditions. Despite the short period of the operation and good fixation, low success rate was attributed not to provide adequate environment in the post-operative period.

Conclusion
This study is showing that calves with the fractures are particularly occur during birth and incongrute helping. It is causes severe economic losses in the calves in our country. Interlocking pin is intended to use widespread application for calf orthopedics.

Keywords: Calves, Fractures, Interlocking Pin

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Evaluation of Pasteurella Multocida Strain’s Efficacy as Possible Vaccine Candidate Against Bovine Hemorrhagic Septicemia

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Purpose
The present study aimed to evaluate the efficacy of a Pasteurella multocida strain genotypically and phenotypically previously characterized to be used as a possible vaccine candidate against bovine hemorrhagic septicemia.

Materials and Methods
100 animals were used to form two groups of 50 mice each, the vaccinated and control. The first group was intramuscularly vaccinated in the thigh with 0.2 mL; the dose was repeated 14 days later. At 21 days, they were divided into ten groups of five animals challenged with the respective dilutions of a fresh culture from 6 to 8 hours in a liquid medium of P. multocida at levels 10^1 to 10^10. The controls were similarly stimulated, daily observing for five days. The mean lethal dose was determined to be sufficient to protect livestock.

Results
A statistical analysis was performed by the method of Reed and Muench.

Conclusion
The study was satisfactory, as noted by the OIE.

Keywords: Pasteurella Multocida, Septicemia, Efficacy

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**BM-0082**

**Phenotypic, Genotypic and Immunological Characterization of Pasteurella Multocida Strains Proposed as a Vaccine Candidate**

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**Purpose**
The objective of this investigation was to study the phenotypic, genotypic and immunological characteristics of the Pasteurella multocida, PMI, PMII, PMIII and Pasteurella multocida PMIVG strains as potential vaccine candidates against bovine pasteurellosis.

**Materials and Methods**
Isolated from samples taken from the liver, lung, heart and bovine skin with clinical signs of pasteurellosis. The strains under study were analyzed by traditional biochemical methods and multi-substrate gallery API 20NE, antibiogram, Polymerase Chain Reaction (PCR) Electrophoresis in pulsed fields and an immunological characterization by rapid sheet agglutination test. It was confirmed that the four strains belonged to the Pasteurella multocida species, multocida subspecies. The PMI, PMII, and PMIII strains were found to correspond to capsular type A and the PMIVG to capsular type B. All were resistant to penicillin, vancomycin, and sensitive to kanamycin, gentamicin, and ciprofloxacin. The PMI and PMIII strains were genetically indistinguishable from each other and the PMII had a 91% similarity to the first two.

**Results**
The immunological studies evidenced the antigenic similarity between the four strains and the high percentage of protection conferred on the animal.

**Conclusion**
The results obtained in this research with the strains of Pasteurella multocida as possible vaccine candidates against bovine pasteurellosis will allow a correct selection of the strain (s) to be used in future vaccine formulations.

**Keywords:** Pasteurella Multocida, PCR, Electrophoresis

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Ameloblastic Fibro-Odontoma in Brazilian Cattle

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Purpose
To report the diagnosis of an ameloblastic fibro-odontoma (AFO) in a Brazilian bull, taking into account that odontogenic tumors are rare in humans and animals, their correct diagnosis is challenging, and it requires histopathological evaluation.

Materials and Methods
A 3-year-old Nellore crossbred bull had a mandibular swelling during ante e post-mortem procedures in a Brazilian abattoir. Grossly, the lesion was well circumscribed, and the affected tissues had hemorrhagic focuses along with ulcerative areas with expansile smelling. Samples of the lesion were excised and sent into a formaldehyde 10% solution to the Department of Veterinary Pathology of Rio Grande do Sul Federal University (UFRGS), Porto Alegre, Brazil.

Results
Microscopic examination showed narrow cords and small islands of odontogenic epithelium in a loose primitive appearing connective tissue that resembled the dental papilla. Foci of enamel and dentin matrix formation in close relation to the epithelial structures were also presented, allowed the final diagnosis of an ameloblastic fibro-odontoma (AFO).

Conclusion
The AFO is a benign, slow-growing, expansile epithelial odontogenic tumor with odontogenic mesenchyme. Malignant transformation is rare, and the recommended treatment is conservative surgery with enucleation. On the other hand, efficient meat inspection procedures are critical not only to keep the credibility of veterinary services in a more and more competitive and globalized world but also to either detect rare pathologies and cooperate to animal disease surveillance.

Keywords: Bovine Diseases, Cancer, Meat Inspection

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The Serological Investigation of Bovine Respiratory Syncytial Virus (BRSV) in Calves Showing Symptoms of Respiratory Tract Infection in a Cattle Farm

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Purpose
Bovine respiratory infections can be caused by many pathogens in cattle. Viral pathogens are the most commonly seen factors.

Materials and Methods
In this study, we worked on 63 Holstein calves aged 6-10 months old (13 calves aged 6 months old, 13 calves aged 7 months old, 13 calves aged 8 months old, 12 calves aged 9 months old, 12 calves aged 10 months old) showing respiratory system symptoms. All animals had no vaccination for BHV-1, BVDV, BPIV-3, BRSV and BAV-3 before the study. Respiratory ELISA kit commercial testing product was used in order to detect antibody presence against mentioned infections.

Results
All sera samples were found seronegative against BHV-1, BVDV, BPIV-3 and BAV-3. However, 35 (55.6%) sera samples were found as BRSV seropositive. The same results were obtained in second blood sampling after 21 days. The female calves were found seropositive (25/25, 100%). Ten male calves were found seropositive (10/38, 26.3%). Seropositivity rates were detected 3.2% (2/63) in 6 months old, 6.4% (4/63) in 7 months old, 11.1% (7/63) in 8 months old, 15.9% (10/63) in 9 months old and 19% (12/63) in 10 months old.

Conclusion
As result of the study; it was determined that presence of antibody increased with age, seropositivity ratio of the female calves were higher than the male calves. BRSV seropositivity rate was found as 17.3% (63/365) in the semi-outdoor management.

Keywords: Respiratory Infection, BRSV, ELISA

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The Investigation of Bovine Rotavirus Production in Serum Free Media and Microcarrier System

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Purpose
In this study, the production of bovine rotavirus (BRV) was investigated in the serum-free media by the microcarrier cell technology.

Materials and Methods
For this purpose, MA-104 cell culture growing on Fibra-cel® discs and bovine rotavirus (BRV) Grup A-B223 VR-1290 were used. The first part of the study was conducted in stationary cultures using 24-well TCPS plates in the presence and absence of Fibra-cel® discs. MTT staining was used for the quantitative estimation of total cell number and the specific growth rate. Also, the average specific glucose consumption and lactate production rates were calculated. These second part of the study, were carried out in the bioreactor which has 0.5 L and 10 L working volume.

Results
The effects of initial cell density, microcarrier concentration and incubation time on cell and virus yields were investigated. The cell yield was estimated by indirect methods. Indirect methods of cell estimation involve the chemical analysis of a culture media for glucose and lactate concentrations and cell dye.

Conclusion
It was seen that cell and virus production in serum-free media were compatible as compared with the media including sera. Furthermore, this method also provides high-yield production of bovine rotavirus with advantages of reduction in the cost of labor and materials, easy-to-operate, low contamination risk and high titer.

(This study was supported by Selcuk University Scientific Research Coordination (BAP, Master thesis, Research No 14202002)

Keywords: Bovine Rotavirus Production, Microcarrier, Serum-Free Media

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The Expression Pattern of Nrf2 Gene in the Milk of Mastitis Dairy Cattle

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Purpose

Mastitis is economically very important disease of dairy cattle. Its adverse effects on animal and human health and the vast economic losses have drawn research attention during the last decades. Escherichia coli often cause acute mammary gland inflammation and severe clinical signs in cattle. In contrast, Staphylococcus aureus and Staphylococcus uberis represent mild or subclinical mastitis. Mastitis causes an increase in free radical formation of milk and oxidative stress, especially in early lactation. Cows with mastitis represent an impressive decrease in superoxide dismutase activity, glutathione concentration, and an increase in erythrocyte lipid peroxides. Nrf2 is one of the genes playing an active role in mammary gland antioxidant response system. This study has aimed the evaluation of absolute quantification of Nrf2 mRNA expression of milk samples from cows with clinical mastitis compared to healthy cows.

Materials and Methods

Milk collected from healthy and mastitis cattle were cultured. After which they milk samples fixed and gram-stained. RNAs from milk samples were extracted and cDNAs were synthesized. Nrf2 gene mRNA expression quantification was evaluated by Real Time PCR.

Results

The results indicated that mastitis caused by Staphylococcus aureus and Escherichia coli leads to up-regulation of Nrf2 gene expression in milk in comparison to healthy cows, although there was not statistically significance. The Escherichia coli-caused mastitis was more up-regulated compared to Staphylococcus aureus-caused mastitis.

Conclusion

Nrf2 mRNA expression in milk of mastitic cattle caused by Escherichia coli was higher than that caused by Staphylococcus aureus.

Keywords: Dairy Cow, Mastitis, Nrf2 mRNA Expression

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Detection of Fever with Subcutaneously Implanted Thermos-Logger in Cattle Administered with Lipopolysaccharide

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Purpose
The purpose of the study was to determine whether subcutaneous temperature (ST) recorded with thermo-sensor tag implanted subcutaneously is correlated with rectal temperature (RT) in cattle with fever induced by lipopolysaccharide (LPS) challenge test.

Materials and Methods
Nine Holstein steers (579 ± 54 kg) were used. As experiment group, six steers were intravenously administered LPS at a dose of 0.5 µg/kg BW. Three steers were injected with 6ml of saline as a control group. ST was recorded in every 10 min by thermo-logger and RT was measured by digital thermometer before administration, and 1, 2, 3, 4, 8 and 24hr after administration.

Results
Mean temperature (°C) of RT and ST was 38.85 and 36.77 before administration, respectively. In experimental group after LPS administration, RT started to increase, peaked around at 3 to 4hr(40.75), and recovered to pre-dose temperature at 8 to 24hr. However, ST showed an unexpected decrease at 1 hr(35.21), increased thereafter and peaked at 4hr(39.45), and recovered to pre-dose temperature at 24hr. There was a positive correlation between RT and ST in animals administered with LPS (r=0.497, p=0.07).

Conclusion
The subcutaneously implanted thermo-logger tag may detect fever and ST fluctuated with RT in cattle administered with LPS, suggesting that the device can be utilized one of the tools to detect cattle with fever. However, further studies are necessary to exclude a variety of factors affecting ST fluctuation including physiological events and ambient temperature.

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None

Keywords: Thermo-Logger, Subcutaneous Implantation, Lipopolysaccharide Challenge Test

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Detection of Adhesin Genes and Biofilm Production Among Staphylococcus Isolated from Bovine Mastitis

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Purpose
The objective was to determine the ability of Staphylococcus aureus and coagulase negative Staphylococcus strains isolated from bovine mastitis cases to produce slime and to detect biofilm related icaA and icaD genes

Materials and Methods
Twenty six milk samples were plated on to blood agar and manitol salt agar. Isolates were identified to the species level based on the standards biochemical and by API Staphy. The presence of slime production of all staphylococcal isolates was evaluated by Congo red agar (CRA) method phenotypically. All plates were examined in terms color changes after 24 to 48 hrs of incubation. A black coloration of the colony was interpreted as a positive test result. Genomic DNA was isolated from each strain carried out according to CTAB method. The PCR amplification product was visualized by electroforesis on 1.2% agarose gel for 30 min at 80 V.

Results
All strains were positive to CRA method forming black colonies. Among the 26 strains of staphylococci 4 strains (one S. intermedius and three S. aureus) were icaA/icaD positive. Fifteen strains staphylococci were positive to icaD (five S. aureus, one S. intermedius, four S. sciuri, one S. simulans, one S. capitis, one S. lugdunensis, one S. saprophyticus and one S. xylosus). S. epidermidis was negative to icaA and icaD.

Conclusion
Slime production and adhesion are considered to be a crucial virulence factor among staphylococci. The presence of icaA and icaD correlate with biofilm in staphylococci isolated from bovine mastitis

Keywords: Staphylococcus, Mastitis, Biofilm

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Applicable SNP Analysis as Alternative Method of Spoligotyping for *Mycobacterium Bovis*

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**Purpose**
Spoligotyping is well known as one of molecular epidemiological methods for *Mycobacterium bovis* (*M. bovis*). In Korea, two kinds of spoligotypes (SB0140 and SB1040) have been identified from livestock and wildlife until now. In this study, we would like to introduce applicable SNP analysis as alternative method of spoligotyping.

**Materials and Methods**
After comparison between Korean *M. bovis* isolates and *M. bovis* BCG Pasteur 1173P2, 87 primer candidates on various genes associated virulence were designed. PCR was conducted to 48 *M. bovis* isolated from cattle, deer and wildlife in Korea and SNP was identified by Sanger sequencing and CLC Main workbench.

**Results**
About 1,300 SNPs from comparison between wild strains and reference strain were identified. Of these, 87 candidates associated virulence factor were selected. And 31 primer sets of all candidates were identified having SNP. Result from SNP typing was divided *M. bovis* isolates into two known spoligotypes in Korea.

**Conclusion**
This SNP analysis also divided *M. bovis* isolates into two groups, which were corresponded to SB1040 and SB0140, respectively. SNP analysis by using some of final primer sets could be used as alternative tool instead of spoligotyping in Korea.

**Keywords**: SNP, Spoligotyping, Mycobacterium Bovis

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Application of Interferon Gamma Test as One of the Eradication Program for Bovine Tuberculosis in Korea

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Purpose
The purpose of this study is to evaluate the IFN-r test as diagnostic method of bovine tuberculosis and as one of the national bTB control program in Korea.

Materials and Methods
We performed the IFN-r test to cattle (n=187) having bTB gross lesion and/or culture positive to evaluate the sensitivity and additionally performed the IFN-r test to cattle from bTB free herds (n=627) to evaluate the specificity from 2013 to 2016. Since September 2013, we collected IFN-r and CFT data from 9 provinces and 8 metropolitan cities to compare the results of two tests (Bionote, Korea & Bovigam, USA).

Results
132 (70.59%) of 187 infected cattle were positive on IFN-Υ test. The specificity of the IFN-Υ test kit was 99.84% (646/647). Since introduction of the IFN-Υ test as national control program, detection of bTB was highly increased in 2014 (376 herds), but it was decreased and fluctuated in 2015 (284 herds) and 2016 (324 herds). The agreement between the IFN-Υ test and CFT test was moderate (kappa index = 0.478).

Conclusion
The most beneficial application of IFN-Υ test to date is as an ancillary test in explosive TB breakdowns or persistently infected herds alongside the skin tests. Our study showed that high levels of sensitivity and specificity in comparison to CFT and IFN-Υ test could be used as large scale to control bTB. We will investigate continuously how application of two tests affects to bTB eradication in Korea.

Keywords: Interferon Gamma (IFN-r), Bovine Tuberculosis (bTB), Caudal Fold Test (CFT)

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BM-0205

Effects of Ruminal pH on the Transcriptomic Adaptation of Rumen Epithelium and Lymphocyte Function of Peripheral Blood in Holstein Calves During Weaning Transition

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Purpose
The relationship between ruminal pH and immune response during weaning transition has not been extensively investigated. We assessed the changes in ruminal pH induced by feeding calf starter with and without forage and its effects on the transcriptomic dynamics of rumen epithelium (RE) and lymphocyte function of peripheral blood.

Materials and Methods
Holstein calves were assigned into a forage provision group (HAY group, n = 3) and non-provision group (CON group, n = 4). Ruminal pH was measured continuously. RE and blood samples were obtained at 3 weeks after weaning. One-color microarray was performed to detect differentially expressed genes. Upstream regulator analysis was conducted using the Ingenuity Pathway Analysis software. Lymphocyte subpopulations and cytokines in the peripheral blood were evaluated.

Results
The 24-h mean ruminal pH were higher in the HAY group, and a higher rumen lipopolysaccharide (LPS) activity was observed in the CON group. Toll-like receptor 4 (TLR4) in the CON group was up-regulated in microarray analysis. NF-κB, AP-1, and IRF3/7 genes were identified as the activated upstream regulator. However, no difference was observed in the peripheral immune function between the two groups.

Conclusion
Dietary forage alleviates the severity of ruminal acidosis, and the lower ruminal pH increases rumen LPS activity. Rumen epithelial TLR4-mediated signaling pathways are stimulated by rumen LPS, regulating immune responses, and these might influence calf immune system at the transcription level during weaning transition.

Keywords: Calf Weaning, Ruminal pH, Immune Response

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**Effects of Castration on Inflammatory Cytokines and Hematology in Hanwoo Cattle**

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**Purpose**

Surgical castration is one of the processes, in removing testes with the objective of making sterile or infertile, and markedly reduce the production of testosterone hormone. Sex hormones regulate immune system through interacting with primary lymphoid organs or peripheral immune cells via cytokine mediators. This study investigated the changes of inflammatory cytokines due to sex hormones deficiency, comparing before and after castration.

**Materials and Methods**

Nine Hanwoo cattle (9 months old with BW of 230kg), confirmed free from five important diseases (Bovine tuberculosis, John’s disease, FMD, Brucellosis, Bovine leukosis), underwent blood collection from jugular vein before castration in day 1, 4, 7, 10, and 14 after surgery. Hematological tests were done and TNF-α, IL-6 and IFN-γ level in serum were measured using ELISA.

**Results**

Serum TNF-α levels after castration revealed significantly lower (p<0.01) than that before surgery. The levels of IL-6 also echoed similar results (P <0.01, P <0.001) on post-operative days samples. Though IFN-γ level decreased after surgery, but there was no significant differences among the days. On hematology, WBC, RBC and hematocrit levels showed a gradual decreased pattern until 4th day and then recovered at 10th day after surgery.

**Conclusion**

Findings showed that pro-inflammatory cytokine levels significantly differs in serum before and after castration. This suggests that testosterone deficiency may induce immunosuppression, although further studies required to determine exact immunological phenomena.

**Keywords:** Bovine Castration, Cytokine, Hematology

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Serum Cortisol Levels Reaction to Parturition and Lactating Period of Dairy Cows in Different Seasons

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Purpose
This study was conducted to investigate changes of the serum cortisol levels reaction to stress of dairy cows in parturition and lactation periods during summer and autumn seasons.

Materials and Methods
To observe blood cortisol level changes depending on periparturition and milk yield, 11 preparturient Holstein cows were selected. Their serum cortisol levels were measured at 7 to 14 days intervals from 10 days before delivery to 119 days after parturition and then compared their milk yield. To observe blood cortisol level changes depending on lactation periods between summer and autumn, 8~31 cows were selected at each lactation period and their serum cortisol levels were measured using ELISA including milk yield in both seasons.

Results
The serum cortisol level elevated at calving day and then gradually decreased during 56 days after parturition. As a result, the serum cortisol concentration varies on lactation periods and seasons(summer and autumn). Cortisol level of early lactation period in summer was significantly higher(p<0.01) than that in autumn.

Conclusion
The serum cortisol level was higher at calving day, but was not influenced by milk yield. The reason why the level of early lactation period in summer was significantly higher was that dairy cows would be more sensitive to stress in hot season. Therefore, serum cortisol level is thought to be more influenced by parturition and high environmental temperature than milk yield.

Keywords: Cortisol, Dairy, Cow

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The Changes of Acute Phase Proteins in Bovine Diarrhea Infected by Coronavirus

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Purpose
This study is to identify the changes of fibrinogen, Haptoglobin (Hp), Serum Amyloid A (SAA) in bovine diarrhea induced by bovine coronavirus.

Materials and Methods
Before and after treatment of diarrhea, feces and serum were collected from 17 Holstein cows. In the laboratory, real-time PCR was conducted for detecting coronavirus. Three APPs (fibrinogen, Hp, SAA) were measured from serum. After diarrhea was recovered, same process was performed and the result was compared.

Results
In total, 17 diarrheic feces and 15 feces after treatment were collected from cows. By real-time PCR, coronavirus was detected from all samples in diarrhea, but only 1 diarrheic-recovered sample was positive. The changes of averages of fibrinogen, SAA and Hp in diarrheic samples and cured samples were 0.7 g/dL to 0.7 g/dL, 46.2 mg/L to 28.5 mg/L and 1.0 g/L to 0.2 g/L, respectively.

Conclusion
Hp and SAA were significantly decreased after coronavirus induced-diarrhea was recovered in 17 cows. APPs in diarrhea might be affected by the onset, degree and duration of diarrhea, the type of causative agent, the further research about the relationship between diarrhea and APP is thought to be necessary.

Keywords: Bovine Diarrhea, Coronavirus, Acute Phase Protein

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Whole-Genome Sequence of Mycobacterium Bovis Strain B-3222, Isolated from the Laryngopharyngeal Lymph Node of Korean Cattle in a bTB-Infected Herd

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Purpose
Mycobacterium bovis is considered an essential agent in the pathogenesis of bovine tuberculosis, an infectious disease of domestic animals, wildlife, and humans. We hereby report the whole-genome sequence of M. bovis strain B-3222, isolated from the laryngopharyngeal lymph node of Korean cattle, together with classification and features of the organism, annotation and analysis of the genome sequence.

Materials and Methods
M. bovis B-3222 was sequenced by two next-generation sequencing methods: 454 GS FLX Titanium and Illumina MiSeq as the reference strain for studying M. bovis isolated in Korea. The results from the whole-genome shotgun project of M. bovis B-3222 have been deposited at DDBJ/EMBL/GenBank under the accession LNOF0000000.

Results
The genome assembly consists of 56 contigs comprising 4,299,850 bp and G+C content of 65.56%. In addition, the genome was annotated to comprise 4,074 genes including 3,916 protein coding genes, 45 tRNA genes, and 3 rRNA genes. The B-3222 genome showed a high similarity with strain 1595, a previously sequenced genome isolated from Korean cattle.

Conclusion
This genome sequence data will provide information used for searching virulence factors and guidelines for diagnosis and preventive strategy.

Keywords: Mycobacterium Bovis, Genome, Sequence

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Molecular Epidemiological Analysis by Single Nucleotide Polymorphism (SNP) Typing for Mycobacterium Bovis Isolates from Animal in Korea

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Purpose
Mycobacterium bovis has been investigated through physiological and molecular studies. However, genome level studies have not been fully demonstrated. Single-nucleotide polymorphisms (SNP) are likely to be a more exact tool for phylogenetic studies. The purpose of this study is to determine differences of SNPs of M. bovis and M. tuberculosis and to define among M. bovis groups in Korea drawing a parallel with spoligotyping and VNTR typing results.

Materials and Methods
A total of 46 M. bovis wild type strains were used to confirm every single SNPs. The PCR primer sets were designed to amplify SNP sites by the results of a M. bovis B-1595 whole genome sequence. The resultant 38 virulence factor SNPs were aligned together with corresponding SNPs and phylogenetic trees were reconstructed by neighbor-joining.

Results
Most SNP results displayed a tendency to follow Spoligotyping results of isolated strains. SNPs of M. bovis strain AN5 and M. tuberculosis strain H37Rv SNPs followed the tendency of spoligotype SB1040. Phylogenetic tree of SNPs were clustered 6 groups and that shown more departmentalized VNTR types.

Conclusion
The results of this study show that spoligotyping and VNTR techniques can distinguish between large groups of M. bovis subspecies. By the analysis of SNPs, more detailed differences in the identical spoligotype appeared. The analysis of SNPs might call for the establishment of a specific division to trace bovine tuberculosis outbreaks through research.

Keywords: Single Nucleotide Polymorphism(SNP), Mycobacterium Bovis, Spoligotyping

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BM-0293

Seroprevalence of Bovine Viral Diarrhoea and Associated Risk Factors in Dairy Herds in Myanmar

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Purpose
The objective of this study was to investigate seroprevalence of bovine viral diarrhea virus (BVDV) and associated risk factors in dairy cattle in three regions of Myanmar.

Materials and Methods
A total of 441 serum samples of cattle were collected from 23 dairy farms from Yangon, Nay Pyi Taw and Mandalay Regions in Myanmar. All cattle were not vaccinated against BVDV. Specific antibody to BVDV was detected by commercially available ELISA (IDvet, France). The data were analysed based on regions, age, and herd size.

Results
Seroprevalence of BVDV at farm level and cow level were 13.04% and 9.98%, respectively. Seroprevalence for BVDV in Yangon, Nay Pyi Taw and Mandalay Regions were 1.91%, 5.82% and 31.58%, respectively. Seroprevalence of BVDV in Mandalay region was significantly higher (p<0.05) than those of the other regions. Seroprevalence of BVDV in dairy cows of ≤2 years, 2-4 years, 4-6 years and >6 years of age were 19.64%, 4.76%, 9.86% and 14.67%, respectively. Seroprevalence of BVD in ≤2 years and >6 years were significantly higher (p<0.05) than 2-4 years group. Seroprevalence of BVDV in herd with <50, 50-100, 100-200 and >200 were 2.48%, 5.39%, 0% and 38.96%, respectively. Seroprevalence of BVDV in herd with >200 was significantly higher than those of smaller herd sizes.

Conclusion
Overall, the finding indicates the presence of BVDV natural infections in dairy cattle in Myanmar. High level of prevalence was observed in Mandalay region, where there is large herd size.

Keywords: Bovine Virus Diarrhoea, Seroprevalence, Risk Factors, Myanmar, Dairy Cattle

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Seroprevalence of Infectious Bovine Rhinotracheitis and Associated Risk Factors in Dairy Herds in Myanmar

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Purpose
The objective of this study was to investigate seroprevalence of infectious bovine rhinotracheitis (IBR) and associated risk factors in dairy cattle in Myanmar.

Materials and Methods
A total of 441 serum samples from cattle was collected from 23 dairy cattle farms from Yangon, Nay Pyi Taw and Mandalay Regions in Myanmar. All cattle were not vaccinated against IBR. Specific antibody to IBR was detected by commercially available ELISA (LSIvet, France). The data were analysed based on regions, age, and herd size and p<0.05 is considered as significant.

