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### 2019 ACVIM Forum Research Report Program
Phoenix, Arizona, June 6 - 8, 2019

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**CARDIOLOGY**

**Coronary Artery CT in 45 Dogs with Heart Base Tumors: Perfusion, Proximity, and Radiation Exposure**

Brian A. Scansen - Colorado State University; Ashley Sharpe - Colorado State University; Carolyne Kruckman-Gatesy - Colorado State University; Susan LaRue - Colorado State University

Heart base tumors (HBT) lie near the coronary artery (CA) circulation, with potential to recruit coronary flow resulting in steal phenomenon. Stereotactic radiation therapy (SRT) is used to treat HBTs, but in people can cause detrimental effects to CAs including endothelial dysfunction and accelerated arteriosclerosis. The aims of this retrospective study were to evaluate CA proximity to HBTs by computed tomography angiography (CTA) and quantify radiation dose to CAs in dogs treated with SRT. Forty-five dogs that underwent CTA for HBT between 2009-2018 had their epicardial CAs categorized as within, adjacent, or distant from the HBT. Twenty-three dogs underwent SRT and their CAs were contoured to quantify radiation dose received by the left vs right CA system. Direct recruitment of CA flow to HBT was seen in 18/45 cases, deriving from circumflex CA (14 dogs), left CA (3), and right CA (1). At least one CA was within the HBT in 24/45 cases, adjacent in 36/45 cases, and all CAs were distant from the HBT in 5/45 dogs. Higher radiation dose was received by the left CA system in dogs whose left CA branches were within or touching the HBT (P < 0.02) while a similar trend was seen for the right CA (P = 0.09). In dogs, HBTs commonly lie in proximity to the epicardial CAs and recruit flow from the CAs. SRT for HBT exposes the CAs to radiation. Taken together, coronary steal and radiation damage may cause risk for myocardial ischemia in dogs with HBT.

**Efficacy of Pimobendan in Cats with Hypertrophic Cardiomyopathy and Previous Congestive Heart Failure**

Karsten Schober - The Ohio State University; Luis Fuentes Virginia - RVC London; John Rush - Tufts University; Tony Glaus - University of Zuerich; Nuala Summerfield - Virtual Veterinary Specialists; Joao Loureiro - North Down Specialist Referrals; Kathy Wright - MedVet; John MacGregor - Bulger Veterinary Hospital; Gerhard Wess - Munich University; Margaret Sayer - Animal Referral and Emergency; Taibinh Nguyen - MedVet

Benefits of pimobendan in the treatment of feline hypertrophic cardiomyopathy (HCM) have not been demonstrated in randomized controlled clinical trials. The objective of this exploratory study was to investigate the effects of pimobendan in cats with HCM and recent congestive heart failure (CHF), with or without left ventricular outflow tract obstruction (LVOTO). The aim was to search for possible endpoints in a larger pivotal study.

Prospective, randomized, placebo-controlled, double-blinded, global multi-center study. 82 client-owned cats with HCM and recently resolved CHF (within the last 60 days) were randomized (29 cats with and 53 cats without LVOTO). Cats received either pimobendan (0.30 mg/kg q12h, n = 43) or placebo (n = 39) in conjunction with furosemide. The proportion of cats reaching the primary endpoint (“success-yes”; \( P < 0.02 \)) while a similar trend was seen for the number of adverse events was comparable in both treatment groups (\( P > 0.05 \)).

Pimobendan administration did not lead to improved 180-day outcome in this study. Additional endpoints for a pivotal study were not identified.

**Treatment with Aptamer BC-007 in Doberman Pinschers with Autoantibodies Against β1-Adrenergic Receptors and Dilated Cardiomyopathy**

Gerhard Wess - LMU University Munich; Sabine Werner - Clinic of small animal medicine; Gerd Wallukat - Berlin Cures; Johannes Müller - Berlin Cures; Niels-Peter Becker - Berlin Cures; Ingolf Schimke - Berlin Cures

Autoimmunity associated with autoantibodies directed against the ß1-adrenergic receptor (ß1-AAB) is increasingly accepted as driving human idiopathic dilated cardiomyopathy (IDCM). A new broad-spectrum neutralizer of these pathogenic autoantibodies, Aptamer BC 007, showed promising results in vitro and in vivo in hypertensive rats.

The objective of this study was to determine the safety and efficacy of treatment with Aptamer BC 007 in Doberman Pinschers with spontaneous DCM.

To evaluate the efficacy of Aptamer BC 007, of 28 Doberman Pinschers in the occult phase of the DoCM with ß1-AAB, 14 dogs got an infusion with Aptamer BC 007 and were compared to the 14 Doberman Pinschers, which received the same background treatment (Pimobendan and antiarrhythmic drugs), but no Aptamer BC 007 infusion. Dogs that developed congestive heart failure (CHF) during the course of the study received furosemide in addition. The primary endpoint was defined as death of cardiac reason.
No adverse events occurred during and after the infusion of Aptamer BC 007, indicating that the administration is safe for use in dogs. The estimated median time to the primary endpoint was significantly longer for dogs receiving Aptamer (572 days, IQR 442-840 days) than for dogs in the DCM control group 1 (266 days, IQR 97-438 days) (Log Rank: P = 0.009).

Given that this is a pilot study with only a small study population, it remains necessary to validate our findings with a large-scale, randomized, double-blind, placebo-controlled study.

**Left Atrial Decompression as a Percutaneous Rescue Therapy for Advanced Mitral Valve Disease**

Justin Allen - VCA West Los Angeles; Kirstie Barrett - VCA West Los Angeles; Anthony Llamas - VCA West Los Angeles; Kevin Phipps - VCA West LA

Left heart failure is common in dogs with advanced myxomatous mitral valve degeneration (MMVD) and is frequently fatal when refractory to medical management or side effects of therapy are manifest. Left atrial decompression (LAD) involves creating an iatrogenic atrial septal defect (IASD) via percutaneous jugular catheterization, which rapidly reduces left atrial hypertension. Case records from one of the authors (JA) were retrospectively reviewed for dogs with advanced MMVD and chronic left CHF that underwent left atrial decompression. Six dogs were identified: 4 were hospitalized for congestive heart failure (CHF) at time of LAD and 4/6 were azotemic at time of LAD.

LAD was successful in all patients, and was successful in reducing left atrial pressure by 50% or greater in 5/6 patients (for one patient, the final LAP was unable to be measured for technical reasons). The size of final balloon dilatation across the atrial septum was 8 mm in the first 3 dogs, 10 mm in the 4th and 5th, and 12 mm in the 6th. Procedural time was 78-150 minutes. Complications of the procedure included self-limiting pericardial effusion (2/6) and arrhythmias (atrial premature complexes (4/6), atrial flutter (1/6)). Arrhythmias were either self-limiting or resolved with therapy in all dogs. Three dogs were discharged the following day, 1 was discharged 2 days post-procedure; 2 died within 36 hours of the procedure (owner-elected euthanasia, respiratory failure).

Signs of left CHF resolved in the surviving dogs following the procedure. Reductions in diuretic doses were achieved in all patients. No evidence of worsening pulmonary hypertension or right CHF was evident in any patient during short-term follow-up (3 months). One dog required an increase in diuretic dose at 45 days; the IASD was noted to be smaller than initially created (4.5 mm vs 1.5 mm at 45 days), suggesting spontaneous closure was responsible for CHF. This dog developed recrudesence of pulmonary edema at 87 days, at which time the defect was not patent; the procedure was successfully repeated, with discharge the following day. The remaining surviving dogs are free of congestive signs with patent iASD at time of report submission.