Results
Overall seroprevalence of IBR at farm level and cow level were 91.30% and 59.41%, respectively. Seroprevalence for IBR in dairy cattle from Yangon, Nay Pyi Taw and Mandalay Regions were 52.87%, 61.38% and 66.32%, respectively. There were no significant differences (p>0.05) in seroprevalence of IBR in cattle among three different regions. Seroprevalence of IBR in dairy cows of ≤ 2 years, 2-4 years, 4-6 years and >6 years of age were 32.14%, 49.40%, 66.20% and 89.33%, respectively. Seroprevalence of IBR in >6 years of age was significantly higher (p<0.05) than those of other age groups. Seroprevalence of IBR in herds with <50, 50-100, 100-200 and >200 cattle were 52.07%, 55.39%, 69.23% and 76.62%, respectively. Seroprevalence of IBR in the larger herd was significantly higher (p<0.05) than those of smaller herds.

Conclusion
The present study indicates IBR infection was present in dairy cattle in Myanmar. The age and herd size were relatively risk factors for prevalence to IBR.

Keywords: Infectious Bovine Rhinotracheitis, Myanmar, Dairy Cattle, ELISA, Seroprevalence

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BM-0115

Digital Dermatitis (Mortellaro Disease) in a Dairy Cattle

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Purpose
In the study, ‘Digital Dermatitis (Mortellaro Disease) in a Dairy Cattle’ was defined as clinically and histopathologically.

Materials and Methods
The study was carried out on a herd of Holstein (n:6) and Swiss Brown (n:15) breed dairy cattle which were suffering from pain and clinical lameness. Lesions were surgically removed under local anesthesia. Tissues were stained with hematoxylin-eosin (H&E) and Warthin-Starry (WS) for isolation of spirochetes.

Results
Histologically, in H&E, thickening of the keratin layer with nucleic fragments was found. The cells of stratum spinosum were increased and the epidermis showed papillary extensions into the dermis. In these areas, inflammatory cells, vascularization, degenerative and necrotic changes in epithelial cells and proliferation of connective tissue were seen. There is no finding of neoplasia. In Warthin-Starry staining of tissue slides black stained spirochetes were seen visible among enlarged keratinocytes and inflammatory cells. Firstly, they were treated only with topical oxytetracycline hydrochloride spray. After one month, four of 21 cattle didn’t heal and lincomycin hydrochloride soluble powder was added to the treatment options. Functional recovery was achieved in all cases and no recurrence of digital dermatitis during a follow up period of 15 months was seen.

Conclusion
As a result, it is known that the economical results of this disease are much greater than the treatment costs. It could be still seen in our country and causes loss of yield in the economy of the country.

Keywords: Dairy Cattle, Digital Dermatitis (Mortellaro Disease), Pathomorphology

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Definitive Diagnosis of Caseous Lymphadenitis in Sheep

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Purpose
The purpose of this study was the isolation of Corynebacterium pseudotuberculosis from animals clinically positive to LC.

Materials and Methods
The caseous exudate samples from hair or half-hair sheep in livestock and stock breeding units that tested clinically positive for CL, this samples were sent to the Microbiology Diagnostic Laboratory (FESC), where the animal data (age, sex and place) were taken. Of the isolated strains, two were chosen, to obtain antigens. Serum was obtained from an animal where one of the strains was isolated, the antigens were evaluated by Western Blot. Serum titration was performed by the hemolysis inhibition.

Results
Corynebacterium pseudotuberculosis isolated strains came from 100% of females, 63.63% from animals older than 4 years, and 45.45% from intermandibular abscesses. The Western blot results were the following bands: 11.66, 16.84, 31.06, 33, 34, 42.19, 50.69, 60.91, 68.89, 77.81, 93.5 and 126.98 kDa. The serum titre was 1/64.

Conclusion
The somatic antigens obtained were recognized by the serum of the clinically ill sheep and bands were found that coincide with those reported by other authors. When using the serum and bacterial strain of the same animal were observed a greater number of bands, unlike that bacterial strain belonging to another animal, being of great importance for the elaboration of probable immunogens.

Keywords: Cutaneous Presentation, Somatic Antigen, Corynebacterium Psuedotuberculosis

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Surveillance of Culicoides Biting Midges in Five Cattle Farms with Different Conditions

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Purpose
Arboviruses are transmitted by Culicoides biting midges and cause various disease outbreaks in cattle. It is not clearly understood their habit depending on the condition of cattle farm.

Materials and Methods
Culicoides biting midges were collected with light traps on five different cattle farms (inside and outside of the farms) located in Jeollabuk-do from May to October in 2016. The basic environmental conditions (temperature, humidity, wind speed, ammonia and methane gas) were monitored at each sampling. Pooled Culicoides species (~30) were subjected to RT-PCR for checking arboviruses.

Results
A total of 4,932 Culicoides biting midges (inside n=3104 and outside n=1828) were collected. Collected Culicoides species were C. arakawai (n=2,988, 60.58%), C. punctatus (n=1497, 30.35%), C. nipponensis (n=246, 4.99%), C. maculatus (n=61, 1.24%) and Culicoides spp. (n=140, 2.84%). Three farms located at high altitudes showed higher collection rate of Culicoides biting midges from inside than outside. Dominant species were C. arakawai in four farms whereas C. punctatus was dominant in one farm with highest altitude. RT-PCR results showed that Bovine ephemeral fever and Akabane virus were positive only in one farm and Chuzan virus was positive in another one farm.

Conclusion
C. arakawai and C. punctatus were dominant species in the Jeollabuk-do. Collection rate could be changed depending on the difference of altitude. Generally Culicoides biting midges were caught more in the highlands and inside of the farms. Finding factors affecting habitat of Culicoides biting midges will be useful for prevention of arboviruses outbreaks.

Keywords: Arbovirus, Cattle Farm, Culicoides Biting Midges

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Feeding Frozen Colostrum to Replacement Calves Does Not Reduce Infection in a Dairy Herd with High BLV Prevalence

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Purpose
To evaluate efficacy of one-time-only frozen and thawed colostrum from BLV+ cows in protecting seronegative calves from becoming infected during their first 5 months of life.

Materials and Methods
Newborn calves from a commercial herd with a high BLV infection prevalence (76%) were enrolled. First and second milkings were collected from BLV+ and BLV- cows. Half of the colostrum was frozen in a commercial freezer (0°F) until its use, and the other half was used fresh. Calves were fed colostrum from the same cow for two feedings, and then fed a commercial milk replacer.

Blood samples were collected before colostrum intake, on day 1, month 3, 4 and 5. Serum was extracted and tested via ELISA for the presence of BLV antibodies.

Results
Of the 50 calves enrolled in the study, 2 had a positive ELISA test before colostrum intake, suggesting in utero infection, and were censored. A total of 37 calves had complete data and showed that 0% (0/11) calves receiving BLV- colostrum, 62% (8/13) calves receiving frozen BLV+ colostrum and 77% (10/13) calves that receiving fresh BLV+ colostrum were positive on ELISA. Although sample size was small, 100% (3/3) calves born to BLV- dams that received frozen BLV+ colostrum were negative at the end of the study.

Conclusion
Our results show that freezing colostrum one single time is not effective in preventing BLV infection in calves after ingesting colostrum from BLV+ cows. However, an interaction with dam status may exist. Other management options need to be studied to prevent BLV infection in newborns.

Keywords: BLV, Leucosis, Calves, Colostrum, Freezing

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Biofilm Formation by *Candida Albicans* Isolated from Bovine Mastitis

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**Purpose**
The purpose was to investigate biofilm production in *Candida albicans* isolated from mastitis bovine

**Materials and Methods**
The material consisted of forty milk samples collected from cows with clinical and subclinical mastitis from one dairy herd located in Queretaro, Mexico. Quarter milk was sampled aseptically and transported at 4 C to the laboratory. The samples were cultured on the blood agar medium and Sabouraud medium. After 48 hrs incubation, the cultures on the mediums were evaluated morphologically, and microscopic preparations were stained by Gram method. Germ tube tests were performed by inoculating 500 ul of fresh bovine serum with a fresh colony and incubation at 37 C for 2 hrs and fermentation and assimilation tests of dextrose, maltose, lactose and galactose were performed. Biofilm formation was assessed in 96-well polystyrene tissue culture microplates. The *Candida* isolates were inoculated into brain heart infusion and incubated at 37 C. Subsequently 200 ul were inoculated per well, the tissue culture plates were incubated for 24 hrs at 37 C. After incubation content of each well was gently removed by tapping the plates. Biofilms were stained with crystal violet 0.1%. Optical density of stained adherent bacteria was determined with a micro ELISA reader at 492 nm. Experiment was performed by duplicate.

**Results**
The eighth isolates were able to form germ tubes, and they were positives in carbohydrate source assimilation and fermentation profiles. Out of the eight *Candida* isolates, 62.5% produced biofilm

**Conclusion**
Bacterial adhesion and biofilm formation are very important concepts in bacterial disease and control

**Keywords**: Candida, Mastitis, Biofilm, Bovine, Adhesion

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Acaricidal Efficacy of Nicotiana Rustica 9Wild Tobacco Against Hyalomma Anatolicum

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Purpose
This study was conducted to elucidate the effect of ethanol extract of Nicotiana rustica (wild tobacco) against hard tick Hyalomma anatolicum.

Materials and Methods
Methods: The study used egg hatchability test and the immersion method. The extract was tested in three replicates of 2, 4, and 8% concentrations. The results were analyzed using SPSS 20.

Results
Highest concentration (8%) of N. rustica completely inhibited egg hatchability. All concentrations of N. rustica were significantly (P ≤ 0.05) lethal to flat stages of H. anatolicum. The LC95 on flat stages of larvae, nymphs and adults was 5.5, 7.59 and 8.41, respectively. All concentrations of the extract exhibited complete deleterious effect on larval- nymphal molting and significantly (P ≤ 0.05) reduced molting of engorged nymphs and inhibited oviposition of the survived engorged females. However, the lethal effect on engorged females was inversely proportional with concentration.

Conclusion
Results of this study showed that, N. rustica is promising botanical acaricide against Hyalomma anatolicum tick.

Keywords: Wild Tobacco, Hyalomma, Efficacy, Acaricide

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A Study on the Effects of Injecting Povidone-Iodine into the Uterus of a Repeat Breeder Cow During the Luteal Phase

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Purpose
Repeat breeder cows are highly caused by uteritis, endometritis, and subclinical endometritis. Therefore, an experiment was conducted to investigate the effects of PVP-I (polyvinylpyrrolidone-iodine), which is a widely used disinfectant, on conception rate by using it on repeat breeder cows.

Materials and Methods
A 2% of PVP-I solution (50mL) was made and injected into the uterus of repeat breeder cows during their progestational phase and follicular phase. Then between 14-23 days after the injection, estrus was induced by using PGF2α.

Results
When fertilization was conducted on these cows, 7 conceived (63.6%). The conception rate according to the phase of estrus cycle was as follows: 5 out of 7 (71.4%) conceived when treated during their progestational phase and 2 out of 4 (50.0%) conceived when treated during their follicular phase. The time it took until the fertilization after treating the subjects with PVP-I was 27.8 days average for the progestational phase (range of 23-37 days) and 48.5 days average for the follicular phase (range of 46-51 days).

Conclusion
Treating cows with PVP-I showed effects in the improvement of conception, shorter estrus time, estrus concentration, etc. And taking into consideration the organic matter (purulent) in the uterus and the phase of estrus cycle, it seems more effective to use 2% of PVP-I solution in the luteal phase than to use in the follicular phase of estrus cycle.

Keywords: Cow, Repeat Breeder, Uterus, Povidone-Iodine, Phase of Estrus Cycle

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Local Surveillance of Persistently Infected Cattle of Bovine Viral Diarrhea Virus in Korea

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Purpose
The identification and eradication of persistently infected (PI) animals might be important for the prevention of bovine viral diarrhea virus (BVDV) infection and the reduction of great economic damage to the Korean livestock industry. This study was undertaken to implement the systematic, well-coordinated control and eradication program in local area.

Materials and Methods
The regional BVD control and eradication program was initiated in Haman-gun of in 2016 and will be promoted until 2018. There are strategies used to achieve control and eradication model 1) initial test to identify individual BVDV-PI cattle in herd status, 2) test-and-removal scheme followed by vaccination or not, 3) continued monitoring to confirm BVDV-free status. The test samples were collected by ear notching and examined by antigen ELISA.

Results
In 2016, a total of 1,656 Korean native cattle’s ear notches were collected from 25 farms. Sixteen cattle were finally confirmed as BVDV-PI. The prevalence of BVDV-PI in Korean native cattle population of Haman-gun was 0.97%. This positive rate was higher than the overall positive rate in Korean cattle. Though the multiple eradication programs that focus on testing and removal of PI cattle, slaughter of PI tends to delay in voluntary control programs. This situation requires an approach to practical biosecurity measures.

Conclusion
In Haman-gun, program for control and eradication of BVDV was initiated voluntarily and the prevalence of BVDV-PI was investigated during the first phase. It is necessary to apply next step for long-term success of eradication program.

Keywords: BVDV, PI, Control, Eradication, Surveillance

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BM-0355

Serum C-Reactive Protein in Calves Born with Neospora Caninum Infection

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\textbf{Purpose}

\textit{Neospora caninum} causes abortion with great economical losses in cattle industry. Several studies have reported that the rate of \textit{N. caninum} infection in Korea was 13~35\% depending on the region. C-reactive protein (CRP) is a representative acute phase protein and was elevated with immune responses. The acute phase response has been evaluated in dogs with parasitic diseases, however only a few studies about CRP response investigated in cattle. The objective was to evaluate the correlation between CRP value and \textit{N. caninum} infection.

\textbf{Materials and Methods}

This study was conducted with total 286 sera of Korean traditional beef cattle born between 2002 January and 2016 April. \textit{N. caninum} infection was confirmed with ELISA detection kit (IDEXX), and CRP level was checked with C-Reactive protein ELISA Kit (Life Diagnostics Inc.)

\textbf{Results}

The nineteen cattle showed positive results in \textit{N. caninum} antibody test. The positive rate of female cattle was 7.76\%. The all of seropositive individuals were born from \textit{N. caninum} seropositive dam or originated from other farm. The CRP values of two seropositive calves were 191.60 and 225.64 \(\mu g/\mu l\). The mean CRP level of normal five calves born in similar period was 56.1 \(\mu g/\mu l\) (SD=50), which correspond with the normal range of manufactural instruction (16~165 \(\mu g/\mu l\)).

\textbf{Conclusion}

In this cattle group, vertical transmission has been main reason of \textit{N. caninum} infection. The calves from seropositive dam showed high serum CRP value suggesting that \textit{N. caninum} infections effect persistently on immune response.

\textbf{Keywords:} Neospora Caninum, Beef Cattle, C-Reactive Protein

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Antimicrobial Resistance in Beef and Pre-Production Dairy Cattle in New Zealand

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Purpose
Antimicrobial resistance (AMR) in food animals is a concern for animal and human health. Food animal studies in New Zealand have focused on production dairy cattle. There are no substantial relevant data on AMR in bacterial isolates from beef or pre-production dairy cattle. This study aimed to collect AMR data to facilitate rational antimicrobial selection for beef and pre-production dairy cattle in New Zealand.

Materials and Methods
A database search of bovine bacterial culture submissions to two commercial laboratories (2003 -2015) was performed. Isolates from beef cattle and dairy cattle prior to the commencement of first lactation were included. AMR and multiple antibiotic resistance (MDR; resistance in three or more antimicrobial classes) data were tabulated and summarised.

Results
4782 cattle samples yielded 2077 isolates. Streptococcus, Staphylococcus, Escherichia, Trueperella, Enterococcus, and Pasteurella spp. were most common. Across these genera, 21.6% of isolates were resistant to amoxicillin/clavulanic acid, ampicillin, cephalosporins, fluoroquinolones, penicillin, tetracyclines or potentiated sulphonamides. Isolates were most resistant to ampicillin (36.5%). AMR of isolates within genera ranged depending upon the antimicrobial class: Streptococcus, (2-36%); Staphylococcus (3-71%); Escherichia (0-87%), Trueperella (0-71%), Enterococcus (0-53%) and Pasteurella (6-50%) spp. MDR was found in 23% of gram-positive and 29% of gram negative isolates.

Conclusion
This study provides baseline data for cattle under non-dairy production conditions. The presence of AMR and MDR has not been previously described in this group in New Zealand, and requires careful review in consideration of the development of antimicrobial use guidelines in food animals.

Keywords: Antimicrobial Resistance, Beef Cattle, Pre-Production Dairy Cattle

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Bovine Spongiform Encephalopathy (BSE) Prevention and Alzheimer’s Disease (AD) Connections?

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Purpose
Neurodegenerative diseases (BSE, AD) are caused by different mechanisms, but the common denominator of neuronal injury, is overstimulation of glutamate receptors, particularly when the N-methyl-D-aspartate (NMDA) receptors are activated. Recent research has shown that increased magnesium (Mg) in the brain, prevents Alzheimer’s in mice. There the Mg acts as a “natural” antagonist of NMDA receptors.

Materials and Methods
From the literature it is known that in British cows at 80s a higher incidence of subclinical (chronic), hypomagnesaemia was found and a new BSE disease appeared. After 1993 began to significantly reduce the incidence of the BSE. The aim of this study was to determine what changes (the period about 1985-1995) have occurred in British cows, in this context.

Results
In the late of 80s were available only commercial Mg-blocks with very considerable variation in palatability, mostly very low Mg-intake. So usually the survey has been; the subclinical hypomagnesaemia was found in about 7-15 % of tested cows. Since the early 90s, it was in Britain gradually implemented, incorporating Mg in concentrates. To achieve the “extra dietary” requirement level 30 g of daily Mg-intake with certainty, Mg was included in feed, thus not leaving any option to the dairy cow, about the Mg intake. In lactating cows at pasture, more palatable Mg-cobs were used. Subclinical/chronic hypomagnesaemia decreased to about 3-4 % in dairy cows.

Conclusion
Based on this interpretation (BSE/Mg), should be similarly preventive do about the AD? When another, although “synthetic” NMDA receptor antagonist (memantine) is a drug in Alzheimer’s patients.

Keywords: Neurodegeneration, Magnesium Deficiency, NMDA Receptors, BSE in Great Britain, Alzheimer’s Di

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Clinical Utility of External Morphometry in Predicting Intrapelvic Parameters in Friesian Cross Dairy Cattle

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Purpose
Pelvic area in cattle is an important contributor to dystocia causing a physical incompatibility with the fetus or fetopelvic disproportion particularly when the pelvis is small. Although pelvic parameters can be measured directly with a pelvimeter, the procedure can be traumatizing to the animal besides requiring a trained person to conduct it. By determining the usage of external measurements as predictors for intrapelvic parameters, farmers will be able to obtain pelvic parameters from easily obtainable external morphometry. Additionally, no studies have been done to determine this relationship in Friesian cross cattle.

Materials and Methods
Thoracic circumference, abdominal circumference, distance between tuber coxae and distance between tuber ischii were obtained as measurements of external morphometry in 50 Friesian cross cattle from 3 farms located in two states in Malaysia. A Rice pelvimeter was used to measure the intrapelvic parameters of pelvic height (PH, cm) and pelvic width (PW, cm). The pelvic area (PA) was calculated using the formula PA = PH × PW.

Results
All external morphometric measurements were correlated with intrapelvic measurements (0.43 < r < 0.60, P = 0.05). Regression analyses showed that the internal pelvic parameters can be predicted from body weight and external morphometric measurements.

Conclusion
There is an association between the intrapelvic measurements and the external morphometry in the cattle. By using the models derived, the intrapelvic measurements can be predicted from the external morphometry.

Keywords: Dystocia, Friesian Cross Cattle, Pelvimetry, External Morphometry

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Effect of $\alpha$-lipoic Acid on Oxidative Stress, Lipid Metabolic and Liver Enzyme in Transition Dairy Cows

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Purpose
This study was conducted to determine whether $\alpha$-lipoic acid has beneficial effects on EB, lipid metabolism, and liver impairment in periparturient dairy cows.

Materials and Methods
Forty-eight Holstein dairy cows 20 d before parturition were allocated into four groups. Except control group, each cow in other three groups was fed with different doses (3, 5 or 8g/d) of $\alpha$-lipoic acid for 50 d. Bloods were collected at the beginning and intervals of 10 d after supplementation to detect lipid mobilization, lipid metabolism and activities of related enzymes.

Results
Serum GSH-Px and SOD activities were significantly higher from d 20 to 50 in group III and d 40 to d 50 in group IV than control, respectively. Serum CAT activities were markedly higher on d 30 in group II and d 30, 40 and 50 in group III and IV serum MDA contents were significantly reduced on d 40 in group III and d 50 in group IV compared with control. Lipid metabolic parameters had no significant differences in $\alpha$-lipoic acid treatment group, only serum NEFA concentrations were markedly changed on d 30 in group IV compared with control. AST activities were significantly lower on d 30 in group III and IV than control. And there was no significant differences in other parameters between treatment groups and control.

Conclusion
$\alpha$-lipoic acid supplementation improves antioxidant capacity, lipid metabolism and protect liver function in transition dairy cows.

Keywords: Cows, $\alpha$-lipoic Acid, Oxidative Stress, Lipid Metabolic, Liver Enzyme

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Purpose
Insufficient feed intake results in a negative energy balance and fatty liver diseases during early lactation. This study was conducted to know the characteristics of metabolic changes.

Materials and Methods
Thirty Holstein dairy cows 30 d before calving were selected for study. Serum were collected at d 30, 20, 10, 5 before and d 0, 5, 10, 15, 20 after parturition. Lipid metabolites, activities of liver enzymes and lipid peroxidation status in serum were measured.

Results
1) serum TG concentrations were declined before calving and followed by a dramatic decline at calving. Change of Chol concentrations were similar to TG prepartum, but increased postpartum. NEFA concentrations were increased at calving and highest at d 5 postpartum, then started to decrease. Serum HDL and LDL concentrations were decreased slightly prepartum and increased gradually postpartum. 2) LDH and AST activities were the highest at d 5 prepartum and calving, respectively. There were no significant difference in serum ALT and AKP activities. 3) CAT and GSH-Px activities were declined prepartum and increased at calving, then was decreased. SOD activity was increased from day -30 to 0, and then was decreased. 4) MDA content was increased from d 5 prepartum and decreased from d 5 postpartum.

Conclusion
Dairy cows undergo fat mobilization and imbalance of oxidative status in the transition period extensively, and liver function is impaired owing to hepatic lipidosis.

Keywords: Cows, Lipid Metabolism, Lipid Peroxidation, Liver Function

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External Unilateral Fixator of Own Design for the Treatment of Some Mandibular Fractures in Horses

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Purpose
To present the construction and usefulness of an unilateral external fixator of own design.

Materials and Methods
The external unilateral fixator consist following components: one rod having a diameter of 10 mm and a length of 20 cm, four booms, 4 connectors and 4 self-threading apex pins with a diameter of 6 mm and length of 100mm. The boom consist three parts; rod with a diameter of 8 mm and a length of 50 mm, clamp, screw M5. One end of the rod has a cone-shaped used to attach the clamp with a screw M5. Connector of the metal implants with main metal rod consists 4 elements; 1. tang, 2. bush, 3. clamp, 4. screw. For production of the stabilizer stainless steel was used. To assemble the stabilizator drilling machine, drill bit 4,5 and set of 3 keys is needed.

Results
Such designed stabilizer was made according to previously prepared drawings and has been used successfully in the treatment of selected open fractures in horses.

Conclusion
1. The above-described stabilizer is simple to use, assembling and disassembling is easy.
2. The stabilizer is light and has the small size.
3. Fixator has the possibility of adjustment in three planes.
4. Device can be used for the treatment of different fractures.

Keywords: Unilateral External Fixator, Mandible Fracture, Horse

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Enterolithiasis in Horses

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Purpose
Enterolithiasis in horses is quite rare problem, that is specific for some geographic regions (USA - California, Texas) and they might be a cause of recurrent colics in this species. The purpose of this research is to present the outcome of the surgical colic treatment, which were caused by enterolithiasis.

Materials and Methods
There were 15 pure arabian horses at the age from 2 to 12 years, various sex, treated in AKF Equine Hospital, Saudi Arabia. The horses were collected from Red Sea and Persian Bay region. All horses were treated surgically by performing the medial laparotomy and enterotomy of the small or/and large colon. In two cases lateral or parainguinal laparotomy was performed additionally.

Results
12 horses were discharged from the clinic as healthy. In three cases euthanasia was performed due to different reasons - 1. necrosis and perforation of small colon, 2. peritonitis, 3. rupture of the rectus abdominis muscle. In four cases the stone was found in the large colon as well as in small one, in two cases there were 3 stones, in the rest - just one. The biggest stone weighed 2034 grams. Only in one case the stone was palpable in rectal exam before the surgery.

Conclusion
1. Enterolithiasis is a typical problem of Red Sea (Jedda) and Persian Bay (Dammam) regions.
2. The treatment outcome is usually good, but depends on the stone localization.
3. The X-ray of abdominal cavity is recommended in suspected cases.

Keywords: Enterolithiasis, Horse, Colics

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Anoplocephala Magna Infection Among Mongolian Horse Population

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Purpose
The objectives of the study were to describe epidemiological indications of Anoplocephala magna infection of Mongolian horses.

Materials and Methods
A total of 755 horses in 134 soums of 18 provinces were examined at necropsy for the tapeworm presence.

Results
Anoplocephala magna (Abildgaard, 1789) was detected in 13.9% of all the examined horses, in 20.8% in Steppe, in 20.3% in Gobi, in 18.5% in Basin, in 9.0% in Altai and in 6.8% in Khangai regions. Then 11.1% of horses examined in summer, 7.9% in autumn, 12.2% in winter and 32.0% in spring were infected. Infection was detected in 52.0% of weaned foals, 19.8% of yearlings, 12.9% of two-year-olds, 4.1% of three-year-olds, 12.1% of four-five-year-olds, 0.6% of caples and 1.9% of mares. Altogether 85.5% of all examined horses were without worms, 9.7% with common, 4.5% with clinical and 0.12% with super tapeworm infections. Common, light, moderate, heavy clinical and super infections detected in 67.6%, 24.1%, 5.6%, 1.9% and 0.93% of the infected horses, respectively. A moderate abundance of the common, light, moderate, heavy clinical and super infections were 2.1, 8.5, 17.7, 28.5 and 50 worms, respectively. Adolescent, mixed and adult worm populations were in 20.4%, 25.9% and 53.7% of the infected horses. Among 485 tapeworms, 26.3% were adolescent and 73.7% - adults.

Conclusion
Two per three horses infected were with common, one per four horses were with light, 5 to 6 per hundred horses were with moderate, 2 - with heavy clinical and one - with super infections.