Left atrial decompression results in rapid reduction of left atrial pressure and may be a promising, cost-effective therapeutic option for dogs in which current medical therapies are limited. Further investigation and long-term follow-up are necessary to determine appropriate timing, patient selection, and size of iASD to optimize outcomes.

**EQUINE**

**Evaluation of Clostridium difficile Antigen Positive Toxin ELISA Negative Results in Foals**

Nathan Slovis - Hagyard Equine Medical Institute; Justine Elam - Hagyard Equine Medical Institute; Scott Weese - University of Guelph

Diagnosis of *C. difficile* infection (CDI) can involve multiple approaches to fecal sample testing, including detection of the organism via antigen (glutamade dehydrogenase) ELISA or culture, detection of toxin genes by PCR or detection of toxin via ELISA.

Discordant antigen and toxin ELISA results may be encountered in horses, complicating diagnosis. The objective of this study was to evaluate the ability to isolate toxigenic *C. difficile* from antigen positive, toxin negative samples. Fecal samples were collected from foals ranging from 1 day to 3 weeks of age that were presented to a referral hospital for diarrhea and/or enteritis, or that developed diarrhea or enteritis during hospitalization. Sampling was performed between 2015 and 2017, inclusive. As part of routine diagnostic testing, fecal samples were tested for *C. difficile* using an ELISA that detects *C. difficile* antigen (glutamate dehydrogenase) and toxin B (C. diff Quik Chek Complete, TechLab, Blacksburg, Va, USA). During the study period, 1354 fecal samples were tested for *C. difficile*. Forty-two (3.1%) yielded antigen positive, but toxin negative results. Samples that were antigen positive but toxin negative were further evaluated by enrichment culture. *Clostridium difficile* was isolated from 22 (54%) antigen positive, toxin negative samples. All 22 recovered isolates were toxigenic, possessing genes encoding toxins A and B. While the clinical relevance of antigen positive, toxin negative results needs further study, foals in this study all had enteritis that could be consistent with CDI, and few had other potential causes of disease identified.

**Influence of Body Condition and Endocrine Status on Blood L-Lactate Concentrations in Horses and Ponies**

Bettina Dunkel - Royal Veterinary College; Edward Knowles - The Royal Veterinary College; Yu-Mei Chang - The Royal Veterinary College; Nicola Menzies-Gow, Reader - The Royal Veterinary College

Blood L-lactate concentrations are higher in ponies with gastrointestinal disease than horses, possibly due to differences in body condition (BC). Therefore correlations between L-lactate concentrations and measures of BC were investigated in healthy horses and ponies. In ponies, the influence of endocrine status on L-lactate concentrations was also investigated. Blood samples were obtained from 101 ponies and 51 horses. Breed, weight, height, measures of BC and L-lactate concentrations were recorded. In ponies, triglyceride and ACTH concentrations were measured and an oral glucose test performed. Correlations between L-lactate and BC parameters were established. The association between L-lactate concentrations, equid type (pony or horse), BC, age, and glucose concentrations was investigated in a multivariable model. Weak (*P* = 0.001) negative correlations were detected between L-lactate concentration and the average BC score (*r* = −0.29), heart girth: height ratio (*r* = −0.27) and age (*r* = −0.27). L-lactate and glucose concentrations were weakly correlated (*r* = 0.15; *P* = 0.036). In ponies, a weak negative correlation between L-lactate and triglyceride concentrations was detected (*r* = −0.21; *P* = 0.045)
but no other significant correlations with endocrine parameters. In the multivariable model, age (−0.02 ± 0.006; \( P = 0.001 \)) and heart girth: height ratio (−1.74 ± 0.53; \( P = 0.001 \)) were significantly associated with L-lactate. This represents a 2% reduction in L-lactate per year increase in age and 10% reduction in L-lactate per 0.06 unit increase in heart girth: height ratio. Age and BC influence blood L-lactate concentrations significantly but weakly.

Identification of a Putative Genetic Mutation for Idiopathic Hypocalcemia in Thoroughbred Foals

Carrie J. Finno - University of California Davis School of Veterinary Medicine; Victor Rivas - UC Davis; Nathan Slovis - Hagyard Equine Medical Institute; K. Gary Magdesian - UC Davis

Clinical signs of idiopathic hypocalcemia in Thoroughbred (TB) foals include hypocalcemic tetany, recumbency and variable seizure-like activity. The condition is invariably fatal. Based on the severity of the clinical phenotype, occurrence only in the TB breed, and similarity to inherited infantile hypocalcemia in humans, a genetic investigation was performed. Clinicopathologic data from two affected TB foals excluded other causes of hypocalcemia. Pedigree analysis revealed a likely autosomal recessive mode of inheritance, with both foals related on the sire’s side within 2 generations and on the dam’s side within 6 generations. DNA samples from both foals and their dams were whole-genome sequenced to ~12x coverage on an Illumina HiSeq2500. Sequence reads were trimmed, mapped to EquCab2.0, and variants identified. Initially, candidate genes implicated in human inherited hypocalcemia (CASR, GNA11, or TRPM6) were evaluated for putative genetic mutations but none were identified. To further investigate other potential variants, a whole-genome association study was performed. A segregating putative functional variant, resulting in a homozygous nonsense mutation in a gene encoding for a guanine nucleotide exchange factor, was highly associated with idiopathic hypocalcemia. The altered base pair is 100% conserved across 100 vertebrate species. Additionally, this genetic variant was not identified in 175 horses from 21 non-TB breeds. Comparative data revealed that, while expressed ubiquitously at the protein level, the transcript is predominantly expressed in parathyroid tissue. Therefore, a nonsense mutation in a guanine nucleotide exchange factor is a likely causative variant for idiopathic hypocalcemia in Thoroughbred foals.

How Long Will My Horse Shed Salmonella?

Brandy A. Burgess - University of Georgia; Helen Aceto - University of Pennsylvania; Emily Barrel - University of Minnesota; Stephanie Brault - Colorado State University; Emily Cook - University of Georgia; Rebecca McConnico - Louisiana Tech University; Nathan Slovis - Hagyard Equine Medical Institute; Paul Morley - Colorado State University

Management of Salmonella enterica in horses typically focuses on clinically affected animals, however subclinical shedding is likely much more common and can greatly exacerbate environmental contamination and potentially increases transmission risks among stablemates. Hospitalized horses are typically discharged within a few days of admission and subsequent detection of shedding, which increases concerns regarding transmission and prevention in home settings. In order to make best-practice, evidence-based recommendations regarding management of individual horses and populations, we must understand the duration of shedding among affected horses, a vital part of the epidemiology of Salmonella in equine populations. To that end, the objectives of this study were to, 1) characterize the duration of Salmonella shedding among culture-positive horses, 2) describe
factors associated with shedding duration, and 3) investigate adverse effects created by exposure of Salmonella-positive horses to their stablemates.

Subclinical and clinically affected horses were cultured weekly for 8 weeks, isolates were phenotypically characterized, and surveys were conducted to determine factors associated with shedding and its impact on stablemates.

In general, horses that had clinical salmonellosis were more likely to intermittently shed *S. enterica* in their feces; and to do so for a greater median duration. Mare-foal pairs tended to parallel each other's shedding pattern irrespective of clinical or subclinical shedding.

The results of this study indicate that horses with clinical salmonellosis likely pose a greater risk for environmental contamination, but that subclinically affected horses may contribute to ongoing transmission, and that on-farm precautions should be taken irrespective of disease status.

**Ultrasound Approach to Diagnose Aortopulmonary Fistulation in Friesian Horses**

Gunther van Loon - Equine Cardioteam, Ghent University; Dominique De Clercq - Equine Cardioteam, Ghent University; Annelies Decloedt - Equine Cardioteam, Ghent University; Lisse Vera - Equine Cardioteam, Ghent University; Glenn Van Steenkiste - Equine Cardioteam, Ghent University; C.M. de Bruijn - Wolvega Equine Clinic; Margreet Ploeg - Department of Pathobiology; Willem Back - Utrecht University; Catherine Delesalle - Ghent University; Koen Chiers - Ghent University

Aortopulmonary fistulation (APF) is extremely rare in the general horse population but relatively common in Friesian horses. Typically, a transverse tear in the aorta occurs near the remnant of the ligamentum arteriosum, with formation of a fistula towards the pulmonary artery. The fistulation often is a relatively long and irregular shaped tract. A wide range of clinical signs may occur. Tachycardia and right heart failure with pulmonary hypertension are usually present. Often a holosystolic and early diastolic murmur are found dorsal to the aortic valve area. Obtaining good quality cardiac ultrasound images may be challenging in some Friesians due to their body conformation. The purpose of this study was to describe how to take diagnostic ultrasound images of APF, applicable in equine practice. Data from 9 Friesian geldings and 3 mares (mean body weight 591 ± 54 kg) were included. Age ranged from 4 to 12 years (median 5.8). All horses underwent a full echocardiographic exam including 2D, M-mode and color flow Doppler. In addition, specific views were acquired to visualize the APF. All horses showed ultrasound changes compatible with severe pulmonary hypertension and a decrease in aortic size. From the right parasternal left ventricular outflow tract view and right ventricular (RV) inflow-outflow view (Figure 1), APF could be visualized in the triangle between right atrium (RA), aorta (Ao) and pulmonary artery (PA) in 8 horses, but lesions were sometimes subtle. Color flow examination from these views did show abnormal flow suggestive for APF in 6 horses. One horse showed an aortic dissection with a circular pseudo-aneurism (cuff) of the entire visible aorta. Left-sided long-axis views of the PA from the third intercostal space were superior to visualize the APF. With cranial (45°) and slight dorsal (10°) angulation (no rotation) (Figure 2), the PA and Ao were visualized. Subsequently, the course of the PA was followed until its bifurcation by gradually rotating the probe clockwise and simultaneously angling it dorsally and caudally. From these views, the APF could be clearly identified between PA and Ao in all horses. Color flow Doppler indicated turbulent flow in the APF and PA in all horses. The actual tear in both vessels could be identified in 11 horses. In all horses APF was confirmed on postmortem. In conclusion, Friesians with APF show ultrasound findings that indicate pulmonary hypertension and right heart failure. However, for accurate diagnosis specific ultrasound views from aorta and pulmonary artery are needed. Longitudinal images from the PA, taken from the left third intercostal space clearly showed the APF in all horses.

**Comparison of Long-Acting Injectable Omeprazole and Oral Omeprazole in Equine Squamous and Glandular Gastric Disease**

David I. Rendle - Rainbow Equine Hospital; Sarah Gough - Rainbow Equine Hospital; Gayle Hallowell - University of Nottingham

The study was performed to investigate whether 2-4 weeks of treatment with long-acting injectable omeprazole (IO; BOVA UK) was as effective as treatment with 4 weeks of licensed buffered omeprazole paste (OO) administered PO SID in treating equine squamous and glandular gastric disease. A retrospective review of clinical cases was performed. Horses were aged 5-22 years and represented a wide range of breeds and disciplines. Gastroscopy was performed prior to
treatment and after 4 weeks of treatment. A further gastroscopic examination was performed at 2 weeks in IO horses. Anonymised gastroscopy images were reviewed blind to details of treatment. Non-inferiority statistics were performed to compare the two treatments with a significant difference between groups being 20% or more. Forty-five horses with EGGD met the inclusion criteria; 33 received IO and 12 received OO. All lesions were within the pyloric antrum and there was no difference in severity between groups. Fifty-six horses with ESGD met the inclusion criteria; 29 received IO and 27 received OO. There was no difference in squamous lesion distribution or grade between the two treatment groups. Treatment failure rates for EGGD and ESGD are presented in Table 1. Injectable omeprazole was found to be non-inferior to oral omeprazole for healing, but not improvement, of glandular ($P = 0.003$) and squamous lesions ($P < 0.0001$). In the IO group there was a significant improvement compared to pretreatment in both EGGD and ESGD lesions within 2 weeks. Given the large differences in failure rates between the two treatments, further investigations with larger numbers are justified to determine whether IO is superior to OO.

**Critical Illness Related Corticosteroid Insufficiency (CIRCI) in Adult Horses**

Allison J. Stewart - University of Queensland/Swedish University of Agricultural Sciences; Eileen Hacket - Colorado State University; Taylor Towns - Auburn University; François-René Bertin - University of Queensland School of Veterinary Science

Illness-associated stress results in cortisol concentration elevations; however, in critical illness related corticosteroid insufficiency (CIRCI), cortisol concentrations and adrenal response to ACTH stimulation are inadequate. The clinical features and consequences of CIRCI have not been described in adult horses.

Medical records from horses admitted on emergency in which stimulation with 0.1 μg/kg of synthetic ACTH was performed were reviewed. CIRCI was defined as baseline cortisol cortisol <2.6 μg/dL or delta cortisol < 1.9 μg/dL. Variables associated with CIRCI, survival, systemic inflammatory response syndrome (SIRS) and presence of ischemic lesions were investigated with $P < 0.05$ considered significant.

Sixty-nine horses were included of which 29% had CIRCI at admission, an additional 14% developed CIRCI during hospitalization, 25% died, 67% had SIRS and 32% had ischemic lesions. At admission, horses with CIRCI had higher ACTH concentrations, lower cortisol, delta cortisol and insulin concentrations and were less likely to survive ($P < 0.03$). Survivors had lower ACTH and cortisol concentrations at admission and on day 2, higher post-stimulation cortisol on admission and on day 2 and were less likely to have CIRCI, ischaemic lesions or reflux ($P < 0.04$). Horses with SIRS had higher glucose concentrations ($P < 0.01$). Horses with ischaemic lesions had higher ACTH, cortisol and glucose concentrations at admission, lower insulin concentrations on day 2 and were more likely to have SIRS and not survive ($P < 0.05$). No multivariable analysis could be fitted for any outcomes. CIRCI in adult horses is common, characterized by high ACTH, but low cortisol and low delta cortisol and is associated with non-survival.