Keywords: Mongolian Horse, Anoplocephala Magna, Common, Clinical, Super Tapeworm Infection

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Development and Evaluation of the Performance of an Indirect Elisa for the Screening of Equine Infectious Anemia

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Purpose
An effective diagnostic program is the only way to control Equine infectious anemia (EIA). This work presents the results of an indirect ELISA- synthetic peptide system validation for the antibodies detection of equine infectious anemia virus. All the components for the system were also produced.

Materials and Methods
The components were standardized and the cut value was established using the estimate of the ROC curve. The performance evaluation against 365 sera tested by AGID included the specificity, sensitivity, accuracy and consistency regarding the AGID and VMRD system. The components stability was also studied during 16 months were estimated the precision samples with different reactivity, calculating the coefficients of variation and building graphics Lewey-Jennings.

Results
The system was standardized with 1 mg/mL of gp 45 peptide and the conjugate produced in 1:10000 titers. The optical density 0.30 was established as the cut value. Sensitivity level obtained to AGID and VMRD system were 99.4% and 95.5%, specificity of 95.1% and 98.3% respectively as well as a very good concordance. The diagnostic exactness was of 97.57%. The variation coefficients were less than 15% and Lewey-Jennings charts at both levels of evaluation showed an adequate precision, in all sera the ranged values were around the limits of the median and two standard deviations values. The system components comply with the all quality specifications designed for the evaluated time.

Conclusion
These results demonstrate an adequate execution and stability of the developed system. This ELISA could be used in diagnostic algorism to the EIA virus.

Keywords: Equine Infectious Anemia Virus, Synthetic Peptide, ELISA.

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Cell-Free DNA as a Potential Synovial Fluid Biomarker for Orthopaedic Disease in the Horse

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Purpose
Data on equine cell-free DNA (cfDNA) is limited with no published information on synovial fluid (SF) cfDNA and its relation to orthopaedic disease. Our objectives were to determine baseline concentrations of SF cfDNA in young healthy Thoroughbreds, and to determine the effects of a carpal chip model of osteoarthritis (OA) on SF cfDNA levels over time.

Materials and Methods
On the first day SF was collected from the middle carpal joints of 17 Thoroughbred fillies. A carpal chip was then surgically created in one middle carpal joint in nine fillies (OA), while eight had a sham operation. All horses commenced a treadmill exercise program on day 14 that continued for seven weeks. SF was collected weekly aseptically and cfDNA concentrations measured by fluorimetry.

Results
On day 0 the median SF cfDNA was 504 $\mu$g/L (IQR= 236). The temporal effects of traumatically induced OA on SF cfDNA concentrations were sustained compared with controls. The OA joints had SF cfDNA levels that were significantly higher than at day 0, and higher than Sham joints on days 28 and 63. The SF cfDNA concentrations were significantly higher in Sham joints on days 7, 14, 21, 35, 42 and 63 compared to pre-trial values.

Conclusion
This study is the first to report equine baseline SF cfDNA concentrations and changes following orthopaedic trauma. Validation of SF cfDNA measurement with naturally occurring orthopedic disease is necessary before clinical utility can be determined.

Keywords: Cell-Free DNA, Synovial Fluid, Trauma-Induced Osteoarthritis

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Antimicrobial Susceptibilities of *Staphylococcus Aureus*, *Streptococcus Equi Zooepidemicus* and *Escherichia Coli* Isolates from New Zealand Horses

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Purpose
Clinically relevant data on antimicrobial susceptibility (AMS) of equine bacterial isolates in New Zealand is limited. These are critical for development of regionally specific antibiotic use guidelines. This study aimed to collect AMS data to facilitate rational antimicrobial selection for three commonly cultured pathogens: *Staphylococcus aureus*, *Streptococcus equi zooepidemicus* and *Escherichia coli*.

Materials and Methods
A database search of equine bacterial culture submissions to a commercial laboratory (August 2003 - September 2014) was performed. Culture and AMS of isolates as defined for the Kirby-Bauer disk diffusion susceptibility test were examined for *Staphylococcus aureus*, *Streptococcus equi zooepidemicus* and *Escherichia coli*.

Results
*S. aureus* was most commonly cultured from skin or wounds, *S. equi zooepidemicus* from respiratory and *E. coli* from urogenital samples. For *S.aureus*, 99.6% of isolates were susceptible to cephalothin, 98% to enrofloxacin, 83% to gentamcin, 50% to penicillin, 71% to tetracycline and 94% to trimethoprim/ sulphonamide. For *S.equi zooepidemicus* corresponding values were 99%, 66%, 78%, 94%, 25% and 64%. For *E. coli* the values were 53%, 98%, 93%, penicillin not tested, 69% and 76% respectively.

Conclusion
The low sensitivities of *S. aureus* isolates to penicillin, and *S.equi zooepidemicus* to tetracycline are concerning. Submission for susceptibility testing is recommended prior to their use. The moderate susceptibility of *S.equi zooepidemicus* isolates to enrofloxacin is problematic as it is not formally registered in New Zealand for equine use. These data highlight the need for regionally relevant guidelines for antibiotic use in horses.

Keywords: Antimicrobial Susceptibility, Antimicrobial Resistance, Equine

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Equine Behavioural and Biomechanical Responses to Trailer Transport and Braking

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Purpose
To the authors’ knowledge, few studies have sought to model the interaction between the transport vehicle, driver inputs, and forces acting upon the horse with its behavioural and adaptive responses. This project aimed to describe and characterise behavioural and biomechanical responses of the horse to the forces associated with trailer transport and braking.

Materials and Methods
8 healthy adult horses were instrumented with accelerometers on the head, rump and limb, and a heart rate monitor. The horse trailer had 4 wireless video cameras internally mounted at the front, side and roof. A standardised driving course was used to collect video and biomechanical data up to 70 kph. Horses were the subjected to mild to moderate acceleration and braking trials from 20, 30 and 60 kph to zero.

Results
At speeds of up to 70 kph, horses unaccustomed to trailering showed behavioural and mechanical signs of failing to adapt. One behavioural variable, ear twitching rate, has potential as an objective measure of acoustic dampening in design. Braking trials highlighted the risk of injury to hindquarters. Deceleration and energy of braking absorbed by horses is greater than that of the vehicle.

Conclusion
These pilot data suggest a focus on the horse is more critical to trailer design and operation, rather than meeting simple load-carrying requirements. This novel approach using smart camera and biomechanical sensor technologies for modelling welfare outcomes has potential for improving the transport experience of animals, through research led vehicle design, and increased driver awareness.

Keywords: Horse Welfare, Transport, Trailer

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Identification of *Taylorella Equigenitalis* from Genital Tract of Thoroughbred Horses from Inland Area of South Korea

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**Purpose**
To verify the existence of *Taylorella equigenitalis* and identify the origin of *Taylorella equigenitalis* from Korea inland Thoroughbred horses.

**Materials and Methods**
External genital swabs (n=38) from Korea inland Thoroughbred horses at the breeding season in 2016 were examined by classical bacteriological culture, Polymerase chain reaction (PCR) and 16S rRNA gene sequencing analysis. In addition, the phylogenetic tree was constructed among 16S rRNA sequences of seven *Taylorella equigenitalis* strains including Korea inland strains and other regional reference strains.

**Results**
Among 38 genital swabs, 2 (5.26%) were positive for *Taylorella equigenitalis* by classical bacteriological culture, PCR and 16S rRNA gene sequencing analysis. The phylogenetic tree showed close phylogenetic relation of Korea inland *Taylorella equigenitalis* strains with those of Japanese strains.

**Conclusion**
From our study, the existence of *Taylorella equigenitalis* was obviously verified and two Thoroughbred horses infected with *Taylorella equigenitalis* were identified as the carrier animals which shed the causal agents without any clinical signs. Our phylogenetic tree confirmed Korea inland *Taylorella equigenitalis* strains may be originated from the importation of Thoroughbred horses infected with Japanese *Taylorella equigenitalis* strains.

Conflicts of interests: None

**Keywords:** Taylorella Equigenitalis, Polymerase Chain Reaction, 16S rRNA Sequencing

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EM-0406

Cardiac Troponin I (cTnI) Level and Heart Rate Variability (HRV) after Cobra Envenomation in the Horse

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Purpose
Cardiac toxicity in horse after cobra envenomation remains to be elucidated. This study aims to detect cardiac injury by measuring cTnI level and to evaluate cardiac autonomic function by HRV analysis in horses injected with monocellate cobra’s venom.

Materials and Methods
In a prospective study, 12 horses were divided into 2 groups; control (saline injected; n=4) and envenomed horses (25 mg of cobra’s venom injected; n=8). Blood samples were collected and ECG recordings were obtained at selected time points (0, 6, 12, 24 and 48 hours, respectively). Complete blood count and serum cTnI measurement were done. cTnI analysis was based on immuno-chromatographic assay. HRV analysis was performed using a computer software (SCM510™). Repeated measurement ANOVA was used to determine the difference between the two groups.

Results
Envenomed horses had an elevation of white blood cell counts (P<0.01 vs. control) at 24h and 48h after venom injection indicating an inflammatory response. Eighty-seven percent of envenomed horses (7/8) experienced myocardial damage as indicated by an increase in serum cTnI (>0.03 ng/ml). cTnI were gradually elevated and peaked at 12h after venom injection. cTnI level returned to the baseline after 48h. HRV analysis revealed an activation of sympathetic nervous system (SNS) since parameters of SNS was increased including LF/HF. Heart rate was elevated (P<0.01) at all-time point in the envenomed group. However, parasympathetic index was unchanged.

Conclusion
Cobra envenomation in horses resulted in an acute myocardial damage and an increase in sympathetic tone possibly due to pain.

Keywords: Cobra Venom, Horse, Cardiac Troponin I, Heart Rate Variability

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Expression and Localization of Equine Tissue-Specific Divalent Ion Transporting Channel Proteins

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**Purpose**

Divalent ions such as calcium, iron and copper play a role in the physiologic functions such as ionic homeostasis and feedback mechanism. Ion channels are present in the membranes of all cells, controlling the flow of ions across the cell membrane. Hyperkalemic periodic paralysis (HYPP), Iron deficiency anemia and horse colic are representative diseases related with ions channels.

**Materials and Methods**

In our study, we focused on expression level and localization of divalent ion channel such as Nckx3, Trpv2, Ireg1, Heph, Ctr1 and Atp7a in equine duodenum, heart, kidney, liver, lung, ovary, uterus and testis tissues. Gene expression of divalent ion channel was quantified by real-time PCR, protein expression by western blotting, immunohistochemistry for identifying the localization of each channel.

**Results**

Localization of proteins was expressed in the respective structure including gland, epithelium, and myocyte. Gene and protein level of Nckx3 and Trpv2 were high at uterus. Ireg1 was quiet different but protein level was exclusively high at liver. Heph mRNA level was high at duodenum and protein level was high at liver. Nckx3 was highly expressed in ovary, uterus, and testis. Trpv2 expressed in duodenum, liver, lung, kidney. Ireg1 was expressed in duodenum liver, ovary, and uterus. Heph was expressed in duodenum ovary, testis. Ctr1 was expressed in all tissue. Atp7a was expressed in duodenum, kidney, and testis.

**Conclusion**

Taken together, each ion channel differ from development location as well as development level respectively on every organ. This discovery could be the first step of divalent channel related diseases in horse.

**Keywords:** Equus Ferus Caballus, Divalent Channel, Ion Channel

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Relationship Between Sociability Toward Humans and Physiological Stress in Dogs

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Purpose
1. To confirm the relationship between sociability and physiological stress in dogs.
2. To compare companion dogs and shelter dogs in terms of their levels of sociability and physiological stress.

Materials and Methods
A total of 37 healthy dogs were included in the study.
• The dogs were divided into four sub-groups based on their sociability behavioral test results and their status as a companion dog or a shelter dog, as follows: companion dogs with high sociability (group 1) or low sociability (group 2) and shelter dogs with high sociability (group 3) or low sociability (group 4).
• The results of the behavioral scoring in the sociability assessments and the calculated salivary cortisol levels were statistically analyzed.

Results
• Age, weight, sex and breed type of the subjects included in this study were not found to have a significant effect on hormonal results (P >0.05).
• Dogs with low levels of sociability became more physiologically stressed.
• Among the dogs with low sociability, the shelter dogs showed significantly higher stress levels.

Conclusion
1. More sociable dogs experience less physiological stress and consequently have a greater ability than less sociable dogs to adapt to various human-based environments.
2. The sociability of shelter dogs may need to be confirmed using behavioral and physiological assessments to predict their ability to successfully adapt to a new environment and to reduce the likelihood of a failed re-adoption.

Keywords: Dog, Sociability, Salivary Cortisol

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Hepatitis E Virus: The Time Course of Infection in a Swine Herd Followed by Serological and PCR Investigations

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Purpose
Hepatitis E virus (HEV) is a hepatotropic RNA virus causes mostly acute, self-limiting hepatitis in humans. Genotype 3 and 4 of Orthohepevirus A are zoonotic with swine being the main reservoir.

Our aim was to follow the time-course of HEV infection at a Hungarian pig farm.

Materials and Methods
Altogether 100 serum samples were collected in different age groups for serological investigations. The collection of the faeces samples in 180 randomly chosen animals for the detection of HEV RNA started on the first day of the fattening stage and lasted for 91 days.

Results
The ELISA results indicated that the HEV infection occurs around the time when piglets are moved from the nursery to the first fattening stage of production.

We detected the presence of the virus in 79% of the faeces samples on Day 1. After a short decrease period followed by a marked increase in positivity, all samples were positive between Day 20 and 49. Virus shedding was detected to decrease from Day 56. At the time of slaughter, some animals were still shedding the virus in their faeces.

Conclusion
HEV infection in swine occurs around the end of nursery / start of fattening stage of production. Because of the slow spread of the infection, virus shedding can last for over 3 months. The slaughter of swine from HEV-infected farms may pose a food safety risk to the consumer.

The study was supported by Grant OTKA112730.

Keywords: Hepatitis E Virus, Zoonosis, Food Safety

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**Food Safety and Occupational Health Significance of Hepatitis E Virus Infection in Rabbit and Hare**

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**Purpose**

*Hepatitis E virus* (HEV) causes mostly acute, self-limiting hepatitis in humans. Genotype 3 and 4 of *Orthohepevirus A* are zoonotic, reservoir animals are swine, wild boar, deer, roe deer and rabbit.

The aim of our study was to detect HEV in rabbit and hare, and estimate the food safety and occupational health significance of the infection.

**Materials and Methods**

By serological investigation, 231 blood samples of domestic rabbit (0.5-1 year of age) and 44 blood samples of hare shot at 3 hunting areas were tested. All samples were collected in Hungary.

Liver, meat and faecal samples of 30 rabbits (4-13 weeks of age) sent for pathological investigation and 100 animals (11 weeks of age) sent for slaughter were investigated by qRT-PCR method.

**Results**

HEV infection was detected in 5/11 investigated rabbit farms. Seroconversion was not detected in any of the hare samples.

By qRT-PCR, 23% of the necropsied and 42% of the slaughtered rabbits were found to be HEV-positive. Viral RNA was detected in liver and faeces as well as in meat samples. By sequencing, in 48 samples 15 different virus variants were identified; all were rabbit-specific HEV-strains.

**Conclusion**

According to our results, farmed rabbits generally got infected around 9-11 weeks of age, and the animals may shed the virus at the time of slaughter. Moreover, HEV RNA was detected also in the meat of the slaughtered rabbits, which raises food safety and occupational health concerns.

The study was supported by Grant *OTKA112730*.

**Keywords**: Hepatitis E Virus, Zoonoses, Food Safety

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Serological Investigation on the Prevalence of Hepatitis E Virus Infection in Food-Producing Animals

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Purpose
The main reservoirs of the zoonotic genotypes of Orthohepevirus A are domestic swine, wild boar, wild ruminants and rabbit. Humans got infected through contact with virus-shedding animals or their manure and not-well-heat-treated meat.

Our aim was to survey swine, wild boar, rabbit, hare and goat samples by ELISA in order to recognise the occurrence of the virus in populations of species proven or suspected to be reservoir of HEV.

Materials and Methods
By ELISA, we investigated 473 serum samples of swine collected at 14 farms, 35 serum samples of wild boar, 231 serum samples of domestic rabbit, 44 samples of hare and 333 samples of goat. Liver juice and meat juice samples were obtained from 80 slaughtered pigs, 30 necropsied and 100 slaughtered rabbits. All samples were collected in Hungary.

Results
HEV is endemic in all the investigated swine farms with more than 50% seroprevalence among the animals. The virus is endemic at the investigated wild boar farm as well, where all 35 tested animals were found to be positive. Anti-HEV antibodies were detected in samples originating from 5 out of 11 rabbit farms. All of the hare and goat samples were found to be negative by serological investigation.

The results of juice samples have revealed that the majority of the investigated pigs had seroconverted before slaughter, while rabbits become infected right before slaughter.

Conclusion
The results raise questions regarding food safety risks.

This study was supported by Grant OTKA112730.

Keywords: Hepatitis E virus, Zoonoses, Food Safety

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Italian Street Food: Technology and Security

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Purpose
The "street food" puts hygiene problems in relation to: the often informal production and distribution in the absence of food safety procedures. We are going to illustrate the production technology and the investigation of microbial contamination levels of "Arrosticini" of Abruzzo, the most famous Italian street food.

Materials and Methods
Technology: boning the carcass, removing tendons, cutting into cubes of 1cm side, and infixion on 20cm long wooden stumps. The process can be manual or partly mechanized. The little skewers of mutton of about 25 grams are cooked on the grill.

The total bacterial count (TBC) depends on the hygiene of carcasses, on the reduction in small pieces and on ranging from impact of the stick. The application of dry heat heals from germs contaminants, but it is not the same effective on meat of sick animals. The immediate consumption eliminates the risk of secondary contamination.

Microbiological investigation was conducted on samples derived from 4 CE approved establishments seeking: CBT (petrofilm); E. coli O:175 (isolation) Salmonella spp (isolation); E. coli (TBX-plates); Listeria monocytogenes (isolation and plate count)

Results
In the refrigerated arrosticini: TBC is an average of 1,800 cfu/g; E. coli (hygiene indicator) <10 cfu/g; Absent: Salmonella spp., Listeria monocytogenes, E. coli O:157, Staphylococcus positive coagulase

Conclusion
The street food is often associated to the risk of foodborne illness. In establishments which apply GMP, GHP for the control of hazards not manageable on the level of individual process steps and HACCP procedures, microbial contamination is lower and pathogens are absent.

Keywords: Street Food, Meat, Food Security

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Research on Distribution of the Aedes Albopictus, Zika Virus Vector (Military Units in Incheon Harbor)

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**Purpose**
WHO declared a state of world public emergency toward Zika virus infections. We started to conduct a study to grasp the inflow of foreign mosquito species from international port of mass distribution and inhabitation density of Aedes Albopictus infected by pathogen for surveillance on vector-borne infection disease.

**Materials and Methods**
We installed mosquito trap to collect mosquitoes for 5 months (from May to September) and classified their species. Classified Aedes Albopictus bundled up under 50 individuals were sent to pathogen inspection conducted with Real-Time PCR method.

**Results**
The total number of collected mosquitos was 9,351 individuals composed of Culex (6,908 individuals, 73.9%), Aedesal Bopictus (2,001 individuals, 21.4%), Anopheles Sinensis (262 individuals, 2.8%), Aedes Vexans Nipponii (131 individuals, 1.4%), Aedes Koreicus (40 individuals, 0.4 %). Foreign mosquito species were not collected. The result of Zika virus inspection toward Aedes Albopictus came out negative. According to the result of the inspection, compared with the average distribution ratio of Aedes Albopictus in ROK, distribution ratio of troops near Inchon Harbor stood at 21.4% which was very high.

**Conclusion**
Soldiers of ROK carrying out military action for long time in the open air are highly exposed to vector-borne infection disease. If disease are broken out, it may come to loss of fighting power and national strength may become weak. Therefore commanders in related area are anticipated to recognize the necessity for continuous surveillance toward vector-borne infection disease and establish countermeasures for prevention.

**Keywords**: Mosquitoes, Aedes Albopictus, Zika Virus

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Genetic Relations of Vaccine Strains of Pasteurella Multocida Through Electrophoresis of Pulsed Fields in Minigeles

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Purpose
Gram-negative bacteria Pasteurella multocida exhibits a wide range of hosts, causing hemorrhagic septicemia and death by different particular serotypes. Molecular characterization, through the exploration of the genome, is one of the most useful and precise strategies for the classification of microorganisms. Pulsed Field Electrophoresis (ECP) has demonstrated greater power of discrimination for the identification and differentiation of Pasteurella multocida strains. The objective of the present investigation is to determine the genetic relationship between four strains of Pasteurella multocida vaccine by the ECP technique in minigels.

Materials and Methods
Four strains of Pasteurella multocida isolated from rabbits identified with codes 129, 248, Thelma and PM2Granma were used for which ECPs were performed using restriction enzyme Apal. The restriction fragments were separated by ECP using the GUEFAST System (Neuronic SA, Cuba), in 1.5% agarose gels. The electrophoretic runs were performed in the CHEF configuration chambers by applying 145V.

Results
The four bands patterns obtained by analyzing the genome of Pasteurella multocida strains showed differences in the amount of DNA bands and their positions in the gel. P. multocida 129 and P. multocida 248 presented the highest genetic similarity coefficient (46%).

Conclusion
It is considered that the strains are genetically related if they present ≥80% similarity in the dendogram. The four strains studied are not genetically related to each other and therefore can be used as vaccine candidates for the prevention of this disease.

Keywords: Pasteurella Multocida, Pulsed field Electrophoresis, Genetic Relationship

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Surveys/Research on Carrier Status and Molecular Epidemiology of Salmonella in Livestock

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Purpose
To research carrier status of Salmonella in cattle and swine through veterinary inspection in an slaughterhouse, and compare isolated strains with that from patients and livestock.

Materials and Methods
1) 15 years swab test data of bovine/swine carcass were reviewed.
2) Salmonella were collected from feces in the bovine/swine rectum in 2015-2016.
3) MLST, PFGE pattern and drug susceptibility of isolates Salmonella 1,4,[5],12:i:- strains were characterized.

Results
Salmonella were collected from bovine carcasses (0.17%, Serotype: 1,4,[5],12:i:-) and swine feces (8.16%, Serotype: 1,4,[5],12:i:- and Brandenburg). All of MLST type of the strain was ST34. Same PFGE pattern strains were also indicated same pattern in drug susceptibility testing.

Conclusion
The ratio of carrying Salmonella in swine was higher than that in cattle, and the identical Salmonella strain was obtained from bovine and swine. Those facts may indicate that proper livestock management would be needed to control cross infection from swine to bovine. Result of MLST and PFGE implies that most of the Salmonella 1,4,[5],12:i:- strain isolated in this research was identical with epidemic strain from patients developed salmonellosis and livestock bred in Hokkaido Japan area. We consider that it is critical to continue to control sanitary management on contamination with Salmonella in slaughterhouses, and risk communication with other administrative organs sharing information of the strain with molecular epidemiology data is also very significant for controlling salmonellosis in humans and livestock.

Keywords: Salmonella 1,4,[5],12:i:-, Dressed Carcasses Inspection, Molecular Epidemiology

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Methicillin-Resistant *Staphylococcus Pseudintermedius* from Animal Hospitals in Taipei

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Purpose
The aim was to study the genotypic and phenotypic characteristics of clinical isolated methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) from pet animals in Taipei.

Materials and Methods
MRSP isolates were recovered from 2 veterinary referral centers and National Taiwan University Animal Hospital. Clonal relationships were determined by *Staphylococcus* chromosome cassette mec (SCCmec), direct repeated unit (dru) and multilocus sequence typing (MLST). The mecA gene, exfoliative toxin and Panton-Valentine leukocidin (siet, lukS-PV and lukF-PV) gene were screened by PCR. Antimicrobial susceptibility tests were performed using VITEK 2 compact method. Biofilm formation ability was determined by microliter plate test. Statistical differences were considered while a P value of <0.05.

Results
There were 72.3% of SP isolates carried the mecA gene. Two major dru clusters (11a/9a clusters) and two MLST groups (ST71/ST335) were identified. Isolates with dru cluster 11a carried SCCmec II-III, III and V, and those with dru cluster 9a carried SCCmec II-III. However, isolates with dru type dt11y carried SCCmec III only. The majority of the MRSP (83.3%) were strong/moderate biofilm producers and negative for lukS/F-PV but positive for exfoliative toxins (siet). Most MRSP showed multiple antibiotic resistance.

Conclusion
MRSP isolates from the infected skin of animals were strong/moderate biofilm producers and had exfoliative toxin gene that may have community infectious potential.

Keywords: Biofilm, Methicillin-Resistant Staphylococcus Pseudintermedius, Taipei

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Prevalence and Dynamics of Antibodies Against *Toxoplasma Gondii* in Kids Born from Naturally Infected Goats

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**Purpose**
The objective was to assess the importance of endogenous trans-placental infection for maintaining the endemicity of *T. gondii* among populations of domesticated goats. We investigated the prevalence and dynamics of antibodies against *T. gondii* in goats in Bangladesh.

**Materials and Methods**
Serum samples were collected from 155 goats from Rajshahi, Bangladesh. Testing for *T. gondii* antibodies was performed using a commercially available diagnostic kit for humans, Toxotest-MT (Eiken Kagaku, Japan), a system which detects anti-*Toxoplasma* antibodies via latex agglutination. F distributions with 95% confidence interval were used to analyze the data.

**Results**
Antibodies to *T. gondii* were found in 51.6% (80/155) of the goats tested. The seroprevalence among goats aged <1 year, 1-2 years 2-3 years and ≥3 years were 36.7%, 66.0 %, 59.1 % and 100%, respectively. The results demonstrated that seroprevalence increased with age. Among the seropositive goats, a subsample of eight free-ranging female goats with access to male goats was placed under continuous observation. These seropositive female goats delivered 11 kids, all of which were found to be seronegative before suckling colostrum.

**Conclusion**
This finding strongly suggested that trans-placental infection rarely occurs in female goats that have acquired an infection before pregnancy. Our results indicate that infection via ingestion of oocysts plays a more important role than endogenous trans-placental infection in maintaining the endemicity of *T. gondii* among goats in Bangladesh.