**FOOD ANIMAL**

**Determination of Zika Virus Pathogenesis in Pregnant Sheep Model**

Andrea S. Lear - University of Tennessee, College of Veterinary Medicine; Marc Caldwell - University of Tennessee; Ky Pohler - Texas A & M University; Aaron Baumann - University of Tennessee

Zika virus (ZIKV) is a member of the *Flaviviridae* family, is an emerging pathogen in humans. During gestation, ZIKV induces placental inflammation and insufficiency, limiting effective nutrient exchange and directly damages fetal tissues. The increasing spread of the virus along with lack of knowledge on mechanisms of viral pathogenesis have demonstrated a need to develop an appropriate animal model for pathogenesis investigation. The main objective of this study was to develop a relevant model to study the viral dynamics at the maternal-fetal interface including fetal congenital abnormalities using sheep.

Our research hypothesis was that pregnant sheep inoculated with ZIKV would be permissive to viral replication resulting in transmission to the developing fetus. In order to critically test this hypothesis, 12 pregnant ewes carrying 45-day fetus were enrolled in the study and randomly distributed between 3 treatment groups receiving intra-amniotic (IA; $n = 4$), intravascular (IV; $n = 4$), and intravaginal (VA, $n = 4$) inoculation of $10^4$ FFU of ZIKV strain PRVABC59. Clinical illness score was assessed daily. Blood was collected daily until 14 days post inoculation (dpi) and PCR performed to identify viremia in the dams. Transabdominal ultrasound was performed weekly, assessing fetal viability. Clinical illness was not observed in any sheep following inoculation. Following 7 dpi and 14 dpi, fetal wastage occurred in all sheep in the IA and IV group, respectively. Molecular techniques were performed, confirming ZIKV. This study demonstrates a feasible animal model to investigate aspects of maternal-fetal ZIKV infection including trans-placental spread and placental cell tropisms.
Understanding the Nasopharyngeal, Tonsillar and Lung Microbiota of Pre-Weaned Dairy Calves with and without Bronchopneumonia

Sarah M. Raabis - University of Wisconsin-Madison; Theresa Ollivet - University of Wisconsin-Madison; Chelsea Holtschbach - University of Wisconsin-Madison; Garret Suen - University of Wisconsin-Madison

There is significant interest in understanding the respiratory microbiome; specifically, how certain phenotypes may relate to disease susceptibility in dairy calves. Potential relevance of this data includes improved screening diagnostics and development of respiratory probiotics. While many survey studies have been performed, this is the first study, to the authors’ knowledge, to investigate short term effects of an experimental bacterial challenge on the upper and lower respiratory tract microbiota. The purpose and primary objective of this study was to evaluate whether bacterial composition and diversity characteristics of the nasopharyngeal (NP) microbiota were protective against pneumonia following experimental challenge with Pasteurella multocida. The secondary objectives of this study were to evaluate the effects of bacterial challenge and ampicillin therapy on the NP, tonsillar, tracheobronchial lymph node and lung microbiota of pre-weaned dairy calves.

A convenience sample of 39 healthy, pre-weaned Holstein bull calves (mean age: 52 ± 6 days) was enrolled in July of 2017 and housed in a BSL-2 laboratory. The experimental protocol was approved by the Institutional Animal Care and Use Committee. After 7 days of acclimation, calves were examined by clinical respiratory score (RS) and lung ultrasound score (USS); 30 calves with a normal RS and USS were selected for bacterial challenge. On the day before the challenge, a deep NP swab was collected. The next day, calves were sedated and a video laryngoscope was used to pass a catheter into the distal trachea; 25 mL of 10^10 CFU/mL P. multocida was administered. Calves were monitored at intervals by RS and USS; when USS showed significant consolidation, a second deep NP swab was collected and calves were randomized to receive either ampicillin trihydrate (n = 17, label dose x 3 days) or an equal volume and duration of sterile saline (n = 11). Two calves did not develop significant lung consolidation following bacterial challenge, so they were not included in the treatment randomization. Calves were monitored daily via RS and USS for 14 days. On day 14, a third deep NP swab was collected. Calves were then euthanized; postmortem samples of the NP tonsil, lung, and tracheobronchial lymph nodes were collected.

NP swabs and tissue samples were stored at −80°C and were processed simultaneously. Following DNA extraction of swabs and 1x1x1 cm sections of tissue, the V4 region of the 16S rRNA gene was amplified using PCR. Libraries were sequenced using Illumina’s MiSeq platform and sequences were clustered into Operational Taxonomic Units using Mothur 1.40.0. Taxonomy and diversity data were analyzed in RStudio 1.0.44. Alpha diversity was compared using ANOVA and Wilcoxon rank sum tests. Beta diversity was compared using PERMANOVA tests. Linear regression models were fit to evaluate the effects of pre-challenge NP diversity characteristics on health outcomes. Mixed linear regression models were fit, with calf as a random effect, to evaluate the effect of sample type and ampicillin treatment on diversity metrics of the microbiota. Relative abundance (RA) of bacterial genera were compared using Kruskal Wallis tests.

Calves with higher NP alpha diversity pre-challenge were RS positive less days (P = 0.03) following bacterial challenge, when controlling for serum total protein at 7 days of age and ampicillin treatment during the trial. NP alpha diversity pre-challenge was not associated with USS (P = 0.31). There were no significant differences in alpha diversity among the 3 NP swabs collected (P = 0.30), but alpha diversity was different between the third NP swab and postmortem tissue samples (P = 0.004). The alpha diversity of the third NP swab and postmortem tissue samples was not affected by ampicillin treatment (P = 0.71) or bacterial challenge (P = 0.90). Beta diversity was different between the three NP swabs (P = 0.002) and between the third NP swab and tissue samples (P = 0.001).

Prior to challenge, the top 5 NP genera were Bacteroides sp, Mycoplasma sp, Escherichia sp, Prevotella sp and unclassified Microbacteriaeae sp. Following bacterial challenge, the top 5 genera included Prevotella sp, Pasteurella sp, Mycoplasma sp, Acinetobacter sp and unclassified Microbacteriaeae sp in both the third NP swab and the postmortem tissue samples. The second and third NP swabs had a higher RA of Pasteurella sp compared to the pre-challenge NP swab (P < 0.01). The first NP swab had a higher RA of Escherichia sp compared to both post-challenge NP swabs (P < 0.02). The RA of Mycoplasma sp in the first NP swab was higher in calves that developed lung lesions faster following the challenge (P = 0.008). The RA of Sharpea sp in the first NP swab was higher in calves that took longer to develop lung lesions after the challenge (P = 0.01). The RA of Pasteurella sp in the third swab and tissue samples was not affected by antibiotic therapy (P > 0.12). The RA of Pasteurella sp was different between post-challenge swabs and tissue samples (P < 0.001). The tonsillar samples had the lowest RA of Pasteurella sp and highest RA of Acinetobacter sp compared to all swabs and tissue samples (P < 0.01).

In conclusion, increased NP alpha diversity was protective against clinical respiratory disease in challenged calves in this study. Increased RA of Mycoplasma sp was associated with more rapid development of lung lesions, while increased RA of Sharpea sp was associated with slower development of lung lesions following bacterial challenge. Interestingly, the RA of Pasteurella sp was not affected by antibiotic treatment in NP swabs or postmortem tissue samples. The increased speed of lung lesion development in calves with higher RA of Mycoplasma sp may be related to synergy between the challenge strain and the resident strain of Mycoplasma sp within the NP. Sharpea sp are lactic acid bacteria with potential antagonistic activity against Gram negative infections; this bacterial population may have partially inhibited the growth of the challenge strain.