**Keywords:** Bangladesh, Goat, Seroprevalence, Sheep, Toxoplasma Gondii

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Purpose
Salmonella serotypes have been widely incriminated as the most important zoonotic pathogens in several countries in the world and are responsible for significant mortality and morbidity in both humans and animals. Products such as amoxicillins, tetracyclines and fluoroquinolones could be effective for the treatment of this microorganism, although they are not able to eliminate the infection completely. The objective of the present investigation is to determine the susceptibility of three strains of Salmonella against a panel of antimicrobials by the Bauer-Kirby method.

Materials and Methods
All bacterial strains (Salmonella cholerae-suis, Salmonella typhimurium and Salmonella oritamerin) used came from the Department of strains of the Production Company of Vaccines Viral and Bacterial, LABIOFAM, which were lyophilized at 2ºC. For the performance of this test the disc diffusion method (Bauer-Kirby) was used according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) of 2016, using nine antimicrobial drugs recommended by the CLSI for the family Enterobacteriaceae.

Results
It was shown that the three strains of Salmonella investigated have a total resistance against ampicillin and tetracycline, although they exhibit a lower resistance to the rest of the antibiotics except chloramphenicol for which all the strains were susceptible.

Conclusion
The strains of Salmonella presented resistance to antimicrobials of wide use, demonstrating in one of them multiresistance pattern.

Keywords: Salmonella, Antimicrobial Susceptibility, Multidrug Resistance

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Survey on Distribution of Vector Ticks in Military Training Field

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Purpose
Severe Fever with thrombocytopenia syndrome (SFTS) is an infectious disease which the virus was first discovered in China in 2011. In Korea, the first case was discovered in 2013 May since then, 36 in 2013, 55 in 2014 was reported and of which 17, 16 cases, respectively, proved to be fatal The army has repeated trainings and is stationed in shrubby area. Therefore, the army's major outdoor training grounds were selected to study the disease mediating flea distribution and its carrier rates to be used as basis date for prevention of army specific disease.

Materials and Methods
From August to October, 5 October, 5 shrubby areas near 6 army camps, fleas were collected by flagging and dry-ice. The collected fleas were studied by PCR with the help of Korean Centers for Disease Control and Prevention's disease mediating insectology department for their virus carrier state. Fortunately, no virus which causes the disease was found in the collected fleas.

Results
Excluding shrubby areas where attention, such as mowing management, was given, various area in which the army facilitates, almost all of them were collected to fleas, From the collected fleas, no SFTS causing virus was found.

Conclusion
In the future, collecting fleas from nationwide scattered training grounds to study their virus carrier state, can be used for preventive measures or the basis data for disease prevention.

Keywords: Dragging Method, Flagging Method, Severe Fever with Thrombocytopenia Syndrome

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Specific Monoclonal Anti-Bear IgG (Ursus Thibetanus Ussuricus)

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Purpose
As endangered animals, the asiatic black bear is under the species restoration project and there are many attempts of artificial proliferation for years by Korea National Park Restoration Institute. Various examinations and research data are collected as a part of species restoration projects. Meanwhile, basic diagnosis for disease management is not intensively performed yet. This study was performed for presenting serological diagnostic methods of asiatic black bear diseases.

Materials and Methods
Bear IgG specific monoclonal antibody (MAb) is developed for diagnostic material. Bear IgG was purified by affinity chromatography with Protein G resin and was employed immunizing BALB/c mouse. Hybridoma cell lines secreting specific anti-bear IgG were attained with spleen cells from overimmunized BALB/c mouse and SP/2 myeloma cells.

Results
Mab type secreted by 7 of attained hybridoma is IgG. Among these hybridoma lines, 1C2, 1G9 and 2F7 showed cross reactivity with dogs and cattle. 3C2 and 5D10 showed no cross reactivity with serum of 11 different species.

Conclusion
The 3C2 and 5D10 specific to bear IgG are considered helpful for serological detection of diverse infectious disease of asiatic black bear.

Keywords: Asiatic Black Bear, Bear IgG, Monoclonal Antibody

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**Study on Isolation, and Molecular Detection of tetA and sulI Gene of Escherichia Coli Isolated from Raw Chicken Meat in Surabaya, Indonesia**

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**Purpose**
The study was undertaken to isolate pathogenic *Escherichia coli* isolated from raw chicken meat, and detection of tetA and sulI gene by using Polymerase chain reaction (PCR).

**Materials and Methods**
During 6 months duration of study a total of 140 raw chicken meat samples were collected from different places of wet market located in Surabaya, under aseptic precautions. For the enrichment of the organism from the collected samples, MacConkey broth was used and inoculation was carried out on MacConkey agar and EMB agar, to confirm the isolates, various biochemical tests such as IMViC test were performed. Antibiotic sensitivity test of *E.coli* to antibiotic tetracyclines and sulfonamides was evaluated by disk diffusion method. Molecular detection was used for deoxyribonucleic acid extraction, and the presence of tetA and sulI gene was detected using PCR techniques.

**Results**
The result of present study revealed that out of 140 samples, 56 samples were found contaminated with *E.coli*. Antibiotic sensitivity test revealed all isolates resistance against tetracyclines (100 %), and high resistance was observed for sulfonamides (91 %). Molecular analysis revealed that 52 tetA gene and 21 sulI gene.

**Conclusion**
Our findings suggest that Surabaya urgently needs an integrated surveillance system within the entire chain, for antimicrobial-resistant *Escherichia coli* isolated from raw chicken meat.

**Keywords:** Escherichia Coli, Raw Chicken Meat, tetA and sulI Gene

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Molecular Epidemiological Analysis of Brucella Strains from Various Animals in Thailand

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Purpose
The objective of this study is to assess the epidemiological features together with bacteriological findings of brucellosis in Thailand.

Materials and Methods
A total of 65 Brucella(B.) isolates from animals in 14 regions of Thailand confirmed to be sero-positive were identified by Brucella-specific multiplex PCR and biotyping assay. Molecular epidemiology was analyzed by MLVA-16.

Results
Brucella isolates were differentiated by 4 B. abortus S19, 15 B. abortus and 46 B. melitensis, and biotyping of B. abortus isolates from beef and dairy cattle were identified as biovar(bv.) 1 and 3, and all B. melitensis from goats, sheep and beef cattle were confirmed as bv. 3. MLVA data revealed 19 B. abortus and 46 B. melitensis were divided into 9 and 28 genotypes, respectively, and they had the adjacent genetic profiles according to provinces and animal species; different provinces, at least 2 ones, have a close genetic relationship in the same cluster. Moreover, B. melitensis isolates tend to be grouped into the same cluster between sheep, goats and cattle. Compared with other foreign isolates, 2 Thai B. abortus were closely similar with Brazilian strain (83.34% similarity). Many kinds of Thai B. melitensis isolates from goats and sheep showed high genetic similarity (88.75~100%) with Chinese strains.

Conclusion
Molecular Epidemiological findings indicate that Brucella strains circulate among various animal species and provinces in Thailand and they are also possible to be transboundary spreads. Taken together, this study underscore to arrange the appropriate control strategies for brucellosis in Thailand.

Keywords: Brucella, MLVA, Thailand

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Seroepidemiology of Severe Fever with Thrombocytopenia Syndrome Virus in Shelter Dogs in the Republic of Korea

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Purpose
Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne infectious disease. Although there is no evidence that the virus causes disease in animals, previous studies reported antigens and antibodies against SFTS virus (SFTSV) in various animals including dogs. Thus, the purpose of the present study was to investigate seroprevalence of SFTSV in shelter dogs in the ROK using indirect immunofluorescent assay (IFA).

Materials and Methods
Sera were collected from 426 dogs in animal shelters throughout the ROK. Sampling regions were classified into four groups according to the administrative divisions of the ROK (northern, central, southern, and Jeju-do). To detect antibodies against SFTSV, IFA was performed. In brief, Vero E6 cell was inoculated with SFTSV (previously isolated in a goat) and fixed in 12-well microscopic slide. Fluorescence-labeled goat anti-dog IgG was used for secondary antibodies. Positivity was determined for sera that were reactive at a dilution of 1:20.

Results
Of the 426 tested dog sera, 86 (20.2%) showed positivity to IFA against SFTSV. In detail, 14.6% (23/158), 15.9% (18/113), 26.4% (33/125), and 40.0% (12/30) of seropositivity were observed in northern, central, southern, and Jeju-do, respectively. In addition, statistical significance was observed according to the regions (P<0.05).

Conclusion
Our results revealed that nationwide distribution of antibodies against SFTSV in shelter dogs in the ROK. Considering the fact that dogs can contact closely with humans as companion animals, continuous monitoring is required.

Keywords: Severe Fever with Thrombocytopenia Syndrome, Shelter Dog, IFA

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Causes and Origin of the Bovine Spongiform Encephalopathy (BSE) Incidence in the United Kingdom?

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Purpose
Epidemiological incidence of neurodegenerative diseases in a certain period, was detected only in cattle in the UK, as the BSE. The official statement about the ca 5-years incubation period of the BSE is based on the feed ban (1988) of meat and bone meal (MBM) in the UK cattle, and the BSE incidence significantly decreased (after 1993).

Materials and Methods
However, it has never been proven MBM feeding in British cows, in the finding of the BSE incidence, in the respective herd of cows! On the other hand, there are scientific publications from that time (with detailed description of the cows nutrition) when BSE disease was detected in cows, without the MBM feeding (however, high protein intake). However, if in the 80s was among British cows found long-term Mg-deficiency, then after significantly higher Mg-supplementation should be the incidence of BSE significantly reduced.

Results
National agency of the Great Britain, which has monitored (1982-1992) the incidence of clinical-subclinical hypamagnesaemia (hypo-Mg); found subclinical hypo-Mg at about 7-15% of tested cows. Data has been collected monthly from 200-250 farms, with an average herd size of 110-130 cows.

Conclusion
Why Mg-deficiency should be related to neurodegeneration and the emergence of BSE? If the lower the Mg2+ level in the animal tissue cells, the more marked is “Ca2+ effect excitotoxicity” (neurodegeneration-BSE), because this condition overactivates glutamate receptors, specifically the N-methyl-D-aspartate (NMDA) receptor. Prolonged Mg deficiency (subclinical hypo-Mg) leads to an excess of Ca2+ in animal tissues, and NMDA receptor overstimulation.

Keywords: Bovine Spongiform Encephalopathy, Meat and Bone Meal, Magnesium Deficiency, Neurodegeneration, Magnesium

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Characteristics of Single Intradermal Tuberculin Test and Enzyme-Linked Immunosorbent Assay for Diagnosis of Bovine Tuberculosis

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Purpose
To estimate the sensitivity (Se) and specificity (Sp) of diagnostic tests for bovine tuberculosis in Thailand, including single intradermal tuberculin (SIT) test and a commercial enzyme-linked immunosorbent assay (IDEXX® ELISA) in dairy cattle under field conditions using a Bayesian approach.

Materials and Methods
In 2015, 278 dairy cows (> 1 year old) from 35 herds in northern Thailand were diagnosed for bovine tuberculosis using the caudal fold SIT test. Serum samples were collected and determined for antibodies against Mycobacterium bovis using IDEXX® ELISA. The tests characteristics were estimated using a Bayesian modeling. A one-population Bayesian model was implemented assuming conditional independence between the SIT test and IDEXX® ELISA.

Results
The 95% posterior probability interval (PPI) of Se of the SIT test ranged from 50.3 to 85.1% (median = 69.4%) while the Sp was higher and (median = 94.3%, PPI = 89.6-99.0%). The Se of IDEXX® ELISA was low, with 95% PPI ranging between 21.9 and 72.3% (median = 42.7%) although its Sp was high (median = 92.5%, PPI = 88.8-95.5%).

Conclusion
These results demonstrate that the SIT test can be used effectively in this area, and IDEXX® ELISA can be applied as ancillary techniques. (Funding sources: Chiang Mai University and University of Minnesota, The National Bureau of Agricultural Commodity and Food Standards (ACFS); Conflict of interest: None)

Keywords: Bovine Tuberculosis, Diagnostic Tests, Bayesian Modeling, Dairy Cows, Thailand

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Reemergence of Rabies in Perlis, Malaysia 2015-2016

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**Purpose**
This report will provide the information about re-emerging rabies outbreak in Malaysia since the last cases in 1999. It will help to understand about rabies introduction, spread and also assessing the relationship between animal rabies and public health. All the information will develop the future control program to eradicate and preventing reemerging of rabies outbreak.

**Materials and Methods**
Data were obtained from Department of Veterinary Services State of Perlis and Department of Health State of Perlis. The data was collected from July 2015 until December 2016.

**Results**
During the observation period, 70 dog bite cases were reported for the year 2015 with 17 (24.28%) cases involving the rabid dog. In 2016, 65 dog bite cases reported with only one (1.53%) case bitten by rabid dog. For the surveillance programme in 2015, there were 57 samples collected with 14 (24.56%) samples tested positive. A total of 51 surveillance samples collected in 2016 with only two (3.92%) samples tested positive.

**Conclusion**
There are risks for spread of rabies from endemic neighbour country instead the disease had been free for certain period of time. Introduction of immune belt area since 1955 and well coordinated national rabies control program should be continuous to prevent and control rabies in Perlis, Malaysia.

**Keywords**: Rabies, Malaysia, Outbreak, Surveillance, Dog

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Wildlife Disease Risk Assessment for Korea

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Purpose
Korea has been impacted by emerging diseases of wildlife origin and is in the process of developing capacity in wildlife health science. We conducted a risk assessment to identify potential hazards from wildlife diseases, and determine priorities for disease surveillance, management, and research.

Materials and Methods
We followed the IUCN/OIE Guidelines for Wildlife Disease Risk Analysis, and methodologies used included retrospective analysis of the published literature, an online questionnaire survey, and a subject matter elicitation workshop.

Results
Diseases identified as high priority included rabies, severe fever thrombocytopenia syndrome, foot and mouth disease (FMD), and highly pathogenic avian influenza (HPAI). Foreign diseases that were assessed as high risk for introduction were Chikungunya virus, Dengue virus, Ebola virus, MERS-Coronavirus, African swine fever, FMD, and HPAI. We also identified a general lack of awareness of health threats to wildlife populations. The pathways for exposure of concern included human movement and travel, the illegal wildlife trade, and migration of wildlife. The risk factors for disease spread of concern included proximity of humans, livestock, and wildlife; high density of livestock populations; habitat loss and environmental degradation; and climate change.

Conclusion
Recommendations based on these results included initiation of wildlife morbidity and mortality surveillance, focusing active surveillance on migratory birds, coordination among the relevant Ministries (formation of an One Health Committee), management of the legal and illegal wildlife trade, development of risk communication tools, and focusing research on HPAI, and climate and land use change in relation to disease emergence.

Keywords: Korea, Questionnaire Survey, Risk Assessment, Wildlife Diseases

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Analysis of Insecticides in Dead Animals in Korea

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Purpose
Insecticides have been widely used in agricultural practices worldwide since the middle of the 20th century to prevent the colonization and feeding of insects on plants and to inhibit outbreaks of diseases carried by insects to humans and animals. In the case of animals, poisoning is often caused by accidental exposure. Direct insecticide poisoning also occurs in wild animals via ingestion of insecticide-contaminated water, seeds, and foliage. In this study, we investigated pesticides in the gastric contents of dead animals requested to the Animal and Plant Quarantine Agency in Korea in 2016.

Methods
We analyzed residual pesticides in the gastric contents from the dead animals which were suspected pesticide poisoning based on the necropsy. Pesticides analysis was done using gas chromatography with flame photometric detector and mass spectrometry.

Results
A total of 153 samples of 13 species were analyzed in this study and pesticides were determined in 11.1% of the total samples analyzed. Diazinon and phorate were the most common pesticides identified. Other organophosphates, organochlorines, and pesticides were also found in various concentrations from dead animals.

Conclusion
In this study, results indicate a poisoning status of animals in Korea and suggest that pesticides poisonings will continue to be a cause of death in some animals in Korea. More attention should be paid to pesticide poisoning and future efforts to reduce the number of pesticide-related deaths are needed to help preserve the animals.

Keywords: Insecticides, Dead Animal, Korea

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**Quantitative Determination of Flumethasone in Pig Kidney by QuEChERS-Based Extraction and LC-MS/MS**

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**Purpose**
Flumethasone is one of the most administered corticosteroids to treat inflammatory reactions in farm animals. Residues of corticosteroids in farm animals cause a public health risk since they may have pharmaceutical and toxicological effects for consumers. Therefore, we need to monitoring its residue. In addition, application of rapid and effective samples preparation process and analytical method are necessary. The purpose of this study aimed to establish the analysis method of residual flumethasone in pig kidney.

**Materials and Methods**
Kidney tissues were homogenized (10g) and spiked with flumethasone at the concentration 5ng/g (n=3). The modified QuEChERS extraction method was used for samples preparation of kidney tissues. Analysis was performed by Shimadzu Nexera LC interfaced to an ABsciex QTrap 6500 mass spectrometer (LC-MS/MS). The chromatographic column was a C18 (2.1 mm x 100 mm, 3.5μm. The source conditions were optimized to obtain two identification points (precursor: 411.3, product: 253.2, 121.2 m/z).

**Results**
Concentration response showed linearity within the concentration range (R² >0.999). Recoveries of flumethasone were between 97.7 to 103.84%. The coefficient of variation observed was 3.2%. The limit of quantification (LOQ) and detection (LOD) were 0.38 and 1.16ng/g with external standard curve.

**Conclusion**
The QuEChERS-based extraction method was applied to save time and effort in the samples preparation process of flumethasone analysis for pig meat samples. According to the obtained results, This method may be applied for the analysis in residues monitoring for flumethasone in pig kidney tissues.

**Keywords:** Flumethasone, QuEChERS, Residue

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Results of Monitoring and Surveillance Testing of Veterinary Drug Residues in Domestic Meats in 2014

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Purpose
The Korean National Residue Program (NRP) consists of three sampling plans for domestic foods of animal origin: monitoring, surveillance and exploratory testing. Monitoring and surveillance testing programs are routinely implemented by 17 Provincial Veterinary Service. The NRP for domestic residue testing serves as a control system for chemical residues and contaminants in foods of animal origin in Korea. In this study, we analyzed the results of monitoring and surveillance of veterinary drug and pesticide residues in 2014.

Materials and Methods
According to monitoring sampling plan, tissues from slaughtered animals were randomly collected at the slaughterhouse and sent to AHLs to test for veterinary drugs. Veterinary drug residues were analyzed using a bioassay for screening purposes and by LC-MS/MS for confirmation and quantification on the basis of the “Korean Food Standards Codex”. The results from monitoring and surveillance tests were collected and investigated annually according to animal species, class of veterinary drugs.

Results
Total 416 residue violations occurred mainly in pigs (344 violations) and cattle (67 violations). Major violated compounds were antimicrobials such as aminoglycosides, penicillins, tetracyclines, fluorquinolones. In pig violations came from mainly fluorquinolones and penicillins, while most residue violations in cattle resulted from aminoglycosides and penicillins. The overall rates of violations were 0.21% in 2014.

Conclusion
The results of KNRP provide information on agricultural chemical residues and it help the control and prevention of residue violations for the food safety.

Keywords: Korean National Residue Program (NRP), Veterinary Drug, Residue Violation

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Development of Rabies Inactivated Vaccine Using Bio-Molecule Expression Technique

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Purpose
Rabies is the most deadly zoonoses to humans and animals, so vaccination is essential to effectively control it. KOMIPHARM has developed a new concept of rabies killed vaccine (Rabies-Fc vaccine) by applying bio-molecule expression technique to current rabies inactivated vaccine.

Materials and Methods
In order to develop a Rabies-Fc inactivated vaccine, a retroviral vector system is used to create a new cell line of BHK21-Fc which is expressing Canine's IgG Fc molecules, and Rabies-Fc virus is developed using this line.

Results
The potency of the Rabies-Fc inactivated vaccine is confirmed by NIH test. It shows that not only suitability of international standard but also superior protection ability than current other Rabies inactivated vaccine. The immunogenicity test result on dogs shows that at least 0.5 IU/ml of neutralizing antibody is maintained more than 1 year after 1 time vaccination.

Conclusion
By applying the bio-molecule expression technique to current rabies-inactivated vaccines, the efficacy of the vaccine can be successfully improved.

Keywords: Vaccine, Fc Molecule, Rabies Virus

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Distribution of Extended-Spectrum Beta-Lactamase (ESBL)-Producing Bacteria Isolated from the Diseased Animal in Jeju, South Korea

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Purpose
The increases of clinical isolates producing ESBL are the most problem for infectious disease control. This work is conducted to investigate the ESBL-producing bacteria from the diseased animals and to identify the species of Gram-negative bacteria from clinical cases submitted to the Animal Teaching Hospital.

Materials and Methods
The bacteria were isolated from various with variety diseases from 2002 to 2015. The pathogens were identified by API kits. ESBL-producing bacteria were screened by antimicrobial double disk test. The specific genes of ESBL were identified by multiplex PCR.

Results
Of 96 bacterial agents, Escherichia coli were prominent as 61 isolates, and 25 different bacterial species were identified. E. coli was isolated from 20 intestinal specimens of cattle (9), dog (3), horse (7) and roe deer (1), founding in 5 skins, 3 ascites fluid, 2 respiratory, 5 urinary, 8 genital, 6 ear and 2 buccal cavity of dogs. ESBL-producing bacteria were found in 10 (10.4%) including 1 A. salmonicida from snake, 6 E. coli from cattle (1), dog (3), horse (3), 3 K pneumoniae from dog (1), dolphin (1), iguana (1). A. salmonicida and 3 E. coli had both blaTEM and blaCTX-M-9, 3 K. pneumoniae harbored blaCTX-M-9, and other 3 E. coli possessed blaTEM.

Conclusion
This study indicates that ESBL-producing bacteria were distributed in various animals since 2002 in Jeju. Our next concerns are that these ESBL-producing genes can transfer among bacterial strains.

This work was supported by IPET funded by MAFRA (2015300160).

Keywords: Extended-Spectrum Beta-Lactamase (ESBL)-Producing Bacteria, Animals, E. coli, K. Pneumoniae, Aeromonas

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Antimicrobial Characteristics of Livestock-Related
Enterococcus Faecalis and E. Faecium

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Purpose
Enterococci are gram-positive bacteria that mainly colonize on the mucosal surfaces of human and animals gut. Generally, E. faecalis is not pathogenic bacteria but in case of extraordinary infection, they represent virulence. Furthermore E. faecium belongs to group called ‘ESKAPE’ pathogens that represent paradigms of pathogenesis, transmission, and resistance. Therefore, it is necessary to monitor enterococci which can be easily found in the surrounding environment.

Materials and Methods
In this study, we collected 512 samples in Korea during 2015-2016. Isolates were screened for susceptibility to 12 antibiotics on Mueller-Hinton agar by disk diffusion method (CLSI). Polymerase chain reaction (PCR) was performed to investigate presence of antimicrobial resistance associated genes.

Results
In this study, 291 E. faecalis and E. faecium were isolated by culture based method and screened by PCR from 512 samples. Relative proportion of E. faecalis and E. faecium were 73.10% and 26.90%, respectively. Some isolates from cattle and pig sources had resistance against ampicillin and amoxicillin/clavulanate. Resistance rate against ciprofloxacin from chicken sources, 2.96 ~ 4.29 folds higher than isolates from cattle and pig sources. Among 227 E. faecalis, most of tetracycline resistant isolates had tetracycline resistance genes especially tet(M), tet(L), or both (111/131, 84.73%).

Conclusion
In conclusion, despite effort to reduce amount of the antibiotics usage, isolated enterococci in this study showed resistance against various antibiotics. To prevent spreading antimicrobial-resistant enterococci to human, thorough surveillance and continuous monitoring are needed.

Keywords: Enterococcus, Antimicrobial Resistance, Antimicrobial Resistance Gene, Livestock, Surveillance

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Evaluating the Feasibility of Porcine Reproductive and Respiratory Syndrome Risk Assessment Program Based on Scoring Systems

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Purpose
Porcine reproductive and respiratory syndrome (PRRS) is considered to be one of the most important diseases of swine in Korea and worldwide. The American Association of Swine Veterinarian developed a web-based program named by Production Animal Disease Risk Assessment Program (PADRAP) to evaluate the risk of PRRS at farm level. We have developed Korean version of PADRAP

Materials and Methods
The survey questionnaires composed of a list questions about risk factors for the occurrence of PRRS outbreaks. Random forest and group lasso method were used to estimate the relative importance for each question. Support vector machines were employed for evaluating the accuracy of prediction model. Dependent variable was whether the farm was PRRS positive or not. Independent variables were every single question in the process of importance estimation. Data from 130 farrow-to-finish farms were selected for analysis.

Results
The highest weight was assigned to the group 1 (the first rank), and ranks from the group 2 to 4 were given the weight in order. No weights were given in the group 5. Questions in group 6 were identified as unimportant.

Conclusion
This study indicated that periodic monitoring for risk factors with higher ranks coupled with biosecurity awareness should be practiced to reduce the risk of future PRRS outbreak.
This research was supported by "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012612)"*, Rural Development Administration, Korea.

Keywords: PRRS, Biosecurity, Risk Analysis

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Differential Identification of \textit{Brucella} Species Using Specific SNPs

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\textbf{Purpose}
There are several diagnostic techniques for brucellosis, but they are difficult to differentiate \textit{Brucella} species, especially in case of PCR, due to highly homologous DNA similarity. We here reported specific SNPs of \textit{Brucella} genus and 10 species for rapid identification.

\textbf{Materials and Methods}
Fifty-three (22 references, 4 vaccines and 27 isolated strains) \textit{Brucella} whole genome sequences (WGSs) were collected at the NCBI web site. Specific single nucleotide polymorphisms (SNPs) were analyzed to the CLC workbench software version 6.0. Specific sites were amplified about 200 bp size. SNPs in the amplified products were confirmed by sequence analysis via assembling and alignment.

\textbf{Results}
The \textit{Brucella} genus-specific SNPs were identified in BCSP31 gene. \textit{Brucella} species-specific and vaccine strain-specific SNPs were found in 9 (\textit{fbaA}, \textit{omp25}, \textit{gap}, \textit{entE}, \textit{RS00005}, \textit{glk}, \textit{clpX}, \textit{BAb} and \textit{rpsL}) genes. As a result of analysis in 23 references, 3 vaccines and 14 isolate strains, all species of \textit{Brucella} were distinguishable to specific SNPs and consistent with classical biotyping results.