Comparison of Disk Diffusion, Broth Microdilution, and WGS for Susceptibility Testing of Mannheimia haemolytica

Brenton Credille - University of Georgia

Multi-drug resistance (MDR) is an emerging issue with Mannheimia haemolytica and a variety of susceptibility testing methods are available for clinical use. The objective of this study was to compare the results of disk diffusion, broth microdilution, and whole genome sequencing (WGS) for susceptibility testing of 48 M. haemolytica isolates collected before and after mass medication with tulathromycin. Each isolate was subjected to antimicrobial susceptibility testing with disk diffusion, broth microdilution, and whole genome sequencing.
For a given antimicrobial, the percentage of isolates classified as susceptible, intermediate, or resistant were compared between the three methods using an exact test of marginal homogeneity. Overall categorical agreement between testing methods ranged from 42-100%, with the lowest agreement being for comparisons of broth microdilution and WGS for penicillin in cattle prior to mass medication. Perfect agreement was found for ceftiofur, enrofloxacin, florfenicol, oxytetracycline, and gamithromycin for all testing methods in isolates prior to mass medication and for ceftiofur, enrofloxacin, oxytetracycline, gamithromycin, and tilmicosin for all testing methods after mass medication. Very major errors (false susceptible) were detected in 3/18 possible comparisons prior to mass medication and 1/18 comparisons after mass medication. Major errors (false resistant) and minor errors (all other errors) were detected in 0/18 and 6/18 comparisons prior to mass medication and 1/18 and 6/18 comparisons after mass medication, respectively. The results of this study show that discrepancies in the results of different susceptibility testing methods exist and suggest a need for harmonization of susceptibility testing methods.

**Continuous Sampling of Healthy and Mastitic Quarters to Determine Ceftiofur Concentration after Intramammary Infusion**

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Pharmacokinetic studies of drugs in the milk are often limited due to infrequent sampling associated with milking. Alternatively, frequent sample collection with repeated milking may increase drug elimination. Previous work with intravenously administered flunixin has demonstrated that continuously sampling the udder using ultrafiltration is an effective means to address these concerns. The objective of this study was to continuously sample both a healthy and mastitic quarter from cows with naturally occurring mastitis to compare the drug concentrations of intramammary infusions of ceftiofur. An ultrafiltration probe was placed with ultrasound guidance into the gland cisterns of normal and mastitic quarters of ten mature mid-lactation cows (9 Holstein and 1 Jersey cows) with naturally occurring subclinical mastitis. The ultrafiltration probe was secured to the caudal or lateral aspect of the udder depending on the quarter being sampled. Ceftiofur hydrochloride (125 mg, Spectramast LC) was infused into both sampled quarters at time 0 and 24 hours. Ultrafiltrate samples were collected at 0, 2, 4, 6, 8, 12, 18, 24, 26, 28, 30, 32, 36, 48, 60, 72, 84 and 96 hours post initial drug administration. Each cow was routinely milked by machine every 12 hours, and milk weights were collected. An aliquot of milk was collected from the treated quarters immediately prior to milking, during milking, and at the conclusion of milking. Equal volumes of these aliquots were mixed for assessment of drug concentrations in whole milk. Ceftiofur and its desfuroyceftiofur-related metabolites were analyzed by ultrahigh-performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS). Milk samples were aseptically collected from both treated quarters immediately prior to treatment and on days 5, 7, and 14 after treatment for aerobic culture. Samples were obtained from 9/10 healthy quarters and 8/10 infected quarters. In the infected quarters, 3 of 8 were positive for coagulase-negative Staphylococcus spp. (CNS), 2 of 8 were positive for S. aureus, and 1 of 8 were positive for Streptococcus uberis, Aerococcus spp., or Escherichia coli. Six of 8 mastitic quarters were culture negative at the conclusion of the study with one CNS and one S. aureus infection not being eliminated. All healthy quarters remained negative throughout the study. At the conclusion of the ongoing drug analysis, we will determine the impact of mastitis on active ceftiofur concentrations in the udder, and the correlation between pharmacokinetic parameters and treatment outcome. We conclude that collection of ultrafiltration samples from the mammary gland of cows provides a viable means to continuously assess drug concentrations in the milk while continuing to milk the cow normally, and more accurately associate pharmacokinetic parameters with treatment outcomes.

**NEUROLOGY**

**Brain Diffusion Tensor Imaging in Canine Degenerative Myelopathy**

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Canine degenerative myelopathy (DM) shares similarities with superoxide dismutase 1-associated human amyotrophic lateral sclerosis (ALS). Brain microstructural pathology has been quantified using diffusion tensor imaging (DTI) in ALS patients. Our objective was to use DTI to characterize neurodegenerative changes in the brain of DM dogs longitudinally and to determine the relationship to clinical severity.

Brain DTI was performed at baseline and every 3 months. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) were calculated on regions of interest in specific white and grey matter areas. Gait scores (0, normal to 14, tetraplegia) were assigned at the time of each scan. DTI values were compared to gait scores and evaluated over time using Pearson correlation and univariate split-plot approach, respectively.

Sixteen DM dogs were included; mean age was 9.7 years (1.4) and mean time from onset of signs to first MRI was 5.7 months (3.3). Mean baseline gait score was 4 (1) and mean score change from baseline to last scan was 4.82 (2.67). Fifty-four scans included 9 dogs with ≤3, and 7 with >3 scans. DTI values poorly correlated with gait scores (R² < 0.1 all comparisons). Among dogs with >3 scans, FA of the internal capsule decreased over time (P = 0.04).

DTI detected longitudinal changes in brains of DM dogs but showed no consistent relationship to disease severity. Further study is warranted in a larger cohort of dogs to clarify the relationships between brain DTI values and progression of DM.

**Analgesic Effects of Gabapentin Alone or in Combination with Meloxicam in Dogs with Neuropathic Pain**

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Beauchamp - Université de Montréal; Paulo Steagall - Université de Montréal

This study aimed to evaluate the analgesic effects of gabapentin alone (G) or in combination with meloxicam (GM) in dogs with naturally-occurring neuropathic pain. Twenty-nine client-owned painful dogs were included in a prospective, randomized, clinical trial after physical and neurological examination, and lesion confirmation via MRI. Dogs were allocated to receive G/placebo/GM or GM/placebo/G during 21 days (7 days/treatment including resting). Client specific-outcome measures (CSOM) and Canine Brief Pain Inventory (CBPI; pain, function and overall impression), and dynamic and interactive visual analog scale (DIVAS; mm) and Glasgow pain scale-short form (CMP-SF) were evaluated by owners (blinded to treatments) or a single veterinary neurologist (blinded to treatment order), respectively, at presentation and at days 7, 14 and 21. Statistical analysis included mixed linear models and Cochran-Mantel-Haenszel followed by adjustment of alpha level when appropriate (P < 0.05).

Both G and GM decreased pain scores significantly compared with presentation, but not placebo using CBPI (pain) (mean ± SD: G: 15.6 ± 8.1; GM: 14.4 ± 9.0; placebo: 17.5 ± 8.5; presentation: 20.1 ± 9.0), CMPS-SF (G: 2.9 ± 2.2; GM: 3.0 ± 1.8; placebo: 4.1 ± 2.7; presentation: 5.0 ± 2.4) and DIVAS (mm) (G: 34 ± 26; GM: 37 ± 28; placebo: 44 ± 31; presentation: 58 ± 28). GM decreased CBPI (function) significantly compared with presentation (G: 16.7 ± 9.0; GM: 16.1 ± 10.4; placebo: 17.0 ± 8.4; presentation: 21.5 ± 9.1). CBPI (overall impression) were significantly better after G (G: 3.2 ± 1.0; GM: 3.0 ± 1.2; placebo: 2.7 ± 1.0; presentation: 2.3 ± 1.1). CSOM were significantly lower after all treatments including placebo than at presentation (G: 7.5 ± 3.1; GM: 7.4 ± 3.1; placebo: 8.3 ± 2.8; presentation: 10.3 ± 2.8).

Based on owner and veterinarian assessments, gabapentin alone or with meloxicam provided pain relief for neuropathic pain.

Characterizing Canine Glioma as a Naturally Occurring Model for Immune Evasion In Human Glioma

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Human gliomas appear to exploit immune inhibitory pathways to maintain an immunosuppressive microenvironment. Binding of programmed cell death protein 1 (PD-1) to its ligand (PD-L1) promotes activated T-cell exhaustion. Tumor cells (TCs) evade host’s immune attack by expressing PD-L1 and stimulating PD-1 expression on tumor-infiltrating lymphocytes (TILs). Accumulation of intratumoral FoxP3+ regulatory T-cells (Tregs) also results in effector T-cell inhibition and decreased anti-tumor response. Little is known about whether canine gliomas share these immunomodulating mechanisms.

We analyzed immunohistochemical expression of FoxP3, PD-L1 and PD-1 in a retrospective series of canine gliomas. Formalin-fixed paraffin-embedded tissue sections of 43 gliomas of all types and grades were immunostained for FoxP3, CD3 and CD20. Of these, 18 tumors were additionally stained for PD-L1 and PD-1. Accumulation of FoxP3+ TILs within the tumor was more pronounced in astrocytic than in oligodendrogial tumors. Mean FoxP3 expression was greater in high-grade gliomas, with significant differences between low-grade oligodendrogliomas and glioblastomas (P = 0.0187).

All tumors contained PD-L1-expressing cells (>1% positive-TCs). Mean expression increased with grade and was considerably higher in astrocytic tumors. PD-1+ TILs were observed in 16 cases; however, only three tumors with high PD-L1 expression and CD3+ TIL density were considered positive for PD-1 (>1% positive-TILs). There was correlation between PD-L1 and FoxP3 expression (r = 0.47; P = 0.054).

This is the first study to immunohistochemically characterize FoxP3+ Treg expression and the PD-1-PD-L1 axis in canine gliomas, demonstrating that spontaneous canine glioma models immune evasion in its human counterpart. This supports the use of canine gliomas in translational immunotherapeutic studies.

Severe Degenerative Myelopathy is Associated with Reductions in Fractional Anisotropy of the Spinal Cord

Philippa Johnson - Cornell University; Erica Barry - Cornell College of Veterinary Medicine; Sarah Stephan - Cornell College of Veterinary Medicine; Wen-Ming Luh - Cornell College of Human Ecology; Andrew Miller - Cornell College of Veterinary Medicine; Ashish Raj - University of California San Francisco

The diagnosis of degenerative myelopathy (DM) is limited by the lack of lesion visibility on conventional MRI and alternative diagnostic techniques are needed. Diffusion tensor imaging (DTI) is an advanced MRI method that is highly sensitive to the detection of white matter lesions. We hypothesized that the DTI measure of fractional anisotropy (FA) would be altered in the spinal cord of dogs with white matter lesions caused by DM.

Twelve dogs with clinical DM (eight were subsequently histopathologically confirmed) and 15 healthy control (HC) dogs were neurologically graded, anesthetized and scanned with a 3-T MRI to obtain DTI data of the whole spinal cord. Data were post-processed and mean FA's documented for the spinal cord at cervical, thoracic and lumbar sites. Data were assessed for normal distribution. Thoracic data were highly variable and non-normally distributed due to artefact and were excluded. Dunnett t-test was performed to detect differences between HC, mildly affected, and severely affected DM cohorts.

Statistically significant differences were identified between HC and severe DM cohorts at C6 (mean +/- sd, HC 0.587 +/- 0.036, Severe 0.522 +/- 0.022 P = 0.028) and L1 (HC 0.650 +/- 0.013, Severe 0.593 +/- 0.021 P = 0.04). A trend towards significance was identified at L2 (HC 0.627 +/- 0.012, Severe 0.580 +/- 0.018 P = 0.058).

This study identifies that although statistically significant alterations in FA are present in the spinal cord of dogs with severe DM, this method of diagnosis may be insensitive for detection of milder lesions in the earlier stages of disease.

Initial Assessment of Canine Degenerative Myelopathy with Positron Emission Tomography Spinal Imaging

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Canine degenerative myelopathy (DM) is a progressive adult-onset neurodegenerative disease that has similarities to human amyotrophic lateral sclerosis (ALS). Temporal post-mortem DM dog central nervous system (CNS) tissue studies have demonstrated neuronal degeneration and astrogliosis in the mid to lower thoracic spinal cord, among other locations. We recently identified a c.118G > A transition in the superoxide dismutase 1 gene (SOD1) that predicts an E40K missense mutation and underlies most cases of canine DM. Similarly, rodent (mouse and rat) G93A SOD1 mutants are known ALS models with related CNS pathological manifestations. A hallmark of CNS tissues within ALS patients, DM dogs and SOD1 mutant rodents is the loss (30-70% over time vs healthy controls; disease and species dependent) of the excitatory amino acid transporter (EAAT2) protein found on CNS astrocyte (glial) cells. Regional CNS excitotoxic injury, promoted by elevated neurotransmitter L-glutamate concentrations, is thought to be a result of altered EAAT2 activity and/or astrocyte availability. Recently, a new positron emission tomography (PET) imaging fluoride-18 radiolabeled tracer, known as 18F-FAA, which targets the CNS EAAT2 protein has been synthesized. The purpose of the study was to initially evaluate whether EAAT2 changes in male and female DM dogs relative to healthy controls can be detected by utilizing 18F-FAA spinal PET imaging, coupled to magnetic resonance (MR) and computed tomography (CT) anatomical tissue data.

Animal studies adhered to IACUC approved protocols utilizing facilities that are AAALAC accredited. Recruitment of dogs was reliant on pet owner participation, and cohorts included healthy controls (HC) and recent diagnosed DM dogs. Routine automated production of the 18F-FAA tracer was performed in which homogenous ~18.5-55.5 MBq (0.5-3 mCi) doses were formulated in ethanol and saline (1-3 mL). An intravenous catheter was placed in a saphenous vein. Dogs were anesthetized, then placed within an imaging cradle to enable MR scanning (T2W, Toshiba/Canon 3 T MR scanner) of cervical and thoracic spine regions. The cradled dog was transported to the PET-CT scanner (wide bore Toshiba/Canon Celestion PUREVISION TOF), injected with tracer (intravenous), then dynamic PET scanning of cervical and thoracic regions for 40-90 min was performed. Attenuation correction was by CT scan. For some dogs test-retest data was collected on different days. The reconstructed PET data were framed and co-registered with the CT-MR data (AMIDE software). Spinal cord regions of interest (cervical, C1-C3; thoracic T5-T12) were defined based on landmarks and atlases. Regional radioactivity curves over time were determined as standardized uptake values (SUV). Similarly, SUV profiles for heart were established to define an imaging derived tracer input function, which was used with other tracer kinetic modeling parameters to afford regional spinal 18F-FAA tracer distribution volume (V_t) estimates.