\textbf{Conclusion}
The specific SNPs of 10 genes were able to distinguish the genus \textit{Brucella}, 10 species and 3 major vaccing strains. In the future, we will try to find SNPs that can discriminate biovar levels of \textit{Brucella} species.

\textbf{Keywords}: Brucella, SNP, Identification

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NIH Potency Test for Rv-Fc and Rv-K Based Candidate Anti-Rabies Vaccine Produced at Komipharm International Co., Ltd. Korea

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**Purpose**

Potency test for rabies vaccine is required to determine the vaccines potential to induce protective antibody response following vaccination. NIH potency test is the most widely used and internationally recommended potency assay for testing of inactivated rabies vaccines.

**Materials and Methods**

Komipharm International Co., Ltd. Korea has produced candidate anti-rabies vaccine from Rv-Fc strain expressing Fc region of Immunoglobulin G (IgG) originated from canine which enhance virus uptake by immune cells.

**Results**

This vaccine was tested for its potency according to NIH potency test protocol in the presence of Rv-K as a comparison, and 13.49 IU/ml and 3.16 IU/ml potency result obtained for Rv-Fc and Rv-K strain respectively. According to OIE recommendation for rabies vaccine, the potency should not be less than 1 IU/ml to be used for animal vaccination and both vaccines pass the requirement for animal vaccination. The modified rabies vaccine harboring canine Fc (Rv-Fc) has a high potency and can enhance antibody response after vaccination compared to PV strain based vaccine.

**Conclusion**

Therefore, these vaccines can be used for animal vaccination at lower cost as it has more than recommended potency value.

**Keywords:** Vaccine, Fc Molecule, Rabies Virus

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Antimicrobial Profiling of *Escherichia Coli* Strains Isolated from Farms, Slaughterhouses, and Retail Meats

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**Purpose**

*Escherichia coli* (*E. coli*) is particularly important pathogen because it is the most common gram-negative bacteria. Antibiotics are used in meat industry to prevent and control pathogenic bacteria. Antimicrobial resistance is of concern to *E. coli* because they can easily acquire antimicrobial resistance and transfer it to other microbes. This study was conducted to determine antibiotic susceptibility patterns and analyze resistance gene profiling of livestock-related *E. coli*.

**Materials and Methods**

In this study, 264 samples were collected from cattle farms, slaughterhouses, and retail meats in Korea during 2015-2016 and 197 *E. coli* were isolated. A standard disk-diffusion test was performed to determine antimicrobial susceptibility for the 17 antibiotics. The presence of antimicrobial resistance genes was verified by polymerase chain reaction (PCR).

**Results**

The highest percentage of drug resistance in *E. coli* isolates were detected from chicken meats (nalidixic acid 79%, ampicillin 72%, tetracycline 67% and ciprofloxacin 67%) followed by pig meats (ampicillin 72%, chloramphenicol 69%, and tetracycline 50%) and cow meats (ampicillin 24%, tetracycline 23%, and chloramphenicol 14%). A total of three (1.5%) extended spectrum β-lactamase (ESBL) producers were detected, one from cow feces and two from retail chicken meats. The antimicrobial resistance genes, *AmpC* (94%), *tetA* (39%), *tetB* (16%), and *floR* (31%) were detected.

**Conclusion**

Antimicrobial resistant *E. coli* isolated from meat production system might represent a significant threat to public health and therefore, constant surveillance of antimicrobial resistant *E. coli* would be required for controlling and preventing multidrug resistance.

**Keywords:** Livestock-Related *E. Coli*, Antimicrobial Resistance, Antibiotics

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Gut Microbiota of Dogs Fed Natural Diet and Commercial Feed

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Purpose
We investigated the change of gut microbiota of dogs according to the dietary difference, natural diet and commercial feed, moreover discussed the possibility of correlation between microbiota and health status in dogs.

Materials and Methods
A total of 11 dogs were randomly recruited from dog owner group who fed their dogs with natural diet (n=6) and veterinary college students who fed their dogs with commercial feed (n=5). All samples were divided into two different groups (natural diet group and commercial feed group) and their fecal metagenomic DNA samples were analyzed using the Illumina Miseq platform.

Results
There were significant differences in alpha diversity, beta diversity, and taxonomic composition of core gut microbiota between two groups. Each group was almost clustered together according to the diet type based on beta diversity. In alpha diversity, the microbiota of natural diet group had significantly higher richness estimates and diversity indices compared to the microbiota of commercial feed group. In addition, the differences in taxonomic composition between the two groups were identified. Particularly in species level Clostridium ramosum (p=0.004) was higher in commercial feed group, while Clostridium perfringens (p=0.017) and Fusobacterium varium (p=0.030) were higher in natural diet group compared to each group respectively.

Conclusion
Our study suggested that the composition of the gut microbiota was more likely to be affected by diet type compared to other individual factors. Therefore, dietary types could play a key role in health by changing the composition of the gut microbiota.

Keywords: Next Generation Sequencing, Microbiota, Natural Diet, Commercial Feed

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Risk Factors Associated with Highly Pathogenic Avian Influenza H5N8 Outbreaks on Broiler Duck Farms in Korea 2014-2015

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Purpose
Highly pathogenic avian influenza (HPAI) virus outbreaks have occurred 6 times in Korea, with 4 subtype H5N1, 1 subtype H5N6 and a HPAI H5N8 outbreaks since 2003. In this study, we conducted a retrospective matched case-control study in broiler duck farm to identify and evaluate potential risk factors for HPAI H5N8 occurrence in Korea.

Materials and Methods
Risk factors associated HPAI were investigate in previous studies and disease control plan of EU, USA and etc. Data was collected by a questionnaire which was consisting of 55 binary, multiple choice and short answer type question. 43 farms were selected as case group from broiler duck farms and control farms were selected which located within 0.5-1km from case farms. Univariate and multivariate logistic regression were conducted to identify possible risk factors.

Results
Four variables were identified as risk factors for the HPAI infection on the poultry farms by final multivariable logistic model: ‘Farms having more than 7 flocks’ (OR= 6.99, 95% CI 1.34-37.04), ‘Farm owner having ≥ 15 experience in poultry industry (OR=7.91, 95% CI 1.69-37.14), ‘Any poultry farms located within 500m of farms’ (OR= 6.30, 95% CI 1.08-36.93) and ‘No Disposal company for feces’(OR=27.78, 95% CI 3.89-198.80).

Conclusion
This is first case control study which were performed to identify the risk factors associated with H5N8 HPAI. We believe that this study highlights the importance of carcass and feces treatment and biosecurity reinforcement for poultry farms to prevent H5N outbreaks.

Keywords: Highly Pathogenic Avian Influenza, Korea, H5N8, Risk Factor, Case-Control Study

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Establishment of ADI and AOEL for Oxathiapiprolin Through Evaluation of Toxicological Data

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Purpose
Registration of pesticides in agriculture is needed to assess safety for protection of human and animal health through safety reference dose.

Materials and Methods
Toxicological evaluation and establishment of safety reference dose for Oxathiapiprolin was conducted with acute toxicity, short term (rats, mice, dogs), long term (rats, dogs), carcinogenicity (rats, mice), reproductive toxicity, teratogenicity, genetic toxicity and metabolism study.

Results
In metabolism study, oral absorption was about 31% in low dose and 5.4% in high dose. Main excretion route was feces. Category of Acute toxicity was 4. In 90-day dietary study for rats, mice, and dogs, there was no adverse effect in high dose then the lowest NOAEL (No Observed Adverse Effect Level) was 1096 mg/kg bw/day. In 1-year dogs study, there was no adverse effect in high dose then the lowest NOAEL was 1242.2 mg/kg bw/day. In chronic/carcinogenicity study, there was no neoplastic finding and no adverse effect, then the lowest NOAEL was 735.2 mg/kg bw/day. In reproductive toxicity study, there was delay of preputial separation in F2 male then the NOAEL was 104 mg/kg bw/day. In teratogenicity and genotoxic study, there was no teratogenic and genotoxic effect.

Conclusion
The lowest NOAEL was 104 mg/kg bw/day in reproductive toxicity study. The Korean ADI (Acceptable Daily Intake) was established as 1.04 mg/kg bw/day (safety factor 100). The most appropriate NOAEL among short term and teratogenicity studies was 1096 mg/kg bw/day in 90-day dog study then the Korean AOEL (Acceptable Operator Exposure Level) was established as 1.1 mg/kg bw/day (safety factor 100 and correction factor 0.1). ADI and AOEL of oxathiapiprolin were likely to be high among the safety reference dose of pesticides and toxicity was low. These could be used for dietary and occupational risk assessment as a safety reference dose for consumer and operator health protection then both of the risk was expected to be low through estimation of TER (Toxicity/Exposure ratio).

Keywords: ADI, AOEL, Oral Absorption, Oxathiapiprolin

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Histology and Histochemistry of Hedgehog (*Erinaceus Europaeus*) Adrenal Gland

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**Purpose**
The Hedgehog (*Erinaceus europaeus*) is a small mammal of the family Erinaceidae. More recently, as a laboratory and pet is a lot of attention. Many anatomical and histological characteristics of this animal organs are unknown yet. In this survey histological structure and histochemistry properties of hedgehog adrenal gland were studied.

**Materials and Methods**
Then samples passed in routine histological techniques and parafinized block were prepared. 7µ histological sections were stained by H&E, PAS and trichrome methods

**Results**
Results showed that hedgehog adrenal gland has relatively thick capsule that it composed from peripheral adipose tissue and a thin layer of dense irregular connective tissue with well-developed collagen (blue in trichrome stinging method) fibers. Inner layer of capsule was PAS positive and had a few smooth muscles cells. Hedgehog adrenal gland parenchyma was observed in an extensive network of fine reticular fibers and cortex and medulla were observed in it. Cortex composed from three cellular layers as glomerular, fascicular and reticular layers. Glomerular cell layer was PAS negative. Fascicular cells cytoplasm were spongiform because existence fat vacuoles, and a little PAS positive. Reticular cells were PAS negative too. Medulla composed from two type cells, PAS positive and PAS negative, PAS negative cells were surrounded by PAS positive Cells

**Conclusion**
Glomerolar and reticular layers cells were PAS negative, it was meaning they didn't have any glycoprotein in themes cytoplasm but fascicular cells were PAS positive, therefore their cytoplasm had glycoproteins molecules.

**Keywords**: Hedgehog, Adrenal, Histology, Histochemistry

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**Purpose**
The aim of the study was to quantitatively estimate the gene expression of the transcriptional factors (T-bet, STAT-1, STAT-4) and the cytokines (IL-2, IL-2, IFN-γ, TNF-α) involved in the activation and development of intestinal mucosa Th1 lymphocytes in clinically healthy baby alpacas from three age-groups of 1-8, 10-21 and 22-47 days-old, which were bred in flocks with natural grazing in the Southern Peruvian highlands.

**Materials and Methods**
Two centimeters-intestinal samples were obtained from the jejunum middle portion of the alpaca cria at IVITA Marangani (Cusco), which were stored at -196°C and processed at the School of Veterinary Medicine from San Marcos University (Lima, Peru). Total RNAs were extracted and RT-PCR real time was performed. The quantitative mRNA expression was estimated by comparing to the expression profiles in the calibrator (fetus) by the $2^{-\Delta\Delta CT}$ method using GAPDH as endogenous control.

**Results**
STAT-1, T-bet, IL-2 and TNF-α expression were shown increased with age in the eldest group, while IFN-γ expression exceed in a hundred-fold the calibrator’s ($p<0.05$). STAT-4 and IL-12 expression were not significant.

**Conclusion**
The expressions of both transcriptional factors activated by IFN-γ, T-bet ($p<0.05$) and STAT-1 ($p>0.05$), increased with age, likewise the cytokines involved in the Th1 immune response. Nevertheless, the participation of NK, cytotoxic and γδT cells cannot be excluded in IFN-γ production. The effect of intracellular parasites and commensal bacteria which promote the stimulation of DCs through TLRs activation and hence the production of IFN-γ might be considered.

**Keywords:** Gamma Interferon, Intestinal Mucosa, Alpaca
Immobilization of Asiatic Black Bears (*Ursus Thibetanus*) with Medetomidine-Zolazepam-Tiletamine in South Korea

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**Purpose**
The Asiatic black bear (*Ursus thibetanus*; ABB) is a globally endangered species, for which a restoration program has been ongoing in South Korea since 2001. However, there is little information on immobilization protocols for ABBs. We evaluated the use of medetomidine-zolazepam-tiletamine for their immobilization.

**Materials and Methods**
During 2005-13, we anesthetized 60 ABBs (32 males, 28 females; 7 mo to 12 yr old) with medetomidine 0.03-0.045 mg/kg and zolazepam-tiletamine 1.54-2.3 mg/kg, with atipamezole 0.15-0.225 mg/kg administered intravenously alone or intravenously and intramuscularly (50:50) for reversal of anesthesia.

**Results**
Mean (and SD) for physiologic collected for 373 immobilizations of at least 60 min were: time to sedation, 7.8 (5.4) min; anesthesia induction time, 13.7 (8.1) min; complete recovery time, 14.8 (12.4) min; respiratory rate, 14 (7) breaths/min; heart rate, 51 (16) beats/min; rectal temperature, 37.3 (1.3)°C hemoglobin oxygen saturation, 88% (6%).

**Conclusion**
Few cardiopulmonary side effects occurred during immobilization and adequate depth of anesthesia was maintained for >60 min without need for supplementation. The dosage and drug combination used was effective for immobilization of ABBs with minimal adverse effects on vital signs, and can be recommended in most clinical applications.

**Keywords**: Alpha-2 Agonist, Anesthesia, Asiatic Black Bear, Immobilization, Medetomidine

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Blood Chemistry Reference Intervals of Captive Asiatic Black Bears
(Ursus Thibetanus)

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Purpose
Blood chemistry values can provide fundamental data to understand physiological changes. However, there is little information about blood chemistry in Asiatic black bears (Ursus thibetanus). Thus, the objects of this study were to determine reference ranges for 29 blood chemistry variables, and to evaluate differences between age groups and between seasons.

Materials and Methods
Blood samples (n = 138) were collected from 44 (20 males, 24 female; age range, 1-15 years) clinically healthy, captive Asiatic black bears (Ursus thibetanus) in the Korea National Park Service, Korea. Samples were stored at 4°C and analyzed within 24 hours using a blood chemistry analyzer.

Results
Young and adult bears showed significantly higher levels of creatinine (CRE) and total cholesterol (TCHO), and lower levels of blood urea nitrogen (BUN), U/C (BUN/CRE) ratio, lactate dehydrogenase (LDH), and creatine kinase MB (CKMB) during hibernation compared to that during non-hibernation. During hibernation, the U/C ratio and levels of ALT, LDH, and CPK in young bears were significantly higher than that in adults, whereas creatinine levels were lower than that in adults. During non-hibernation, the U/C ratio and levels of calcium (Ca), ALP, LDH, CPK, and CKMB in young bears were significantly higher, whereas CRE, total protein (TP), albumin (ALB), gamma-glutamyl transferase (GGT) and haemoglobin (Hb) levels were lower than that in adults.

Conclusion
The results in this study provide reference values to understand the physiology of Asiatic black bears and assess the health of these animals in captive environments.

Keywords: Asiatic Black Bear, Blood Chemistry, Reference Ranges

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Chronic Renal Failure with Renal Medullary Fibrosis in a Siberian Tiger (Panthera Tigris Altaica)

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Purpose
Siberian tiger (Panthera tigris altaica) is now classified as one of severely endangered species by the International Union for Conservation of Nature. Here we report an extremely rare case of renal medullary fibrosis in a male Siberian tiger.

Materials and Methods
A 12-year-old male Siberian tiger began to exhibit depression, anorexia and weight loss with abnormally high level of BUN (>140mg/dl) and ammonia (101ug/dl) in the blood, suggesting severe renal failure and uremic condition. Finally, the tiger died with severe hematemesis 9 days later. Necropsy was performed at college of veterinary medicine, Kyungpook National University. Organ tissue samples were fixed in 10% neutral buffered formalin and processed routinely for histopathological observation.

Results
Grossly, the kidney showed white-yellowish lesion in the medulla and mild petechia in the cortex. Lung revealed multifocal hemorrhages and edema lesions. Stomach exhibited severe gastric hemorrhages as well as multiple mucosal ulcerations. Microscopically, marked renal fibrosis characterized by tubular atrophy, degeneration, necrosis, calcifications and chronic inflammatory cell infiltration was found in the medulla. The renal cortex showed moderate interstitial inflammation.

Conclusion
Present case was diagnosed as chronic renal failure accompanying uremic condition. To author’s knowledge, present case is the first case of chronic renal failure by renal medullary fibrosis in Panthera tigris altaica, although chronic renal disease is quite common in domestic felines.

Keywords: Chronic Renal Failure, Kidney, Uremia

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Hepatic Myelolipoma and Renal and Hepatic Amyloidosis in a Whooper Swan

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Purpose
The aim of this report is to discuss the hepatic myelolipoma and renal and hepatic amyloidosis in a whooper swan, which died following the short history of lethargy and ataxia.

Materials and Methods
An adult whooper swan showed symptoms of lethargy and ataxia. Physical examination and blood test were performed. Despite routine medical treatment, the animal's physical condition continued to deteriorate and finally died.

Results
Postmortem examination was performed by referring veterinarian and liver and kidneys were sent for histopathological interpretation. Grossly, liver was swollen and firm. The kidney was also bilaterally enlarged. On histopathology, amyloidosis was noted in both kidney and liver mainly involving the renal glomeruli and hepatic space of disse. In addition, neoplastic cells consisted of lipocytes and hematopoietic cells as well as marked osseous metaplasia was also noted throughout the liver.

Conclusion
Based on these results, the whooper swan was diagnosed as concurrent hepatic myelolipoma with osseous metaplasia and renal/hepatic amyloidosis. Studies indicate that chronic inflammatory reaction associated with bacterial infection play some role at least amyloidosis in avian species. Renal visceral gout was also noted. The brain was not observed microscopically so the exact cause of ataxia and lethargy was not clarified.

Keywords: Myelolipoma, Amyloidosis, Osseous Metaplasia, Liver, Whooper Swan

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A Survey of Marmoset Wasting Syndrome: Prevalence and Contributing Nutritional Factors in Wildlife Reserves Singapore

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Purpose
Marmoset Wasting Syndrome (MWS) has been described in captive callitrichids worldwide and results in significant morbidity and mortality. Two major clinical signs include chronic diarrhoea and weight loss. Exact aetiology is unknown although many causes have been postulated. This study aimed to investigate contributions of pathogenic bacteria, endoparasites and nutritional factors on MWS occurrence. Changes in clinical pathology of MWS suspects were compared to a group of non-diarrhoeic animals.

Materials and Methods
Twenty three dens, comprising fifty nine callitrichids, of eight species were studied. All animals from dens with abnormal results were anesthetized for health checks and testing. The fibre was increased for the entire callitrichid collection. Nutritional intake and fecal scores (1= firm/dry to 5= watery diarrhoea) were recorded before and after the diet change.

Results
Thirteen animals (22\%) were suspected to be affected with MWS. Five dens were positive for \textit{Campylobacter jejuni} but only three suspects tested positive. Compared to the control group, suspects presented with monocytosis (n=10), inverted calcium: phosphorus ratios (n=9), serum albumin <35 g/L (n=9), toxic neutrophils (n=5) and isolation of fecal \textit{Serratia} sp. (n=3). Neutral detergent fibre inversely affected fecal scoring whereas acid detergent fibre directly affected it; except in MWS suspects.

Conclusion
High fibre diet is encouraged in callitrichids to prevent occurrence of diarrhoea and avoid chronic intestinal inflammation associated with MWS. Lack of ultraviolet light and proximity to other family groups may be associated with higher stress and development of MWS.

Keywords: Tamarins, Fecal, Colitis, Diarrhoea, Fibre

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Experimental Oral Transmission of Chronic Wasting Disease to Sika Deer (Cervus nippon)

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Purpose
Chronic wasting disease (CWD) was introduced into Korea through the importation of a CWD-infected elk from Canada. Additional cases of CWD were subsequently detected in Korean elk, as well as other cervid species including farmed Sika deer. Farmed and feral Sika deer populations exist in other regions of Asia, North America and Europe, although natural transmission to this species has not yet been documented outside of Korea. Understanding the pathogenesis of CWD in Sika deer is important for the development of diagnostic and disease control strategies.

Materials and Methods
Six Sika deer were orally inoculated with a brain homogenate prepared from a farmed Canadian elk with clinical CWD. Deer were euthanized due to intercurrent disease or following the development of terminal CWD. An array of tissues were collected and stored frozen or in formalin and were tested for the presence of PrP CWD by ELISA, western blot and/or immunohistochemistry.

Results
Primary oral transmission of CWD from elk to Sika deer occurred in all inoculated animals, and was detected by routine diagnostic tests. Two deer were removed at 3.9 and 10.9 mpi due to intercurrent disease. At 3.9 mpi, PrP CWD was restricted to the RPLN and tonsil. The remaining four deer progressed to clinical disease within a mean of 22.2 mpi. Clinical signs were similar to other cervids with CWD (progressive ataxia, bruxism, tremors, sialorrhea).

Conclusion
Widespread detection of PrP CWD by IHC suggests that, similar to other cervid species, infectivity is distributed throughout a wide range of tissues in sika deer with clinical CWD.

Keywords: CWD, Sika Deer, Oral Transmission

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Cardiogenic Shock in a Harbor Seal (*Phoca Vitulina*)

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Purpose
Lethargy and loss of appetite were observed in a Ten years old, female, harbor Seal after transferring into a new aquarium. Death occurred 2 days after commencing of signs. Carcass was submitted to the department of Veterinary pathology, Kyungpook National University for determining of the cause of death.

Materials and Methods
Necropsy with detailed examination of organs was performed. Samples from multiple organs fixed in 10% neutral formalin, were taken for histopathological examination. Blood samples in heparin were taken for detection of poisons.

Results
Upon gross examination, generalized congestion of multiple organs was observed. The lungs were congested, showing hemorrhage and typical bronchopneumonia. Hemopericardium was a prominent finding. Wide areas of congestion were identified on the surface of the liver, with bleeding from cut sections. Congestion was noted in the kidneys, spleen, brain, small intestines, and lymph nodes. Stagnation of blood circulation caused red blood cells to escape from blood circulation that was presented by petechiae in mucosa of stomach, hematuria, and melena. Congestion of the previously mentioned organs was noted on histopathological examination. Fragmentation of cardiac muscle fibers was also noted. Fat droplets were observed in hepatocytes due to poor oxygen supply. Depleted lymphoid follicles in the spleen and lymph nodes were replaced by plasma cells and lymphocytes. Renal impairment was identified by coagulative necrosis of the proximal and distal convoluted tubules.

Conclusion
Circulatory failure due to cardiac shock was the cause of animal death. This research was supported by a grant of the Korea Health Technology R&D Project (HI15C0001).

Keywords: Cardiac Tamponade, Blood Stagnation, Hemorrhage, Congestion, Shock

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Complementary Animal Health and Welfare Role of Veterinary Para-Professionals and Occupations

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Purpose
The veterinary profession began as a response to the devastating effects of Rinderpest in Europe in the 18th Century that led to the founding of a veterinary school to train personnel to control animal disease in Lyons in 1762. However, it took 250 years later that Rinderpest could be eradicated, thanks to the application of Participatory Disease Surveillance impacts efforts especially in Africa and Asia. The presentation highlights the complementary Animal Health and Animal Welfare role of Para-professionals and non-professional occupations to Veterinarians.

Materials and Methods
Review of relevant publications, as well as Case Studies research in Nigeria.

Results
Three occupations and Para-professionals that complemented the Veterinary Profession in the eradication of Rinderpest were Veterinary Technicians/Technologists VTs, Community-Based Animal Health Workers CABHWs and Primary Animal Health Care handlers PAHCHs. The FAO, OIE and AU-IBAR recognize their relevance. Despite their acknowledged roles, veterinarians in many parts of the world including Nigeria, refer to these cadres as ‘quacks’.

Conclusion
Veterinarians do not have exclusive prerogative on animal health and related issues. VTs, CABHWs and PAHCHs are frontline complements and not substitutes for professional level services. They are a need for when and where the existing veterinary service cannot be extended because of financial, geographic, policy and technical problems. Veterinary associations worldwide should recognize and utilize their complementary animal health roles, without professional bias of regarding them as quacks; in the interest of the Veterinary Profession and its diverse contribution to Animal Health, Human Health and Public Health.

Keywords: Veterinary Medicine, Para-Professionals, Complementary Role

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Under the Skin: Donkeys in Crisis

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Purpose
To explore the global breadth of the emerging donkey hide trade, the consequences for animal welfare and for livelihoods of the people who depend on them.

Materials and Methods
Literature review and interviews of key animal welfare partners around the world.

Results
Increased levels of personal wealth in China is fuelling demand for luxury products including ejiao, a Traditional Chinese Medicine ingredient made using donkey hides. Demand is conservatively estimated at 4 million donkeys per year; the global donkey population estimate is 44 million. China’s donkey population has nearly halved in the last 20 years suggesting a highly unsustainable trade, and entrepreneurs are now looking worldwide to satisfy a growing demand.

Despite their essential role in livelihoods and community resilience donkeys are largely invisible in livestock policies, livelihoods and humanitarian projects. Donkeys are frequently stolen from owners across Africa and illegally slaughtered in the bush. Only the skins are removed and carcasses left to rot. Elsewhere, donkeys are bought at less than current market value and are transported in inhumane conditions to recently built slaughterhouses. In the short term donkey owners are facing their livelihood being stolen and donkey prices that have increased up to tenfold within a few years leaving them without the means to replace animals they depended on. Like the donkeys themselves, the trade is largely invisible.

Conclusion
This demand risks the welfare of donkeys, the communities who live with them, and, within a few decades, perhaps the species as a whole.

Keywords: Donkeys, Livelihoods, Welfare

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Relationship Between Coat Colour and Stress Reactions in UK Shelter Cats

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Purpose
Stress in shelter cats can deteriorate their welfare and rehoming prospects. This study examines the relationship between coat colour and stress in cats in two UK rehoming shelters.