The following results were obtained. Signalment of the cohorts included HC: age range, 5-10 years as 1 male (1 Labrador Retriever), 9 females (4 Beagle x Chinese Crested (BCC) dogs, 1 Chesapeake Bay Retriever (CBR), 4 Boxers (BX); and DM: recently diagnosed, age range, 8.9-11.3 years, 3 males (1 CBR, 1 BX, 1 Pembroke Welsh Corgi, PWC) and 4 females (2 BX, 1 CBR, 1 PWC). Multiple tracer doses were afforded per production run, with high molar activity (133-925 TBq/mmol) and purity (>95%). The imaging derived input function used the heart left ventricle. The V_t estimates were derived with a two compartment (one tissue) kinetic model. A significant statistical difference in 18F-FAA tracer V_t was found for T5-T12 thoracic spine, where the DM cohort had a lower V_t value than the HC cohort (ANOVA P = 0.0212). A significant V_t change in cervical spine (HC vs recently diagnosed DM) was not found. We conclude that the HC vs recently diagnosed DM cohort findings demonstrate that 18F-FAA PET imaging is sensitive for the detection of thoracic spinal cord EAAT2 protein target changes. These initial outcomes are consistent with previous DM EAAT2 spinal pathology determined by post-mortem ex vivo measures as reported in the literature (eg, Ogawa et al., 2014, Vet. Path. 51:591-602). Therefore, there is impetus to advance longitudinal PET imaging studies in dogs affected by DM in order to assess progressive EAAT2 changes in various CNS tissues.

**Disposition of Single and Multi-Dose Extended Release Levetiracetam in Healthy Small Breed Dogs**

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The purpose of this two part study was to evaluate the disposition of single and multi-dose extended release levetiracetam (XRL) in healthy dogs with body weight between 5 and 15 kg. Part 1: Timed serum sampling was performed in 7 dogs following administration of a single 500 mg XRL tablet. Median (range) Cmax, Tmax, and AUC were 31.0 (13.3-69.7) μg/mL, 4.6 (2-8) hour, and 173.4 (47.1-354.8) h*μg/mL, respectively. No clinical adverse effects were noted. Based on these data, a 12 hour dosing interval was chosen for the multi-dose study. Part 2: Eight dogs received 1-500 mg XRL tablet q 12 h for 10 days. Timed serum sampling was performed days 3, 7 and 10 at trough 2, 4, 6 and 8 hours after tablet administration to observe for individual variation. Neither median Cmax (P = 0.05) nor AUC (P = 0.11) were significantly different across the 3 sampling days, using a Friedman test. Individual change in Cmax ranged from 0.5 to 21.4 μg/mL, with a median decrease in serum levetiracetam concentrations of 2.8 μg/mL from day 3 to day 7. Median and individual serum levetiracetam concentrations remained above the minimum human therapeutic interval.
Treatment of Acute "Deep Pain Negative" Thoracolumbar Spinal Cord Injury in Dogs by Extended Durotomy

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Currently, dogs that lose "deep pain perception" in the hindquarters following acute thoracolumbar intervertebral disc herniation have ~55% chance of recovering the ability to walk on the pelvic limbs by 3 months. Investigations in laboratory rodents and human patients suggest that extensive durotomy or duroplasty may improve outcome after acute spinal cord contusion.

In this pilot study, 22 consecutive dogs presenting with deep pain negative paraplegia following acute T3-L3 intervertebral disc herniation underwent continuous "extended durotomy" over 4 vertebral lengths (2 cranial and 2 caudal to lesion epicenter) immediately following conventional decompressive surgery.

Delay between onset of paraplegia and presentation ranged from 30 minutes to 6 days (median 20 hours). Nine dogs definitely recovered to walk voluntarily again, within a mean of 29 days. Five dogs definitely did not recover within 3 months (including one that developed progressive myelomalacia at 4 days and was euthanized and one that recovered pain sensation but could walk at 5 months) and 4 were irrevocably lost to follow-up within 3 months (including one euthanized at 14 days because of no change in status). Median delay till presentation was similar for dogs that did (24 hours) and did not (20 hours) recover. Four dogs have not yet completed the 3-month follow-up period.

While not strongly conclusive, the results in dogs for which we know the outcome appear promising. A prospective randomized trial to compare outcome of conventional surgery vs conventional surgery plus extended durotomy will be necessary to define the value of this intervention.

A Mitochondrial Transporter SLC25A12 Gene Mutation is Associated with Inflammatory Myopathy in Dutch Shepherd Dogs

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Similar to human inflammatory myopathies (IMs), canine IMs are a large group of disorders characterized by infiltration of inflammatory cells into muscle. The most common IMs in humans are immune-mediated, and include polymyositis (PM), dermatomyositis and inclusion body myositis. In dogs the immune-mediated IMs masticatory myositis, PM and myositis caused by infectious diseases, usually protozoal infection, are most common. The specific aim of this study was to characterize an IM in a small family of young Dutch Shepherd dogs, with progressive clinical signs of generalized muscle weakness and muscle atrophy, and persistently elevated serum creatine kinase activity. Physical and neurological examinations supported a neuromuscular disease in 5 affected related dogs. Given the early onset of muscle weakness and presence of related affected dogs, an underlying genetic cause was suspected.

Histopathology of skeletal muscle biopsies confirmed an inflammatory myopathy and immune-mediated, infectious or possibly dystrophic disorders were considered. Immunofluorescent staining was used to phenotype cellular infiltrates, determine if the major histocompatibility antigens MHC I and II were overexpressed, and for localization of the dystrophy associated protein dysferlin. A genome-wide association strategy was used to map a chromosomal locus to CFA36, followed by whole genome sequencing (WGS) that identified a homozygous L349P substitution in the SLC25A12 gene, encoding the mitochondrial aspartate-glutamate transporter isoform (AGC1) most prominently expressed in the brain and muscle (heart and skeletal muscle).

Transport studies performed by functionally reconstituting the recombinant wild-type and mutant AGC1 in liposomes confirmed a dramatic decrease of AGC1 transport activity by the mutant carrier demonstrating pathogenicity of the variant. In addition, targeted, broad-spectrum metabolomic analysis of skeletal muscle from affected dogs and controls demonstrated a proinflammatory milieu and strong experimental support for oxidative stress in affected dog muscles.

This is the first description of a SLC25A12 variant associated with an IM in any species and the first report of an underlying mutation resulting in a metabolic defect associated with an IM. To date, 14 human inherited diseases associated with mitochondrial transporters of the SLC25 family have been reported that result in pure metabolic disorders including defective mitochondrial energy production, and well characterized biochemical and genetic defects. The reported diseases in humans are predominantly associated with the central nervous system and include developmental delay, congenital epilepsy, hypomyelination and possibly autism. The explanation for why muscle is primarily affected in dogs with the SLC25A12 variant will be the subject of future investigations.