Materials and Methods
The shelters sampled had low cat population densities and provided enrichment. Seventy-five cats were studied using composite measures of stress including Scan Sampling behaviour, location, food consumption, elimination, and Cat Stress Score (CSS). Maximal six observations per cat were recorded. Coat colour was categorised as black, black/white, ginger, tabby, calico and white. Age, sex, neuter status, admission status, length of stay, and outcome were obtained from shelter databases. Anderson-Darling tests showed non-normal distribution so Kruskal-Wallis tests were used.

Results
No significant differences in stress levels were found between cats of different coat colours. The CSS range was lower than in other studies, with an overall median of 2.24, classified as Weakly Relaxed. Scan sampling scores also indicated low stress. These cats were more active and vocal than in previous studies. Up to 40% of the cats had low stress body scores while meowing.

Conclusion
Reduced stress in low-density catteries probably contributed to lack of significant difference in the demographics, and to elevated activity levels and vocalizations. Although meowing is classified as a sign of stress in the CSS, these data show significant percentages of cats meowing with low stress body postures. Further research is needed to compare these results with high-density catteries and to further investigate vocalizations in relation to stress levels.

Keywords: Domestic Cats, Stress, Shelters

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Stray Dog Population Management with Animal Welfare Perspectives in a Local Authority Area

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Purpose
Mass culling was the method practiced to manage stray population to overcome public health issues. Objectives were to survey the baseline age group structure of population and to assess the impact of innovation, controlling fecundity of population through catch-neuter-release (CNR) method to manage population turnover (PT) instead mass culling.

Materials and Methods
8 of 29 wards were selected randomly as samples for surveillance in the local authority area. Maps were utilized to identify the boundaries and to cover entire routes in the selected wards. Counting and data collection were done using standard protocol on same time of the days. Number of pups and lactating females were used as indicators to assess the impact of innovation. Surveillance was repeated exactly the same way after 3 years. CNR method was applied for females over 3 months and notched ear for identification.

Results
The baseline of the age group structure was 65%, 29% and 6% for adults, Juveniles and pups respectively in 2013 and for lactating females was 16% out of 821 in 2013. 300, 275 and 200 females were CNR in 2013, 2014 and 2015 respectively. After 3 years, age group structure was 75%, 22% and 4% for adults, Juveniles and pups respectively. Lactating females were 1% out of 794 in 2015.

Conclusion
An innovation with social and welfare perspectives are important to bring down fecundity of animals. CNR could make an effective impact to control fecundity and thereby to manage PT rather than culling.

Keywords: Stray Dog, Population, Fecundity

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Community-Based Feral Cat Control Program: 
Results of the First Ten Years and the Local Volunteer Support

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Purpose
In Taito City, Japan, protection and feeding of feral cats had long been a common practice among residents for controlling rodents. This tradition, however, led to a growing number of feral cats and associated problems, and the city introduced a community-based feral cat control program in 2005. The purpose of the study is to describe and evaluate the results of the first 10 years of the program.

Materials and Methods
The feral cat control program is based on capturing, neutering and then returning cats to the community, where local volunteers care for them through hygienic, appropriate feeding and cleaning up feces on a regular basis. Workshops were held every year to train these volunteers. In addition, a financial assistance program started in 2008 to promote neutering. An organizational support program was also introduced in 2012 to encourage neighborhood associations and commercial districts to participate in this community-based effort.

Results
The number of feral cat-related complaints and the number of dead cats found on streets both decreased over the ten years. The community-based program was effective for the management of feral cats and successfully reduced their population.

Conclusion
However, this program takes time to obtain results, long-term support for volunteers from the local government is essential. We also found that financial assistance and, more importantly, motivational support for volunteers were both vital to the program. This community- and volunteer-based approach may also be useful for addressing issues related to cat owners.

Keywords: Community-Based, Feral Cat Control Program, Local Volunteer Support

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Stress-Related Cortisol Level Change of the Dogs During an Animal-Assisted Activity in Vulnerable Group Children

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Purpose
Although interest in the contribution of AAA to human welfare in Korea is increasing, AAA is lacking in research on welfare of participating animals. In this study, we measured the change of stress level in participating dog during the AAA for vulnerable group children with salivary cortisol hormone.

Materials and Methods
The eleven dogs were participating in the AAA supported by Seoul city from October 14 to November 3, 2016 were investigated. The Salivary cortisol samples collected, from before, during, and at the end of each activity were measured using Competitive Enzyme Immunoassay.

Results
In the samples obtained from 7 dogs and 52 sessions, sufficient saliva were obtained for cortisol measurement. The medians of total cortisol values were 0.28, 0.263, and 0.255 ㎍/dL at 0, 60 and 120 minutes after the start of AAA activity, respectively. There showed a trend which cortisol increased after 60 minutes of activity and slightly decreased by 120 minutes. But no statistically significant changes were not noticed.

Conclusion
The study has shown that dogs participating in the AAA for children at a vulnerable group have some change in stress-related cortisol levels during activity. Long-term observation and evaluation of behavior, another criterion of stress, and additional investigations with a control group in daily life seem to be needed to obtain more accurate results.

Keywords: Animal Welfare, Animal-Assisted Activity, Stress, Cortisol, Noninvasive Measurement

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The Effects of Implementing a Feral Cat Trap-Neuter-Return Program in a Taitung County, Taiwan

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Purpose
To evaluate 2 different kind of townships in Taitung county to do trap-neuter-return (TNR) programs for stray cat population management via mathematical modeling.

Materials and Methods
Stray cats assessed from 2008 to 2016 in Chihshang township, Taitung (n = 812), and from 2008 to 2016 in Lanyu township, Taitung (n=109). The result of the TNR programs of the stray cats and modifications to the dynamics was assessed with an elasticity analysis.

Results
In both townships, results of analyses did not indicate a consistent reduction in per capita growth, the population multiplier, or the proportion of female cats that were pregnant.

Conclusion
Stray cat populations have a high intrinsic growth rate, and TNR programs are estimated to be effective at reducing cat populations. Results may be used to suggest monitoring and modification of TNR programs, which could result in useful controlling and reducing stray cat populations.

Keywords: Stray Cat, Trap-Neuter-Return (TNR) Programs, Chihshang Township, Lanyu Township, Taitung County

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Engaging Members in Contemporary Animal Welfare Issues: World Veterinary Association Fact Sheets

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Purpose
The World Veterinary Association (WVA) sought to engage its members with information about contemporary animal welfare issues to compliment other projects undertaken by the association and to keep these important global welfare issues at the forefront of discussion. Recognizing that English is not the first language of many WVA members, the Animal Welfare Working Group developed a new approach to messaging through development of a template to produce short pithy factsheets in plain language.

Materials and Methods
The template describes an outline for a one-page overview on the animal welfare topic of interest. The approach is to provide compelling facts and information that members can use in their own discussions with regional governments, other WVA members, and members of the lay public. The factsheets are supported by key references and regulatory guidance documents, where available.

Results
The WVA Animal Welfare Working Group has produced three factsheets to date dealing with: Stray and Unowned Dogs, Welfare Issues of Working Equids, and Welfare Issues Associated with Food Animal Transportation, with several others planned or under development. Factsheets are disseminated to members via the WVA website and various social networking venues along with regional member distribution. Factsheets also can be printed in hard copy and distributed by member associations.

Conclusion
Member response to factsheets has been positive, suggesting that the factsheets provide quick and available talking points on key animal welfare issues. The WVA Animal Welfare Working Group will continue to work with its member associations to produce additional factsheets of relevance.

Keywords: Animal Welfare, WVA, Communication, Fact Sheet, Membership

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Pilot Project of the High-Quality, High-Volume Spay-Neuter Clinic Model for Community Cats in Seoul

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Purpose
To improve the trap-neuter-return programs for population control of community cats the Seoul city government and the Humane Veterinarians (HV) piloted a high-quality, high-volume spay-neuter (HQHVSN) clinic model on Feb, 2017.

Materials and Methods
The Humane Veterinarians modified the 11-stage HQHVSN clinic model from Operation Catnip in Gainesville, Florida into the 7-stage model. The 7 teams of trained volunteers including 12 veterinarians, 14 veterinary students, and 4 veterinary technicians were assigned to each station, with the cats being transferred from station to station to maximize the efficiency.

Results
A total of 43 community cats (19 females and 24 males) were sterilized and returned safely. The time required from anesthesia to recovery was 41 and 18 minutes for individual females and males. The whole procedures for the 43 cats took 4 hours and 54 minutes (7 mins per cat, approximately 21 mins saved comparing to the sum of separated procedures).

Conclusion
The pilot project proved the effectiveness of the HQHVSN model in community cat population control with shortened procedure time and no complication. For more successful implementation of the HQHVSN model the capacity building of participants through the systemic pre-education for volunteers is needed. This TNR program was supported by the Animal Protection Department of Seoul City and Eunpyeong-gu community cat care takers group, and carried out by the Humane Veterinarians.

Keywords: Community Cat, TNR, High-Quality High-Volume Spay-Neuter

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Effectiveness of Shoe Covers and Hair Caps for Bioexclusion within Animal Holding Rooms in a Research Facility

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Purpose
Department 3 at the Biological Resource Centre, Singapore, is a specific pathogen free rodent facility using individually ventilated cages. Personnel entering are required to remove street clothes, and wear scrub-suits, dedicated facility shoes, hair caps and shoe covers before passing through an air-shower. Additional personal protective equipment (PPE) (hair cap, shoe covers, gloves, face mask, gown) are donned when entering animal rooms. The additional hair cap and shoe covers were thought to be for bioexclusion purposes, but there is a dearth of scientific evidence supporting this practice. It was thus proposed to re-evaluate the necessity of this extra PPE.

Materials and Methods
A case-control study design was used. Consenting husbandry personnel (n=18) were divided into 3 groups. Each participant randomly picked a task (e.g. cage changing) and performed it twice, with and without the extra PPE, hence acting as their own control. Contamination powder that fluoresces under UV-A light was scattered at the animal room entrance and used to track dispersal patterns with a scoring system. Means were analysed using the paired T-test.

Results
There was no significant difference between the amount of contamination on participants’ bodies whether or not extra PPE were used, before tasks were performed. Before leaving the room, participants who wore extra PPE were significantly more contaminated. There was no contamination found in any mouse cage.

Conclusion
Extra shoe covers and hair caps used within animal rooms is not effective for bioexclusion. Personnel wearing additional shoe covers have an increased risk of contaminating their gloves, which may compromise bioexclusion.

Keywords: Shoe Covers, Hair Caps, Bioexclusion

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Complex Adnexal Tumor with Sebaceous and Apocrine Differentiation in a Nulliparous Guinea Pig

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Purpose
In the case, ‘Complex adnexal tumor with sebaceous and apocrine differentiation’ in a nulliparous guinea pig was defined as clinically and pathomorphologically.

Materials and Methods
A-3-year-old, female, nulliparous guinea pig was brought to with suspected of mammary tumor to Department of Obstetrics and Gynecology. After surgery, the mass was sent to Department of Pathology. Then it was fixed in formalin 10%, processed routinely, embedded in paraffin. Paraffin blocks were sectioned at 5μ and slides were stained with hematoxylin-eosin (H&E) and Masson's Trichrome stains.

Results
The mass weighed 36 g and had with dimensions of 25x15x10 mm, soft consistency, homogeneous whitish color on the cut-section. Histopathologically, neoplastic epithelial cells with varying size and shape were noted. They usually had no nucleoli and normocromatic, rounded, oval nuclei. They have with no definite cytoplasm boundaries. Also, myoepithelial cells, had numerous hyperchromatic nuclei, were seen. Sebocytes in the center had small hyperchromatic nuclei and light pink cytoplasm containing vacuoles. Ectatic single-layered tubular glands and ducts were filled with secretory material. In addition, edema around the tubules, inflammatory infiltration of eosinophil leukocytes and plasma cells and hyperemia were noted. Therefore, the case was diagnosed as ‘Complex adnexal tumor with sebaceous and apocrine differentiation’.

Conclusion
This tumor firstly was described by Allison and Moeller (1993) at veterinary literatures and it is seen rarely. On the other hand, the tumor was firstly described at a guinea pig in our country.

Keywords: Complex Adnexal Tumor, Guinea Pig, Sebaceous and Apocrine Differentiation

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TSG-6 Secreted by Human Adipose Tissue-Derived Mesenchymal Stem Cells Reduces Inflammatory Bowel Disease by Inducing M2 Macrophage Polarization in Mice

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Purpose
Previous studies revealed that mesenchymal stem cells (MSCs) alleviate inflammatory bowel disease (IBD) by altering macrophages and modulating inflammatory cytokines in inflamed intestine. However, the mechanisms underlying these effects are not completely understood. We sought to investigate the therapeutic effects of human adipose tissue-derived (hAT)-MSCs in an IBD mouse model and to explore the mechanisms of the regulation of inflammation.

Materials and Methods
Dextran sulfate sodium-induced colitis mice were infused with hAT-MSCs intraperitoneally (1×10⁷ cells/kg) and colon tissues were collected on day 10 for histopathological, quantitative real-time PCR, and immunocytochemical analyses.

Results
hAT-MSCs were shown to induce the expression of M2 macrophage markers and to regulate the expression of pro- and anti-inflammatory cytokines in the colon. Quantitative real time-PCR analyses demonstrated that less than 20 hAT-MSCs, 0.001% of all systemically injected hAT-MSCs, were detected in the inflamed colon. To investigate the effects of hAT-MSC-secreted factors in vitro, transwell co-culture system was used, demonstrating that tumour necrosis factor-α-induced gene/protein 6 (TSG-6) released by hAT-MSCs induces M2 macrophages. In vivo, hAT-MSCs transfected with TSG-6 small interfering RNA, administered systemically, were not able to induce M2 macrophage phenotype switch in the inflamed colon and had no significant effects on IBD severity.

Conclusion
In conclusion, hAT-MSC-produced TSG-6 can ameliorate IBD by inducing M2 macrophage switch in mice.

Keywords: Inflammatory Bowel Disease, Mesenchymal Stem Cell, Anti-Inflammation

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Effect of Human Adipose Tissue-Derived Mesenchymal Stem Cells on L-Arginine Induced Severe Acute Pancreatitis Mouse Models

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Purpose
Severe acute pancreatitis (SAP), a common necroinflammatory disease initiated by the premature activation of digestive enzymes within the pancreatic acinar cells. The use of L-arginine-induced SAP models is nowadays becoming increasingly popular in mice. However, the method has low effectiveness rates in mice compared with rats. The aim of the present study investigated whether human adipose-derived mesenchymal stem cells (hATMSCs), have potential therapeutic effects in newly L-arginine-induced SAP models.

Materials and Methods
SAP models were induced by four times intraperitoneal injections of L-arginine (2.5g/kg) at 1h intervals. hATMSCs (2x10⁷/kg) were infused on 1h after last L-arginine injection via intraperitoneal. The models were sacrificed three days after infusion of hATMSCs. Subsequently, the level of pro-inflammatory cytokines, stem cells migration effects and histopathologic scores were evaluated in pancreas tissues.

Results
Infused hATMSCs more migrated to pancreas tissues in the therapeutic group than those in the sham group, and ameliorated acinar cell necrosis, pancreatic edema, and inflammatory infiltration. Also, infused hATMSCs decreased the level of pro-inflammation cytokines such as TNF-a and IL-1β.

Conclusion
These findings indicate that hATMSCs have a therapeutic effect on L-arginine-induced severe acute pancreatitis mouse models.

Keywords: L-Arginine, Acute Pancreatitis, Mesenchymal Stem Cells

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Therapeutic Potential of Ultraviolet Blood Irradiation in Diabetic Animal Model

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Purpose
The purpose is to evaluate the effects of ultraviolet blood irradiation (UBI) when a low dose of ultraviolet C (UV-C) is directly irradiated to the blood of diabetic rabbit model.

Materials and Methods
Type 1 diabetes was induced by alloxan monohydrate in New Zealand white rabbits. 10 ml blood with UBI by UV-C (4 W intensity, 260 nm wavelength) was autotransfused back 8 times (every week). We used Nova Stat Profile 8 CRT for measuring blood pH, K⁺, Mg²⁺, Ca²⁺, anion gap (AG), and osmolality (Osm). We used Hitachi 2070 for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine (CRE), blood urea nitrogen (BUN), uric acid (UA), total cholesterol (T-CHO), low-density lipoprotein (LDL), triglyceride (TG), high-density lipoprotein (HDL), total protein (T-PRO), and albumin.

Results
After UBI treatment, blood glucose, pH, ALT, AST, LDH, CRE, BUN, UA, T-CHO, LDL, TG, K⁺, AG, and Osm were reduced significantly. On the other hand, body weight, HDL, T-PRO, albumin, Mg²⁺ and Ca²⁺ were increased significantly.

Conclusion
The UBI treatment could have therapeutic potential to alleviate the diabetes.

Keywords: Diabetes, Ultraviolet Blood Irradiation, Therapeutic Potential

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Resolution of Liver Fibrosis Using Decellularized Liver Extracellular Matrix Extract in Mice

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Purpose
To develop antifibrotic therapy for resolution the liver fibrosis using thioacetamide-induced hepatic fibrosis in mice. Liver fibrosis is the excessive accumulation of extracellular matrix (ECM) proteins that distorts the hepatic architecture. Advanced liver fibrosis results in cirrhosis and liver failure that often requires liver transplantation. Decellularized liver tissue contains different structural proteins with growth factors that mimic the natural hepatic environment. We hypothesize that decellularized ECM extract can replace the necrotic hepatocytes and damaged ECM.

Materials and Methods
Mouse liver was decellularized and processed to form a hepatic matrix extract. We tested the ability of the matrix to enhance the migration of hepatocytes and endothelial cells. Then, the matrix gel was injected into liver parenchyma in mouse after induction of liver fibrosis using thioacetamide.

Results
The resulting liver ECM maintained a complex composition, including glycosaminoglycan, collagen, elastin and growth factors content. Hepatocytes and endothelial cells were shown to migrate towards the liver matrix in vitro. The matrix was delivered successfully in a minimally invasive procedure. The results of in vivo tests showed that the matrix gel was able to enhance the neovascularization and cell migration toward the fibrotic regions.

Conclusion
We have demonstrated that liver matrix gel could be utilized as an injectable scaffold for liver tissue engineering to promote neovascularization and reduce the fibrosis degree. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ0110022015)", Rural Development Administration, Korea.

Keywords: Fibrosis, Decellularization, Liver

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Attenuation Values in Computed Tomography of the Abdomen in Healthy Cynomolgus Monkey (Macaca Fascicularis)

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Purpose
Computed tomography (CT) estimates the density of the tissue from the calculation of the attenuation coefficient of the X-ray beam which passes the target tissue. Herein, attenuation values of the abdomen were measured from CT images in cynomolgus monkey (Macaca fascicularis).

Materials and Methods
Abdominal CT images of healthy cynomolgus monkey, weighing 2.5 to 6.4 kg, obtained between July, 2016 and December, 2016 at Korea Institute of Toxicology were reviewed. In pre- and post-contrast (iohexol, 600 mgI/kg) CT images, attenuation values of liver, spleen, kidney, pancreas and lumbar vertebra were measured.

Results
In total 51 monkeys, 51 pre- and 44 post-CT images were reviewed. In pre-contrast CT study, attenuation values were as follows: liver 59.48 ±4.94 (48.27-73.27), spleen 55.53 ±2.44 (50.30-60.00), renal cortex 40.03 ±4.57 (30.20-54.70), renal medulla 40.87 ±3.76 (34.70-50.00), pancreas 56.26 ±6.76 (35.37-67.84), cancellous bone 346.62 ±62.68 (218.00-496.50), cortical bone 1546.42 ±136.36 (1022.10-1779.70). In enhanced images, CT attenuation values were as follows: liver 130.34 ±17.79 (97.26-184.69), spleen 140.82 ±27.91 (95.19-210.70), renal cortex 236.82 ±39.61 (169.21-335.97), renal medulla 281.57 ±56.12 (160.86-414.36) and pancreas 112.01 ±17.12 (80.88-177.66).

Conclusion
CT attenuation values had no difference regardless body weights. This study presented CT attenuation value of abdomen in healthy monkey and can be used as basic information for in biomedical and toxicological research.

Keywords: Attenuation Value, Cynomolgus Monkey, Computed Tomography

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In Search for Biomarkers in Different Stages of Early Osteoarthritis in a Rabbit Model

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Purpose
The objective of this study is to identify protein biomarkers from synovial fluid in early onset of osteoarthritis. The outcome will help in understanding the pathogenesis of early osteoarthritis.

Materials and Methods
Twenty adult New Zealand white rabbits (n=20) were divided into Control group (n=5) and experimental group (n=15) which is subdivided into Week 4 (n=5), Week 8 (n=5), and Week 12 (n=5) groups. The rabbits from experimental group were induced using monoiodoacetate into right stifle joint. At the end each time-points, the rabbits were euthanized. Synovial fluid from the right stifle joint was collected for proteomic analysis via two dimensional gel electrophoresis and MALDI TOF/TOF. The right stifle joint were harvested and subjected to micro-CT and histology analysis as gold standard to stage the osteoarthritis. Statistical analysis from micro-CT data was performed using ANOVA and the histology slides will be qualitatively graded via OARSI Cartilage Histopathology Grading/Staging System.

Results
For proteomic analysis, eleven proteins (ceruloplasmin, fibrinogen, serotransferrin, serum albumin precursor, serpin peptidase inhibitor, α-1-antiproteinase F precursor, haptoglobin precursor, complement 3a, apolipoprotein-IV precursor, immunoglobulin lambda chain C region, immunoglobulin gamma heavy chain) had significantly different expression (>2.0 fold) between synovial fluid samples obtained from the control group and the experimental groups. Changes in parameters of subchondral bone micro-architecture and articular cartilage showed early onset of osteoarthritis and demonstrated different degree of severity of osteoarthritis at different time points.

Conclusion
Findings suggested identification of proteins involved is crucial for future biomarker studies.

Keywords: Osteoarthritis, Biomarkers, Proteomics, Subchondral Bone, Articular Cartilage

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**Beta-Glucan Alleviates Septic Shock Through Attenuating Non-Canonical Inflammasome**

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**Purpose**

β-glucans, which have a long history as non-specific immunomodulators, are heterogeneous polysaccharides of glucose polymers. β-glucans can enhance the functional activity of macrophages as well as the antimicrobial activities of neutrophils and mononuclear cells. In this study, we determined the effect of lentinan, a β-glucan extracted from shiitake (*Lentinula edodes*), on inflammasome activation, a multi-protein platform, in myeloid cells.

**Materials and Methods**

For bone marrow-derived macrophages (BMDMs), bone marrow cells were obtained by flushing tibia and femur bones from C57BL/6 mice and cultured in DMEM supplemented with 10% FBS in the presence of L929 cell-conditioned medium containing granulocyte/macrophage colony-stimulating factor. BMDMs (1.0 x 10⁶ cells per well) were plated in 12-well plates and treated with 10 mg/mL of LPS in RPMI 1640 containing 10% FBS and antibiotics for 3 h. After LPS priming, PBMCs or BMDMs were subjected to various inflammasome triggers. Cellular supernatant was transferred into a new tube, and the remaining PBMCs and BMDMs

**Results**

Lentinan selectively inhibited absent in melanoma 2 (AIM2) inflammasome activation. In addition, lentinan up-regulated pro-inflammatory cytokines and induced expression of inflammasome-related genes through toll-like receptor 4 signaling. Furthermore, we assessed the effect of lentinan on mice treated with *Listeria monocytogenes* or lipopolysaccharide as an AIM2 or non-canonical inflammasome-mediated model. Lentinan attenuated IL-1β secretion resulting from *Listeria*-mediated AIM2 inflammasome activation and reduced endotoxin lethality via inhibition of non-canonical inflammasome activation.

**Conclusion**

Lentinan is suggested as an anti-AIM2 and anti-non-canonical inflammasome candidate despite its up-regulation of cytokine expression.

**Keywords**: Glucans, Inflammasome, Interleukin-1beta, Macrophages

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Computational Bioinformatics Modeling of Quorum Sensing Inhibition

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Purpose
This study determined the quorum sensing inhibition properties of selected medicinal plants and using computational bioninformatics, determined the predicted molecular interaction of the component of the plants with the targeted bacterial quorum sensing molecule synthase enzyme.

Materials and Methods
Selected traditional herbal medicinal extracts were tested for their ability to modulate quorum sensing using Chromobacterium violaceum and Agrobacterium tumefaciens biosensor assay. The following medicinal plants were tested for their quorum sensing inhibition properties: Allium sativum, Allium schoenoprasum, Aloe vera, Artemisia dracunculus, Benincasa hispida, Carica papaya, Chrysanthemum indicum, Cucurbita maxima, Eucalyptus globulus, Jasminum officinale, Mentha rotundifolia, Momordica charantia, Nasturtium officinale, Nelumbo nucifera, Ocimum basilicum, Origanum majorana, Origanum vulgare, Pandanus amaryllifolius, Rosmarinus officinalis, Salvia officinalis, Thymus vulgaris, Vanilla planifolia and Zingiber officinale.

Published mass spectrometry component analysis were utilized to identify potential ligands as the active quorum sensing inhibiting component. Acyl homoserine lactone synthase was selected to be the target protein for the docking experiment using SwissDock and the results were analyzed using UCSF Chimera.

Results
Four of the twenty evaluated plants showed consistent quorum sensing inhibition in the bioassay. These are: Eucalyptus globulus, Rosmarinus officinalis, Salvia officinalis and Allium sativum. Based on literature analysis of key components, 1,8-cineole was selected. Docking experiments using SwissDock revealed docking with FullFitness (kcal/mol) values of -1157.10 and estimated ΔG (kcal/mol) 6.14 of 1,8-cineole with the V groove region of acyl homoserine synthase enzyme which important for the two step synthesis of quorum sensing molecules.

Conclusion
Quorum sensing inhibition is a potential mechanism of action for plant derivatives with antibacterial properties and can be determined using in-vitro and computational bioinformatic methods.

Keywords: Quorum Sensing, Drug Discovery, Computational Bioinformatics

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The PK/PD of Coagulation Aberration Induced by Sodium Dehydroacetate in Wistar Rats

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Purpose
Sodium dehydroacetate (Na-DHA), a preservative, is used in food, animal feeds and cosmetics. Prolongation of coagulation factors and haemorrhage in organs of Sprague-Dawley rats has been reported at oral administration of Na-DHA. We investigated the PK/PD of alterations in coagulation parameters and serum pharmacokinetics upon Na-DHA administration.

Materials and Methods
Wistar rats were administered Na-DHA with 50, 100, 150 and 200 mg/kg p.o. for 2 weeks. Weight gain, food consumption, prothrombin time (PT), activated partial thromboplastin time (APTT), and serum levels of Na-DHA were measured at 5, 7, 9, 11, 13 and 15 days after first administration, and histopathology undertaken. PT and APTT were measured using commercial kits, serum Na-DHA content was detected by high-performance liquid chromatography.

Results
Significant reductions in body weight and food consumption, as well as prolonged PT and APTT, were observed. Serum Na-DHA concentration in females were significantly higher than in males. Congestion in hepatic sinusoids, renal tubules and spleen, as well as haemorrhage in lung alveoli, gastric mucosa and intestinal mucosa was observed in 200 mg/kg by histopathological analyses. The correlation coefficient (R2) between serum Na-DHA via PT and APTT in females was 0.581 (p=0.004) and 0.791 (p=0.000), respectively, Na-DHA via PT and APTT was 0.451 (p=0.203) and 0.601 (p=0.004) in males.

Conclusion
Na-DHA can induce coagulation aberration in Wistar rats. Correlation of serum Na-DHA via PT and APTT, in females was better than that in males. Female rats are more sensitive than males to Na-DHA.

Keywords: Sodium Dehydroacetate (Na-DHA), PK/PD, Coagulation, Wistar Rats, Prothrombin Time (PT)

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The objective of this study is to investigate the effect of horse oil on UVB-irradiated SKH-1 hairless mice and to evaluate its therapeutic potential as topical application to sunburn healing.

Materials and Methods
Horse oil was applied on dorsal skin of SKH-1 hairless mice irradiated UVB (290-320 nm). After topical application of horse oil, the histopathological features of the skin were examined, and the differential gene expression profile was observed using cDNA microarray analysis.

Results
The exposure of UVB (180mJ/cm²) onto SKH-1 hairless mouse skin resulted in thickening of the epidermis, whereas skins from the UVB+horse oil group showed little thickening of the epidermis. cDNA microarray analysis revealed that the expression levels of 18 genes related to cytoskeleton including MAP kinase family were up-regulated (higher than 2.0 fold) in the UVB group, whereas the levels were restored to the control level in the UVB+horse oil group. In contrast, the gene expression levels of 32 genes related to cytoskeleton were down-regulated (less than 0.5 fold) in the UVB group, whereas the levels were restored to the control level in the UVB+horse oil or UVB+guaiazulene group. Overall, the results showed that horse oil restores the gene expression levels involved in cytoskeleton, which were up- or down-regulated by UVB.

Conclusion
Results suggest that horse oil extract restored the gene expression levels, which were increased in UVB-treated mice. Therefore, horse oil can be applied to therapeutic agents for the treatment of skin damaged by sunburn.

Keywords: Horse Oil, Sunburn Healing, UVB, SKH-1 Mice, CDNA Microarray
The Effects of Green Tea Flower (Camellia Sinensis) on Melanin Synthesis in B16F10 Melanoma Cells

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Purpose
The purpose of the present study is to evaluate the effects of green tea (Camellia sinensis) flower extract (GTFE) on melanin synthesis in B16F10 melanoma cells.

Materials and Methods
The anti-oxidant activity of GTFE was determined by measuring the DPPH free radical scavenging activity. Cell viability assay in the presence of GTFE was analyzed using B16F10 melanoma cells. To evaluate the inhibitory effect of GTFE on tyrosinase activity, mushroom tyrosinase activity was measured at different concentrations of the sample. Tyrosinase inhibition and melanin synthesis inhibition assays were performed using B16F10 melanoma cells after stimulating melanin synthesis using α-MSH (melanocyte-stimulating hormone) at different concentrations of GTFE. Differential gene expression patterns in B16F10 melanoma cells were observed using RNA sequencing analysis.

Results
GTFE expressed a significant radical scavenging activity on DPPH from the concentration of 2,000 μg/mL. Mushroom tyrosinase activity was suppressed significantly by GTFE concentration from the concentration of 2,000 μg/mL. In addition, GTFE significantly diminished α-MSH stimulated cellular melanin content and tyrosinase activity from its concentration of 800 μg/mL. Based on RNA sequencing analysis, differential gene expression patterns were observed in B16F10 melanoma cells which were normalized by addition of GTFE.

Conclusion
Overall data indicate that GTFE has a free radical scavenging activity, cellular tyrosinase and melanin synthesis inhibition abilities. The results suggest that GTFE inhibits melanin synthesis in α-MSH stimulated B16F10 melanoma cells by normalizing genes which are important for melanin synthesis including tyrosinase.

Keywords: Green Tea Flower, B16F10 Melanoma Cells, Antioxidant Effect, Tyrosinase Inhibition, Melanin Synthesis

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Restoration of the Gene Expressions by Horse Oil in DNCB-Induced Atopic Dermatitis in Balb/c Mice

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Purpose
The objective of this study is to investigate the effects of horse oil on DNCB-induced balb/c mice and to evaluate its therapeutic potential as topical application to atopic dermatitis healing.

Materials and Methods
Horse oil was applied on dorsal skin of balb/c mice treated with DNCB to induce atopic dermatitis. After topical application of horse oil, the histopathological features of the skin were examined, and the differential gene expression profile was observed using cDNA microarray analysis.

Results
After the application of DNCB, the group showed atopic dermatitis symptoms including severe erythema, hemorrhage, and erosion, whereas the symptoms were alleviated after treatment of horse oil. cDNA microarray analysis revealed that the expression level of 31 genes related to the inflammation were up-regulated (higher than 2-fold) in DNCB group, whereas the levels were restored to the control level in the DNCB+horse oil groups. In contrast, the gene expression levels of 32 genes related to inflammation were down-regulated (less than 0.5 fold) in DNCB group, whereas the levels were restored to the control level in the DNCB+horse oil groups. Overall, the results showed that horse-oil restored the gene expression levels involved in inflammation, which were up- or down-regulated by DNCB.

Conclusion
The results suggest that horse oil normalizes the expression levels of genes related to inflammation perturbed by DNCB. Therefore, horse oil can be applied as a therapeutic agent for the treatment of skin damaged by atopic dermatitis.

Keywords: Anti-Atopy Effect, DNCB-Treated Balb/c Mice, Horse-Oil Products, CDNA Microarray

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Echocardiographic Evaluation of Cardiac Function Based on Myocardial Infarct Size in Rat Myocardial Ischemia Reperfusion Injury Model

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Purpose
Increased incidence of myocardial infarction (MI) has recently emerged as the cause of cardiovascular morbidity and mortality worldwide. Echocardiography is well-established diagnostic tool for invasive and accurate evaluation of cardiac function in clinical practice. Rat myocardial ischemia/reperfusion (I/R) injury model are commonly used as cardiovascular research and the ligation of left anterior descending (LAD) coronary artery mimic the pathophysiology of MI in human. Echocardiographic evaluation of cardiac function was performed based on myocardial infarct size in rat myocardial I/R injury model.

Materials and Methods
Male, 8- to 9-week-old, Sprague-Dawley rats maintained by the guidelines of the animal care and use committee of Daegu-gyeongbuk medical innovation foundation. Rats were anesthetized and the LAD coronary artery was ligated and reperfused. Cardiac function was evaluated by Echocardiography.

Results
The values of FS and EF reflecting the left ventricular systolic function were significantly reduced in the I/R group than in the sham group after MI. The E 'and E / E' values reflecting the left ventricular diastolic function also remained low. There are differences between the 20% and 40% of myocardial infarct size.

Conclusion
Echocardiography is important evaluation factor of myocardial infarction. In addition, rat myocardial I/R injury model and the assessment of cardiac function would be a widely used in the development of new drugs for the MI patients.

Keywords: Acute Myocardial Infarction, Echocardiography, Myocardial Ischemia/Reperfusion Injury, Coronary Artery

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Artificial Induction of Cryptorchidism in Canine Recipient Using Laparoscopy for Spermatogonial Stem Cell Transplantation

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Purpose
Recipient preparation is the key step for the successful transplantation of spermatogonial stem cells (SSCs) which are unique stem cells in adults that can transmit genetic information to offspring. In this study, we investigated the surgically induced cryptorchidism using laparoscopy to deplete the endogenous germ cells of male dogs by exposure to abdominal heat, a step necessary to prepare them for use as recipients for SSCs transplantation.

Materials and Methods
Unilateral cryptorchidism was induced in 7-month-old male mongrel dogs by returning one testis to the abdominal cavity and suturing to peritoneum via laparoscopically assisted surgical procedures. The diameter of seminiferous tubules, histological and immunohistochemistry analyses were measured.

Results
Surgically induced cryptorchid testis was a marked reduction of the diameters in the seminiferous tubules compared to those of the scrotal testis in the histological appearance. Thirty days after the induction of cryptorchidism, sperm were not found in the cauda epididymis of cryptorchid testis compared with scrotal testis. Spermatogonial stem cell and spermatocytes were significantly decreased in the operated testis.

Conclusion
In the present study, we found that the laparoscopic-assisted artificial induction of cryptorchidism in dogs is a safe and feasible method to prepare recipients for SSCs transplantation. Additionally, using laparoscopy results in decreased pain and tissue trauma as well as quicker postsurgical recovery, and improves animal welfare.

Keywords: Spermatogonial Stem Cell Transplantation, Cryptorchidism, Laparoscopy, Canine Recipient

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Purpose
Human rabies remains an important public health problem in many developing countries. Transmission by dog bites is reported to be responsible for 94% to 98% of human cases. Community owned dogs live in close proximity with, and are widely and positively accepted by the resident population. However, should a rabies outbreak occur, the proximity and previous tolerance of these socialised dogs become a human health issue. Dog welfare, human rabies, and dog population management (DPM) are inextricably interlinked. New insights may help develop innovative strategies in the fight against the rabies while protecting welfare.

Materials and Methods
The current literature was reviewed to evaluate the links between DPM, animal welfare and human rabies.

Results
It has been postulated that a 70%-90% sterilization rate is necessary to maintain stable dog populations. To date, an effective and universally acceptable formula for dealing with free ranging dogs through effective & humane DPM has not been found. Human behaviour is one of the powerful forces influencing dog population dynamics. Responsible pet ownership appears one of the key factors as it recognises that good dog welfare can result in less aggression, less biting and thus possibly a reduction in human rabies incidents.

Conclusion
Key issues of DPM, ensuring the welfare of both humans and dogs should include factors like education, spay and neuter programs and vaccination, involving local authorities and the local community. Only a comprehensive local or regional approach can result in a reduction of the number of human rabies cases.

Keywords: Dog, Management, Rabies

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Infodemiology of Rabies in the Philippines Using Google Trends

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Purpose
This study determined the rabies information seeking behavior of Google search users in the Philippines. The penetration rate of smartphones in the Philippines is 30% and hence insights from Google trends can be useful in formulating rabies eradication strategies.

Materials and Methods
Google Trends was used to examine the rabies keyword search data categorized by time, geographic location and keyword ranking. With the interest over time values, numbers represent search interest relative to the highest point on the chart for the given region and time. A value of 100 is the peak popularity for the term. A value of 50 means that the term is half as popular. The same scoring is applied to geographic location as well as the ranking of related queries.

Results
The top ten rabies related searches in the Philippines (2007-2017) are the following: 1) rabies symptoms 2) anti rabies 3) dog rabies 4) rabies vaccine 5) anti rabies vaccine 6) symptoms of rabies 7) rabies in humans 8) cat rabies 9) rabies symptoms in humans and 10) dog bite rabies. Contrary to popular knowledge that rabies cases are attributable to the lack of knowledge- and hence the primary intervention is always educational, keywords search reveal a high rate of search for vaccine related terms.

Conclusion
Access to vaccines and not the lack of knowledge is the key factor in online searches for rabies in the Philippines based on Google trends.

Keywords: Rabies, Google Trends, One Health

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OH-0193

LeishNão Project: A One Health Approach for Visceral Leishmaniasis Prevention in an Endemic Area in Brazil

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Purpose
The "LeishNão" Project started in 2013 as an interdisciplinary project with the One Health approach (OHA) by the collaboration between medical and academic professionals, working directly with the local communities of Campo Grande and Dourados municipalities, one of the highest endemic areas for visceral leishmaniasis (VL) in Brazil. It was implemented due to the lack of knowledge on preventive measures and health education of this neglected tropical disease.

Materials and Methods
LeishNão is held by professors and students of Veterinary, Nursing, Medicine, Biology and other courses from two Universities in Campo Grande and one in Dourados, strategically applying the OHA at schools and community associations. Various methodologies (lectures, theaters, science fairs, games, talk wheels, distribution of printed booklets) are used, focusing in educating the preventive measures against VL applied to animals, people and the environment. LeishNão is evaluated using quali-quantitative methods such as number of participants and activities/year and semi-structured questionnaires.

Results
From 2013 to 2016, there were 161 people involved in OHA, acting in 22 institutions, providing education for 6,000 individuals, and at least 1,137 animal owners and patients attended by health facilities in the three Universities. Most people had none or small knowledge about VL. After these participatory activities they were enabled for animal, human and environmental prevention measures against VL.

Conclusion
Annually we perceive the transformative power of the Project, for both participants and community, in applying OHA for prevention of VL in these endemic areas.

Keywords: Extension Project, Leishmania Infantum, Zoonosis

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OH-0196

"From the Approach To the Concept" - A Successful "Grass Root" One Health Movement in Brazil and Latin America

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Purpose
Latin American countries focus emergency attention on infectious diseases that cross between animals and human beings; many of these diseases occur as manifestations of environmental changes related to land use, climate change, intensification of food production, habitat destruction, human encroachment and wildlife interference. Professionals recently faced high mortality outbreaks such as Zika, Dengue, Yellow Fever and other zoonosis. Academics, clinicians and field workers joined forces by acting immediately together. Chagas Disease, Rickettsia, Ehrlichia, Leishmania, Borrelia, Bartonella, Babesia and co-infections are still undiagnosed and neglected endemic areas.

Materials and Methods
The One Health "approach" in Latin America happened before there was widespread knowledge, a word’s choice (Saude Unica?) and clear definition - hence the appearance of the "grassroots" movement. Since 2007, groups from several universities, private and government institutions have been successfully working together.

Results
The One Health "concept" was officially introduced at graduate and undergraduate curriculum. Training has been disseminated through courses, workshops, symposiums, online videos, conferences, One Health textbook chapters published in Portuguese. Sustainable research have been established through Centers of Excellence for Global One Health which have been created consisting of interdisciplinary teams of professionals from science, humanities to arts. Better communication and national centralization is still needed.

Conclusion
Successful team efforts occurred during the outbreaks through the One Health approach. Antibiotic resistance, food safety, nutrition and other topics are all in scope. Human health must involve creating and maintaining healthier animals and environments.

Keywords: One Health, Research, Training

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An Analysis of Military Veterinary Medicine Programs in United Nations Peacekeeping Operations Based on One Health Concept

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Purpose
The aim of this study was to analyze military veterinary medicine program in UNPKO as humanitarian and civil operation by reviewing reports of ROK armed forces.

Materials and Methods
We analyzed 10 UN PKO military veterinary medicine plans from 2007 to 2017 and interviewed army veterinary officers who served in.

Results
Our analysis revealed that korean veterinary officer as UN Peacekeeper conducted important veterinary medicine plans for deacreasing zoonotic diseases (e.g., surveillance disease, pest control, diagnosis, treatment and vaccinations).

Conclusion
UN PKO military veterinary medicine planes in Korean armed forces are useful to prevent zoonotic disease at operational zone. This study provided useful information for developing korean veterinary medicine program for UN PKO further in korea

Keywords: Zoonotic Disease, Veterinary Officer, Korean Armed Forces, UN PKO, Veterinary Medicine Plan

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**Biorisk Challenges in the Practice of Veterinary Medicine in Ghana**

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**Purpose**
This is to expose the dangers associated with nonadherence of veterinary professionals in Ghana to biosafety and biosecurity regulations. It is also to draw attention to the fact that most of the highly pathogenic agents we work with are potential bio-weapons in this era of international terrorism.

**Materials and Methods**
Non probability purposive sampling was used to target participants. Data Gathering for this study took place between January and July 2016. Data was gathered through observations, interviews, and analysis of secondary data of activities in various veterinary clinics, laboratories across the country.

**Results**
Most of the Institutions had serious biosafety lapses. Procedures were conducted without the observation of the proper sanitary measures and precautions. Post Mortem examinations in the fields were mostly conducted without the appropriate protective equipment. There was improper disposal of carcasses after postmortem with the resulting possibility of the spread of zoonotic pathogens. The bio-containment levels in most of the laboratories fell far below the required biosafety levels. Unauthorized personnel had easy access to most of the Veterinary Laboratories and hence access to pathogens being kept there.

**Conclusion**
Zoonotic Diseases endemic in Ghana and a number of yet unknown diseases pose a high risk to the general population due to the non-adherence of veterinary practitioners and health officers to the required levels of biosafety and biosecurity in conducting their activities. This is not only due to non-complacency but mainly due to the gross lack of adequate resources, appropriate facilities, structures, maintenance, evaluation, and most importantly education.

**Keywords:** Biosafety, Biosecurity, One Health

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**Proposed One Health Interprofessional Veterinary Education in Nigeria: 
Case Study of Eruwa Veterinary Field Station Project 
of the University of Ibadan, Ibadan, Nigeria.**

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**Purpose**
Interprofessional education IPE refers to students from two or more professions in health and social care learning together to understand and value the importance of teamwork, communication and collaborative care as they grow into health professionals. University of Ibadan is the first tertiary institution in Nigeria and is credited with pioneering One Health activities in Nigeria after outbreak investigation into Lassa fever in 1969 by the Rockefeller-funded laboratory known as Arbovirus Research unit, University College Hospital, Ibadan. In 1977, the Faculty of Veterinary Medicine of the University acquired an 810 hectare (2000 acre) Eruwa Veterinary Field Station EVFS. Under the ‘Review of the University of Ibadan Strategic plans and Internationalization strategic plan for 2015-2019’, efforts are being made for the EFVS to be a model Regional One Health Centre in Integrated and Effective Animal Health-Food Safety Surveillance Capacity Development

**Materials and Methods**
Application of Interprofessional Veterinary Education at EVFS

**Results**
a. One Health review of current Community-based rural Veterinary Medicine (Course VPM: 616) to include collaboration with the Ibarapa Project in research into common zoonotic diseases.
b. Development of multidisciplinary Animal-Human-Plant- Environment One Health Curriculum for practical collaboration and partnership with other relevant Departments/Faculties of the University;
c. Developing One Health Master program and a multidisciplinary One Health Residency program;
d. Integrated Commercial Crop: Livestock Production Project.

**Conclusion**
One Health Education Aid from FAO, OIE, WHO, World Bank and other non-government agencies required.

**Keywords**: Inter-Professional Veterinary Education, Eruwa Veterinary Field Station, University of Ibadan Nigeria

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The Role of the Veterinary Statutory Body of Kyrgyzstan in Improvement the Potential of the Private Veterinary in One Health Conception

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Purpose
Kyrgyzstan is an Agricultural country. Our ancestors from ancient times lived among animals and up to this day our people can not live without animals and products of animal origin. However, due to the era of globalization, new biological threats have been accelerating, emerging and spreading.

Materials and Methods
Veterinary Statutory Body of Kyrgyzstan (VSB KR) is a very young and new organization in the post-Soviet countries. Our organization conducts series of trainings and seminars for private veterinarians throughout Kyrgyzstan to train them in new methods of combating dangerous zoonoses. We teach that veterinarians are not only responsible for the health of humans and animals, but also for the whole ecosystem.

Results
During the six years since of establishment the VSB KR, we have trained more than 1,000 veterinarians in more than 20 different topics. At the moment, we have created a mobile application for our vets for assessment of qualifications and validation of knowledge through the use of test questions.

Conclusion
Kyrgyzstan is a developing country. We have to do a lot in veterinary system to meet international standards. We learn from international experts to further extend our knowledge among veterinarians in the country. Constant expansion of agricultural land with the global boom in the field of animal husbandry means that "the domestic and wild animal species re more closely than ever before, that means we are in much closer contact with the animals".

Keywords: VSB KR, Kyrgyzstan, Veterinary System

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Survey of Anti-Rabies Antibodies in Bats, Rats and Chipmunks in Kien Giang Province, Vietnam

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Purpose
Measurement of anti-rabies antibodies in consisting of bats, rats and chipmunks which may be a carriers of rabies.

Materials and Methods
Serum samples were collected from 30 bats, 32 rats and 31 chipmunks. ELISA kit (SERELISA® Rabies Ab Mono Indirect), France
Total antibody levels from serum samples were measured by indirect ELISA method. Epidemiology analysis method was used to analyze, evaluate...

Results
Serum samples of squirrel and rat were negative for anti-rabies antibody. Therefore, the tested rats and chipmunks were not rabies carriers.

Conclusion
Three out of 30 bat serum samples (10%) were positive with anti-rabies antibodies. Therefore, the circulation of rabies virus in Kien Giang province was possible. The result was in agreement with previous report of Scheffer et al. (2007), the proportion of positive samples with rabies was 1.9% (82 out of 4393)
The results have provided more knowledge about rabies and carriers. Among tested carriers, bats were able to spread the rabies. So, we should carefully consider about them which contributes to control and management of rabies in Kien Giang province, Viet Nam.

Keywords: Rabies Virus, Antibodies, Rat, Chipmunk, Bat

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Perceptions of Zoonotic Risks Amongst Veterinary Professionals in the UK

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Purpose
Veterinary professionals play an important role in risk analysis, management and communication of zoonotic diseases. However, zoonotic perception of risks can be influenced by previous episodes of illness. This study aimed to assess the perception of veterinarians working in the UK on occupational zoonotic risks.

Materials and Methods
An on-line questionnaire consisting of 36 questions was distributed to veterinarians working in the UK through social media, UK veterinary education institutions and several UK veterinary associations. Results were collated and analysed; statistical measures used were Fisher’s exact test and two sample Z test. The survey had the ethical approval of the School of Veterinary Medicine and Science of the University of Nottingham.

Results
A total of 190 UK practising veterinarians completed the survey. Of these, 65.4% (n=106) reported a previous personal experience of a zoonosis, with the majority reporting fungal infection (49%; n=73/150). Over 85% of zoonoses reportedly occurred within the first five years of working in practice. The most frequent and serious personal risks were perceived to be ringworm, and bovine tuberculosis, respectively. Biosecurity, lack of time and type of work were perceived risk factors for acquiring zoonoses, while the predominant motivation to use personal protective equipment (PPE) was personal risk of contracting a zoonosis (98.4%; n=115/117).

Conclusion
A high proportion of veterinarians reported having contracted at least one zoonotic disease. However, further analyses are required to better understand the relationship between the different factors influencing veterinary risk perceptions about zoonoses.

Keywords: Biosecurity, Personal Protective Equipment, Veterinarians, Zoonoses, Zoonotic Disease

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**Impact of Brucellosis Control in Korea: An Ecological Study**

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**Purpose**
Since the main target of the eradication of bovine brucellosis was for cattle, the impact on human brucellosis infection has not been studied or quantified. The purpose of this study is to measure the impact of the mandatory nation-wide surveillance program and investigate the relationship between bovine and human brucellosis.

**Materials and Methods**

**Statistical analysis**
We examine the incidence of brucellosis from cattle and humans before and after the implementation of the new surveillance program, and test immediate and gradual effect of the intervention by time series analysis. To limit the anticipatory effect as the confounding of interrupted time series analysis, we defined January to December 2006 as the intervention phase on the incidence of bovine brucellosis.

**Results**
The time series (n=156) analyzed were monthly reported cases. Over the study period, the incidence of human and bovine brucellosis was sharply increased from January 2005 to July 2006 and dramatically decreased afterward.

**Cross correlation**
Since each lag is a difference of 1 month, hence, a cross correlation of a lag 0 explains the concurrent relation between the number of cattle case (per 1,000) and the reported case of human brucellosis (per 100 million).

**Conclusion**
Despite the several aforementioned limitation of this study, the implementation of National wide surveillance program of bovine brucellosis significantly contribute to reduce the human brucellosis. This findings highlights that intervention program with intensive surveillance program in animal is critical to control human zoonotic disease.

**Keywords:** One Health, Brucellosis, Time Series Analysis, Epidemiology, R

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Antibiotic and Metal Resistance in Histophilus Somni from Feedlot Cattle

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Purpose
This study examined prevalence, phenotypes, and genotypes of antibiotic and metal resistance in strains of Histophilus somni isolated from clinical cases of Histophilosis in feedlot cattle in Alberta, Canada.

Materials and Methods
Thirty one historical strains of Histophilus somni from the 1980's, and 63 contemporary strains isolated between 2012 to 2016 were examined. Antimicrobial resistance profiles were generated using micro-broth dilution and disc diffusion methods. The minimum inhibitory concentration of copper sulfate and zinc sulfate were determined using an agar plate assay method. Whole genome sequencing was performed using Illumina Miseq with paired-end 150 base pair reads. Draft whole genomes were assembled using Geneious software. Gene prediction and annotation were performed using Prokka software. The transmissability of the integrated conjugable element was examined using conjugation assays.

Results
A statistically significant increase in antimicrobial resistance (P<0.001) was observed in contemporary isolates as compared to the historical isolates. Sixty two percent of historical isolates exhibited multidrug resistance. More than 50% of contemporary strains exhibited an increased tolerance to zinc and copper. An integrated conjugable element was identified on the chromosome which contained genes associated with tetracycline, copper, and zinc resistance. This element was readily transferable to other Histophilus somni strains, and to Pasteurella multicida strains, and conferred phenotypic resistance against tetracycline, copper, and zinc.

Conclusion
We identified a horizontally transferable integrated conjugable element in contemporary strains of Histophilus somni from feedlot cattle, which confers phenotypic resistance against tetracycline, copper, and zinc.

Keywords: Respiratory Disease, Histophilus, Feedlot, Cattle, Resistance

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One Health Village: A Demonstration Site for Complicated-Problem Solving

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Purpose
The One Health Village was established and maintained as a study model for the One Health approach to solving complex problems in a local community using multidisciplinary team.

Materials and Methods
The chosen site was Klang Pla Kod village, located in Kanchanaburi province, Thailand. Relevant information about the village including background, map and timeline were obtained mainly from interviews with a village leader, board of community, and villagers. During the course of the interviews, the tentative problems were gathered from the villagers. The stakeholders were invited to brainstorming and devised practical solutions. The participants consisted of community leaders, Salakpra Wildlife Sanctuary officers, elephant camp owner, representatives from Zoological Society of London, provincial waterworks authority officer, and people from academic sectors.

Results
We identified and prioritize the top three problems of the community which are composed of human-elephant conflict, water supply, and low income. The solution for human-elephant conflict is to conduct surveys of wild elephants and study their behavior so that villagers can learn to co-exist sustainably with the wild elephants. Moreover, the international experts visited the village and wildlife sanctuary areas and gave valuable suggestions. For the water supply problem, the solution is to improve water supply system. Lastly, the solution for the low-income problem is to develop alternative agriculture for people in the community.

Conclusion
One Health Village was initiated as a demonstration site, where the complicated problems were solved by the multidisciplinary team.

Funding source: USAID

Conflict of interest: None

Keywords: One Health Village, Elephant-Human Conflict, Stakeholders

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Antimicrobial Resistance in *Escherichia Coli* and *Staphylococcus Aureus* Isolates from Goats and Antimicrobial Resistance Awareness among Small Ruminant Farmers in Selangor, Malaysia

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**Purpose**

1. To evaluate the susceptibility of *E. coli* and *S. aureus* isolated from goats against different antimicrobials.
2. To assess small ruminant farmers' understanding and awareness on antimicrobial resistance (AMR)

**Materials and Methods**

Thirty goats were selected with milk and fecal samples were collected from each animal. Isolation, identification, and biochemical confirmation of *E. coli* and *S. aureus* isolates and antimicrobial agent susceptibility testing against amoxicillin, trimethoprim/sulfamethoxazole, penicillin, neomycin, tetracycline, enrofloxacin, ampicillin, erythromycin and gentamicin were performed. A total of 13 respondents from 9 small ruminant farms participated in a survey conducted to assess their understanding and awareness on antimicrobial resistance.

**Results**

A total of 92% samples were positive for *E. coli* and 72% samples were positive for *S. aureus* from 3 small ruminant farms. All (100%) of *E. coli* isolates were found to be susceptible to all of the antimicrobials tested except for amoxicillin and penicillin. One out of 11 (9%) of the *S. aureus* isolates was resistant to erythromycin while 2/11 (18%) isolates were resistant to tetracycline. As for the antimicrobial resistance awareness, 54% (n=7/13) of the respondents did not understand the actual scenario regarding AMR and the impacts of arising antimicrobial issue.

**Conclusion**

The isolates of *E. coli* and *S. aureus* have low susceptibility to different antimicrobials indicating high level of antimicrobial resistance with most of respondents were not aware the prudent use of antimicrobials and its relationship to AMR.

**Keywords**: Antimicrobial Resistance, Small Ruminant, Antimicrobial Susceptibility

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Prevention and Control of Intestinal Parasites Infection in Endemic Area: One Health Application in the Field Observational Research

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Purpose
Intestinal parasites are prevalent in tropical regions, especially among immigrant communities at country borders. This study aimed to determine intestinal parasites prevalence and contributing risk factors in Tha Song Yang district at Thai-Myanmar border, Tak Province.

Materials and Methods
To determine prevalence, we collected 513 human fecal samples from six sub-districts. To assess possible zoonotic transmission, we retrieved 30 domestic animal fecal samples. Knowledge and attitude towards risk factors and prevention were evaluated by questionnaires. In collaboration with Ministry of Interior (MOI) and Ministry of Public Health (MOPH), we facilitated multi-sectorial brainstorming among villagers, health volunteers, health care providers, and public health authorities, to explore perceptions on disease-associated risks and solutions.

Results
Microscopic examination of human fecal samples revealed Endolimax nana (17.5%) as the highest prevalence, followed by Entamoeba coli (11.75%), and Blastocystis hominis (11%). Helminthes species accounted for 10.25%. Interestingly, 90% domestic animal fecal samples showed infective forms of intestinal parasites similar to those of human. At this stage, zoonotic correlation of these parasites was still inconclusive. Questionnaire analysis indicated that 70% respondents have inadequate knowledge about disease-associated risk factors and prevention. Brain storming displayed water-related problems and improper animal waste management as most prevailing risks.

Conclusion
Target-specific solution focused on necessity of assistance from local authorities to improve water quality and waste management. These suggestions, combined with technical findings, were constituted into Policy Brief and notified to MOI/MOPH for strategic planning to reduce disease burden. Further studies include species-specific PCR confirmation and subtyping of samples to assess zoonotic correlation of these intestinal parasites.

Keywords: Intestinal Parasites, One Health, Zoonosis

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Colistin Resistant *E. Coli* from Swine Samples in Various Regions of Thailand

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**Purpose**
In Thailand, colistin has been effectively used for treatment and metaphylaxis in swine more than two decades. Recently, colistin was reported as a last resort of antimicrobial for human therapeutic options against carbapenem-resistant bacteria. To find out the distribution of colistin-resistant isolates, *E. coli* from diseased pigs with different clinical signs isolated from 35 farms located in 18 provinces of Thailand, between 2013 and 2015 were chosen.

**Materials and Methods**
Ninety-two *E.coli* were tested colistin minimum inhibitory concentration (MIC) using broth micro-dilution assay. According to polycationic nature of colistin, three times of each test were compared to agar dilution assay and evaluated their reproducibility. Plasmid mediated colistin resistant genes: mcr-1 and mcr-2 were amplified.

**Results**
The agar dilution method had a median of MIC (median 7.33, 95% CI 4.00 to 8.00) higher than the broth dilution method (median 5.33, 95% CI 4.00 to 6.66). The colistin resistant rate was relatively high at 80.43% (74/92). The MIC values were 1 µg/ml (8 isolates), 2 µg/ml (10 isolates), 4 µg/ml (30 isolates), 8 µg/ml (39 isolates), and 16 µg/ml (5 isolates). All colistin sensitive isolates (18 isolates with MIC £2 µg/ml) did not carried mcr-1 gene but 13 of them had mcr-2.

**Conclusion**
There was not significant different between two MIC tests (p-value >0.05). The more MIC level increased, the higher rates of mcr-1 carried isolates were found. Totally 29.35% and 54.35% of the isolates had mcr-1 and mcr-2 genes, respectively.

**Keywords:** Antimicrobial Resistance, Mcr, Colistin, Pig

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Assessing Quinolone Antimicrobial Residue in Poultry Meat and Eggs of Kathmandu Valley

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Purpose
The purpose of the study was to assess the qualitative and quantitative residue of quinolone group of antibiotics in poultry meat and eggs of Kathmandu valley.

Materials and Methods
Enzyme Linked Immuno Sorbent Assay (ELISA) was employed for qualitative analysis and High Performance Liquid Chromatography (HPLC) was employed for quantitative analysis with residue values above maximum residual limits (MRL).

Results
ELISA test on meat samples (N=80 samples) showed that 88.75 % were found to be positive for quinolones residues. Of which, 12.6 % were found to have residues above MRL (i.e. 100µgm/kg). ELISA test on egg samples (N=80 samples) showed that 85 % were found to be positive for quinolones residues. Of which, 2.94 % were found to have residues above MRL (i.e. 100µgm/kg). The HPLC analysis showed that 3 meat samples were found to have residues of Enrofloxacin above MRL and 1 sample was found to have residue of Ciprofloxacin above MRL. However, out of 3 egg samples analyzed by HPLC, all egg samples were found to have no residues of Enrofloxacin and Ciprofloxacin.

Conclusion
The imprudent and haphazard use of antimicrobials in poultry has led to antimicrobial residues in meat and eggs ready for human consumption. Thus, the policy and standards of antimicrobials use in food producing animals should be implemented by the Government of Nepal to protect the human, animal and environment health.

Funding source:
Colorado State University, USA and United States Agency for International Development (USAID)

Keywords: Antimicrobial Residue, Enrofloxacin, Ciprofloxacin, Meat, Eggs

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A Comparison of Health Science and Non-Health Science Undergraduate Students Regarding Rabies Basic Knowledge

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Purpose
The study compared the basic knowledge of rabies between health science and non-health science undergraduate students in Mahidol University.

Materials and Methods
The cross-sectional study was set up to compare with the rabies basic knowledge from two groups; health science (faculties of Medicine, Dentistry, Nursing, Pharmacy, and Physical therapy) and non-health science (faculties of Liberal Arts, Engineering, and Information and Communication Technology, colleges of Music and Sports Science and Technology). Five rabies questionnaires (the cause of rabies, species of animal that can get infected, clinical signs of a rabid dog, the procedure after the human was bitten by rabies suspected dog, and how to be safe from rabies) were tested using face-to-face interview and Google drive.

Results
The purposive sampling included 629/30,000 (2.1%) of the third and fourth-year undergraduate students in Mahidol University which were composed of 326/629 (51.83%) health science and 303/629 (48.17%) non-health science. The correct answers were mainly gathered from health science group. The basic knowledge of rabies in health science group was significantly different from non-health science group (Chi-square, \( p <0.01 \)) regarding all rabies questionnaires.

Conclusion
The understanding of basic rabies knowledge in health science group is better than non-health science group. The campaign of rabies knowledge implementation should be provided especially in non-health science group.

Conflict of interest: None

Keywords: Rabies, Health Science, Non-Health Science

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Implementation Status of OIE Recommended Veterinary Core Curriculum in Korea

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Purpose
The OIE recommends the Model Core Veterinary Curriculum in order to ensure high quality veterinary education in member countries. In this way, veterinarians in the society can provide quality veterinary service to assure health and wellbeing of animals, people and ecosystems. This study was conducted to find how OIE Core Curriculum was reflected in veterinary educational curriculum in Korea and to make suggestions for future improvement in quality of education.

Materials and Methods
The 6-year-curriculum for 2016 from 10 veterinary schools were acquired and subjected to gap analysis. The each course provided was compared against criteria (course name, sequence in curriculum and course description) of the OIE Core Curriculum.

Results
The gap analysis revealed that 10 courses are fully meet with OIE Guideline (biochemistry, anatomy, physiology, parasitology, pharmacology/toxicology, pathology, transmissible diseases, microbiology, clinical & diagnostic sciences, public health) in Korean veterinary educational curriculum. Some colleges do not provide core courses, which include genetics, immunology, biomathematics, epidemiology, animal welfare & ethology, rural economics & animal production, food safety & hygiene, professional jurisprudence & ethics, herd health management & nutrition, national and international veterinary legislation, while non-of the colleges provide communication.

Conclusion
The results shows the strength and weakness of Korean veterinary education in global standard. Thus each college should make full effort to fulfill OIE recommendation. At the same time, curriculum evaluation for institutional accreditation would accelerate adoption and reform of the veterinary curriculum toward global standard.

Keywords: Veterinary Education, OIE Model Core Curriculum, Competencies

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Creating an Education Pathway in Partnership with a Tertiary Provider to Deliver Internationally Recognised Qualifications for Practising New Zealand Veterinarians.

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Purpose
To create a valued continuing education pathway for New Zealand veterinarians in the production animal sector leading to a recognised international qualification. The pathway will be a partnership between New Zealand Veterinary Association and Massey University. The purpose: to provide a three-tiered structured pathway: 1-certificate, 2-postgraduate certificate, 3-Masters. To provide NZ veterinarians with improved knowledge, skills, confidence, learning direction and incentive and will strengthen the veterinarian’s position at the intersection between animals, people, and the environment.

Materials and Methods
Existing relevant education courses identified and partnership between Massey University and NZVA created. The requirements of the NZ veterinary profession established. Learning outcomes for modules created and relevant university papers identified. The university accreditation system utilised.

Results
The CPD pathway is under development with a planned launch end 2017. The pathway will be a mixture of online courses, seminars, and workshops taking 2-3 years part time.

Conclusion
The NZVA’s strategy is based around One Health. The CPD pathway supports the concept: wellbeing/welfare of animals, people, and environment are inseparable. It will have a positive impact on veterinarians and supports sustainable increase in productivity and profitability of New Zealand’s red meat and dairy products and reducing the footprint of the agricultural sector on the environment.

Keywords: Continuing Professional Development, Red Meat, Education

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Application of Systems Dynamic Approach for Veterinary Workforce Projection in Thailand

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Purpose
The objective of this study was to analyze and project veterinary workforce in Thailand using systems dynamic approach.

Materials and Methods
A mathematical simulation modelling was applied to generate the workforce supply and demand projection in the next 12 years (2016-2027) based on the assumption that all factors involving demands and supplies of veterinary services were not changed. The supply projection model was referred to as a stock and flow model, where DVM graduates entering and exiting the workforce (flows) periodically adjust the initial number of licensed veterinarians (stock). The demand projection models included simulations of demands of veterinarians for; 1) companion animals, 2) food animals in private sectors and 3) food animals in government sectors. Demand/Supply ratios were calculated to explain the expected situation of veterinary workforce market in the future.

Results
Veterinary workforce in Thailand was currently shortage, and the demand/supply ratio was projected to be equilibrium in 2026. However, veterinarians in government sectors were highly shortage through the next 12 years, while veterinarians for companion animals and food animals in private sectors tended to be surplus in 2027.

Conclusion
In the next decade, based on the assumption of constant factors, the supplies for veterinary services in Thailand will meet the demands. Veterinary workforce planning should be done by multi stakeholders, especially policy makers, to provide sufficient veterinary services in Thailand. (Funding sources: National Veterinary Education Committee, and International Health Policy Program, Ministry of Public Health, Thailand; Conflict of interest: None)

Keywords: Veterinary Workforce, Systems Dynamic, Veterinary Services

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Meaningful and Inspiring Feedback to Motivate Students

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Purpose
After graduation, veterinary students face the expectations of the animal owners and the society. Therefore, as first day competencies, veterinarians must own a practical and skillful knowledge to perform the required procedures on their own. How can supervisors give proper feedback to enhance students’ performance and help them to become reliable professionals?

Materials and Methods
Studies were performed recently as part of a “Workplace-based e-Assessment Technology for Competency-based Higher Multi-professional Education” project, where partners from different educational and technical fields participated. The veterinary schools were educating supervisors in giving motivating assessment and measuring the effect of meaningful feedback on their students while using an e-portfolio system (EPASS).

Results
The students emphasized their wish to receive feedback in a clinical work-place from experienced and trusted person, preferably from the supervisor. They also stated that feedback on their performance can come from other sources including peer students. The desired feedback is supposed to be personal, come after the performed task, should include opinion about strengths and weaknesses and give advice on improvement strategies. Both the students and the supervisors liked the idea to use an e-portfolio system.

Conclusion
Providing meaningful and motivating feedback to inspire the improvement is often uneasy. Students are also experiencing difficulties when seeking for performance-relevant information. The final goal is to motivate and guide the students to act unsupervised on different professional activities.

This study was supported within the EU Seventh Framework Programme under grant agreement N° 619349 “Workplace-based e-Assessment Technology for Competency-based Higher Multi-professional Education” (WATCHME).

Keywords: Work-Place Based Assessment, Entrustment Decision, E-Portfolio, Narrative Feedback, Veterinary Education

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Accreditation System for Veterinary Education of Korea

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Purpose
Accreditation for veterinary education (VE) and establishment (VEE) is to evaluate and ensure the quality of VE and VEE. It is important to keep the continuous quality improvement (CQI) for quality assurance. This poster presentation is aimed to introduce the accreditation system for veterinary education in Korea.

Materials and Methods
ABOVEK was established in 2010 as the first NGO and NPO in ASIA. It has begun to evaluate and accredit a VE and VEE according to the accreditation standards from 2012. The accreditation standards are consisted of finances, curriculum, students, faculty, facilities and graduation competencies including OIE recommendation for veterinary services. The cycle of accreditation is classified into five types: full accreditation (5 years), limited accreditation (2 years), provisional accreditation (within 2 years), unsatisfactory accreditation (no-pass) and revocation (withdraw or rescission). Full accreditation is granted when the candidate school fully meets the criteria set by the accreditation standards. However, the accreditation period may be extended or shortened by the degree of compliance with the accreditation standards.

Results
The accreditation process begins from submitting an application of a candidate school to ABOVEK. Next, the candidate school performs evaluation to determine in compliance with the evaluation criteria presented by ABOVEK and submits a self-evaluation report (SER) to ABOVEK. This SER is reviewed by a written evaluation and site-visit. Approval of accreditation shall be in accordance with the results of final evaluation results. At present, four out of ten schools have received full accreditation.

Conclusion
The success of accreditation is ensured by the active participation, reasonable standards, fair and clear procedures and so on. Also, it is important to secure international collaboration. In this regard, it is expected to establish INTERNATIONAL ASSOCIATION OF ACCREDITATION AGENCY FOR VETERINARY EDUCATION, and to play significant role by WVC and OIE.

Keywords: Accreditation, Veterinary Education, CQI

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Improvement of Veterinary Medical Education in Romania

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**Purpose**
This paper presents the progress and the results of four projects co-financed by European Social Funds (N), implemented during 2010-2015 in University of Agronomical Sciences and Veterinary Medicine of Bucharest, Romania. The objectives of the projects were to facilitate and improve labour market insertion process and working skills development through practical stages, upgraded curriculum and flexible learning opportunities.

**Materials and Methods**
The projects achieved their objectives by using 3911 students, 100 university professors, 100 agreements with social - economical partners and a national e-learning platform. For the students involved in the projects, it was monitored the labour market insertion.

**Results**
The labour market insertion monitoring shows a progressive increasing of the students’ employability: 61.15% from the targeted students in 2012, 62.83% in 2013 and 67.49% in 2014. The students approached new compulsory subjects regarding career development, professional ethics, quality management in practice and education, emergency medicine. There were also issued 400 certificates for emergency medicine skills.

**Conclusion**
The study demonstrates that the increasing of the education quality trough these projects results in a real improvement of the practical skills and knowledge for beneficiaries, facilitating the insertion on the labour market.

**Keywords:** Veterinary Education, Labour Market Insertion, Upgraded Curriculum, Employability, Quality

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Comparison of Veterinary Student Perception Regarding Antimicrobial Resistance Between Chiang Mai University and University of Minnesota

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Purpose
This study aimed to compare the perception of veterinary students at the Faculty of Veterinary, Chiang Mai University, Thailand (CMU) and the College of Veterinary Medicine, University of Minnesota, USA (UMN), regarding antimicrobial resistance in small animal practice.

Materials and Methods
Structure questionnaire was used to collect the data from 86 and 83 veterinary students, who have been practicing in small animal clinic, at CMU and UMN, respectively. Collected information included awareness of antimicrobial resistance and antimicrobial selection and use in small animal practice.

Results
Veterinary students from two schools similarly concerned about the increasing prevalence of multidrug resistant bacterial infections in small animal (level 8 and 7 from 10 for CMU and UMN, respectively). However, 82.93% of CMU students felt that antimicrobial resistance was a moderate to severe problem, while 50% of UMN students felt that. First generation cephalosporins and Amoxicillin (22.95% and 22.95%, respectively) were selected, by CMU student, to be the choice of skin infection patients but among UMN student, 86% selected first generation cephalosporins were the drug of choice for skin infection. According, urinary tract infection, Amoxicillin and Amoxicillin with Clavulanate were most frequent choice among UMN students (39% and 33%, respectively). While CMU students selected enrofloxacin and Amoxicillin with Clavulanate to treat urinary tract infection (25.40% and 23.81%, respectively).

Conclusion
Students from different country seems to be aware of antimicrobial resistance. Veterinary schools need to emphasize the important of antimicrobial resistance in global impact.

Keywords: Antimicrobial Resistance, Perception, Small Animal, Student, Veterinary

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Changes in Multisensory Learning Style Among Veterinary Students in the US as They Progress Through the Curriculum

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Purpose
To determine if veterinary students changed in their learning style preference from the time they entered veterinary school until they graduated, given that different school years focus on different areas.

Materials and Methods
Students at Oregon State University were asked to complete the VARK\textsuperscript{(R)} survey during the first week of class each year of their veterinary education and at graduation. Students were categorized as visual, auditory, read/write or kinesthetic if that specific learning style represented $\geq35\%$ of their profile, otherwise they were categorized as "No preference". Distributions of learning style preference were compared between demographic profiles and class. Given the small number of students per class (N=46), significance was set at $\alpha=10\%$.

Results
Most students (40.8\%) were kinesthetic. There was no difference between genders. Auditory students tended to be younger ($P=0.113$). Freshmen had the lowest proportion of visual learners; Sophomores had the lowest proportion of read/write learners; Juniors had a balanced distribution of learning styles; and Senior students had the lowest proportion of auditory learners. Freshmen differed from Sophomores ($P=0.032$), Senior Students differed from Sophomores ($P=0.004$), and New Graduates differed from Freshmen and Sophomores ($P=0.104$ and $P=0.067$, respectively). Kinesthetic learners had the lowest GPA, while auditory learners had the highest GPA.

Conclusion
Learning styles differ between school years. This likely reflects adaptation to teaching styles of different subjects. However, because GPA is associated with learning style, veterinary schools could improve GPA by adapting teaching styles to learning styles.

Keywords: Learning Style, Gender, Age, Grades, Teaching Style

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Students Perceptions on the Relevance of Veterinary Public Health for the Veterinary Curriculum and Profession

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Purpose
Though not always evident for veterinary students, veterinary public health (VPH) is an area of veterinary medicine which has a substantial impact on human and animal health. This study aimed to analyse the perceptions of UK veterinary students regarding VPH.

Materials and Methods
An on-line questionnaire consisting of 33 questions was distributed to students currently registered in veterinary undergraduate courses in higher education institutions in the UK. The analysis of the multiple choice questions of the questionnaire was carried out through descriptive statistics. Open-ended qualitative responses were analysed by basic thematic analysis, grouping and categorising themes. The survey had the ethical approval of the School of Veterinary Medicine and Science of the University of Nottingham.

Results
A total of 578 students responded the questionnaire. An 82.2% (n= 466/568) of the respondents showed an increase in knowledge and awareness of VPH throughout the period of studying the veterinary degree. The veterinary curriculum was found to be one of the biggest influences resulting in raising awareness of VPH (68.22%; 380/557). Veterinary work related to farm animals was considered as highly relevant for VPH (Median= 100; n= 523). However, the majority of students identify only slaughterhouse work as a VPH career path.

Conclusion
The veterinary curriculum has a large impact on student awareness of VPH. However, most emphasis on the wider role of the veterinary profession should be given during the veterinary training in order to highlight potential career pathways to students.

Keywords: Career Options, Food Safety, One Health, Veterinary Curriculum, Zoonoses

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Educational Activity of Asian Society of Conservation Medicine

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Purpose
This presentation is aimed to introduce the educational activity of Asian Society of Conservation Medicine (ASCM).

Materials and Methods
ASCM was established in 2006 and holds annual meetings in several Asian countries including Thailand, Taiwan, Malaysia, Myanmar, Nepal, Vietnam, Indonesia, Singapore and Korea. One of our goals is to promote “One Health” education in Asia Pacific veterinary colleges. Since 2014, we have been holding educational workshops in local veterinary colleges including VNUA (Vietnam), UVS (Myanmar) and NTU (Taiwan), aiming to educate the local veterinarians and students regarding the importance of “One Health”. Efforts to provide capacity building for the education of basic veterinary science (Anatomy and Physiology) have also been made in Cambodia in collaboration with FAVA since 2016.

Results
We have so far received very positive responses and support, and have been able to enthuse the local community that significant future changes to this field can be anticipated.

Conclusion
The annual meeting of ASCM in conjunction with the education workshop will be held in Borneo in October, 2017.

Keywords: Veterinary Education, One Health, Asia

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Brazilian Veterinary Statutory Body Accreditation System

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**Purpose**
Brazil has experienced a significant increase in the number of Veterinary Colleges, associated to broad variability of in-college educational standards. Accordingly, the Federal Council of Veterinary Medicine (CFMV) has established in 2016 the Accreditation System of Veterinary Colleges (ASVC) in Brazil. In addition, ASVC has also aimed to: (1) fulfill the graduation requirements in compliance to Brazilian regulations and international organizations standards; (2) stimulate the continuous improvement of educational quality in Brazilian Veterinary Medicine Colleges; and (3) publicly certify the high-quality of accredited colleges throughout the Veterinary Medicine and general society.

**Materials and Methods**
The accreditation system has been developed based on universality, voluntariness, periodicity and transparency, finally established to provide meritocracy and publicity of accredited institutions. The evaluation instrument consisted of 33 indicators distributed in three dimensions: pedagogical organization, university population, and infrastructure. After a self-evaluation, the institution has been visited for four days by a pair of experienced evaluators.

**Results**
The Brazilian nationwide system has been established and is currently at the implementation phase. Three institutions were evaluated to test and improve accreditation methodology, with promising initial results on discriminatory capacity of the evaluation instrument and the receptivity of the target public.

**Conclusion**
The evidence-based data gathered to date has shown that those involved in the administration of Veterinary Colleges were aware of demand and standards for an accreditation process. The system has developed a discriminatory capacity to standardly improve the quality of veterinary medical education in Brazil.

**Keywords:** Veterinary College Educational Quality, Improve Veterinary Education Quality, Day-1 Competencies

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Effective Veterinarian-Client Communication in a Companion Animal Clinic; Recall of Information

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Purpose
Communication between veterinarians and pet owners is necessary for a good relationship, optimizing patient care and treatment decisions, owner compliance and ultimately improving animal welfare. The aims of this study were: i) How much information is remembered by pet owners from a veterinary consultation; ii) Which information is most likely to be memorized; iii) Which variables influence the quality and quantity of the information recalled.

Materials and Methods
In total 55 pet owners visiting for the first time the division for Oncology and Radiotherapy at the UCCA, with a dog or a cat, were approached by the researcher. They were asked for permission to make a voice recording of their visit and to interview them by telephone a few hours after leaving the clinic to ask them a few questions about their visit. All consultations were performed by the same veterinarian.

Results
The age of the owners ranged from 22 to 81 years, 29% were men and 71% were women. The average recall score was 66.6%. Multivariate analysis showed two significant explanatory variables for recall: age of the owner (P=0.018) and level of education (P=0.031). The older the owner, the less recall and the higher the education, the higher the recall.

Conclusion
Age and education of owner are important regarding the amount of information owners can recall. However this study was done with only one veterinarian and further studies with multiple veterinarians are needed to see what aspects of communication of the veterinarian will make a difference in recall.

Keywords: Communication, Recall, Information

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