ONCOLOGY

Controlled, Randomized Study of Intratumoral Tigilanol Tiglate (EBC-46) for Treatment of Canine Mast Cell Tumors

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Mast cell tumors (MCT), the most common skin malignancy in dogs, can pose treatment challenges for several reasons. With only one currently approved therapeutic for MCT in the US, development of additional treatment options is important to advancing clinical management.
Tigilanol tiglate, isolated from the Australian rainforest plant Fontainea picrosperma, possesses antitumor activity and stimulation of enhanced wound healing of the treatment site via activation of protein kinase C. Tigilanol tiglate may be effective when injected intratumorally as treatment for canine cutaneous or subcutaneous MCT.

One hundred twenty-three dogs with cutaneous or lower limb subcutaneous MCT confirmed by fine needle aspiration cytology were enrolled in the study. Dogs were randomized 2:1 to treatment with a single intratumoral injection of tigilanol tiglate or to sham treatment (untreated controls) in an investigator- and owner-masked multicenter study. The primary efficacy outcome was complete response (CR; disappearance of the target lesion) on Day 28. Other outcome measures included wound healing, tolerability, safety and health-related quality-of-life (HRQoL). Treated dogs with less than CR could receive a second intratumoral injection of tigilanol tiglate on Day 30 and untreated dogs could be crossed over to treatment with tigilanol tiglate on Day 30.

One hundred eighteen dogs were evaluable. Sixty of 80 dogs (75%) randomized to treatment with tigilanol tiglate achieved CR after a single intratumoral injection compared with 2 of 38 untreated dogs (5.3%) by Day 28 ($P < 0.0001$). Eighteen of the 20 treated dogs not achieving CR received a second intratumoral injection. Eighty-seven percent (68/78 evaluable) of treated dogs achieving CR after first injection remained tumor-free at post-treatment day 84.

Of the 38 dogs randomized to the untreated control group, 33 were crossed over to intratumoral tigilanol tiglate and 62.5% (20/32 evaluable) achieved CR by Day 28 and 44.4% (9/20) of these remained tumor-free at post-treatment day 84.

Wounds developed in 92.5% (74/80) of dogs treated with tigilanol tiglate and healed rapidly from Day 7. Wound development is anticipated pathology associated with the mechanism of action of the drug. The most frequent adverse events were transient reactions at the treatment site. Owners indicated that overall HRQoL of treated dogs was similar to that of untreated dogs.

Tigilanol tiglate was highly effective for the treatment of cutaneous and lower limb subcutaneous MCT in dogs and was well tolerated with manageable side effects. Tigilanol tiglate has potential to play an important role in expanding treatment options available for dogs with MCT in both primary care and specialty settings.

Prolonged Remission and Survival in Canine B-Cell Lymphoma After Chemotherapy and Low-Dose Rate Half-Body Radiotherapy

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CHOP-based chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone) has been the standard of care for canine B-cell lymphoma for over 3 decades. The purpose of this study was to investigate whether CHOP combined with low-dose rate half-body radiation therapy (LDRHBI) might lead to improved outcomes for dogs with this disease. Between July 1, 2013 and February 15, 2017 at VCA Western Veterinary Specialist Centre in Calgary, Canada, CHOP-LDRHBI was offered for all dogs with B-cell lymphoma as a potentially superior therapy to CHOP alone. Standardized staging diagnostics were performed in all cases including screening bloodwork, urinalysis, thoracic and abdominal imaging, bone marrow aspiration cytology and incisional lymph node biopsy. A 15-treatment, 25-week CHOP-based protocol incorporating L-asparaginase was used. LDRHBI was inserted at weeks 6 (cranial half) and 8 (caudal half); 700 cGy were delivered using a low dose rate, extended treatment distance protocol that delivered approximately 35-40 monitor units/minute. Treatment delays, chemotherapy drug dose reductions, and choice of concomitant medications were made at the discretion of the attending medical oncologist (GEM). NCSS v. 12.0.4 Statistical Software was used for all statistical analyses.

Twenty-seven dogs were entered into the study on an intent to treat basis. Median first remission duration in this group of dogs is...
1135 days, with a 95% confidence interval of 345 to 1135 days. Median overall survival time has not been reached: 1 dog is lost to follow and 9 dogs have died of other causes still in first remission at a median of 367 days (range, 71 to 1883 days), and 6 dogs are still alive and in first remission at a median of 1436 days (range, 959 to 2017 days).

All 27 dogs experienced generally manageable treatment-related toxicity, including anorexia (27 dogs), vomiting (25 dogs) and diarrhea (21 dogs). All 27 dogs developed neutropenia and thrombocytopenia and received antibiotics during therapy. Neutropenia resulted in treatment delays in all dogs; dose reductions were made as a last resort in 3 dogs. Lengthy neutropenia prompted discontinuation of therapy in 4 dogs before the end of the planned protocol. Mild elevations in blood urea nitrogen and/or serum creatinine were seen in 18 dogs after caudal LDRHBI. Two dogs were euthanized because of treatment-related toxicity.

These results suggest that for owners of dogs with B cell lymphoma willing to accept increased cost and treatment-related toxicity, CHOP-LDRHBI can result in prolonged remissions and survival times.

In Vitro and in vivo Evaluation of High-Dose Ascorbate in Dogs

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Studies in humans have determined that supraphysiological concentrations of ascorbate (plasma concentrations > 20 mM, that is, pharmacological ascorbate [P-AscH−]) readily produce high fluxes of H2O2 via its oxidation; this H2O2 preferentially induces cancer cell death compared to normal cells. The vast majority of in vitro studies have focused on the effects of P-AscH− on human tumor cells. The effects of P-AscH− on canine osteosarcoma, and if high levels of ascorbate are achievable in dogs, are unknown.

The purpose of this study was 2fold: (1) determine the pharmacokinetic (PK) profile of high-dose intravenous ascorbate in healthy Beagle dogs; and (2) determine the effects of P-AscH− on canine osteosarcoma cells.

Eight Beagle dogs were administered two doses of ascorbate (250 mg/lb or 1000 mg/lb) via intravenous infusion over six hours, on separate days. Plasma ascorbate concentrations were measured at 12 time points during and after the infusion for PK analysis. Minor adverse effects were seen in two dogs: mild nausea or vomiting. Plasma ascorbate levels peaked at 9 mM following the higher dose and returned to baseline 6-8 hours after dosing.

Clonogenic assays were performed on 2 canine osteosarcoma cell lines and 1 normal canine fibroblast cell line after exposure to high concentrations of ascorbate. Ascorbate significantly decreased survival in the osteosarcoma cells, while sparing normal fibroblasts. These data indicate: P-AscH− is preferentially cytotoxic to canine-derived cancer cells; and high levels of ascorbate can be safely administered to dogs. Further studies are needed to determine the effects of P-AscH− on canine cancer patients.
Detection of Feline Coronavirus from the Respiratory Tract and Conjunctiva of Cats

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Feline upper respiratory infection (URI) is an important infectious disease complex that affects the feline population, particularly those in densely-housed populations such as shelter and rescue facilities. Clinical signs include conjunctivitis, corneal ulcers, and ocular discharge, as well as typical upper respiratory tract signs such as sneezing. Feline URIs are associated with a number of pathogens including feline herpesvirus-1 (FHV-1), feline calcivirus (FCV), pneumovirus, Chlamydothila felis, Mycoplasma sp., Bordetella bronchiseptica, and Streptococcus equi subsp. zooepidemicus. The role that feline coronavirus (FCoV) has as a primary causative agent of feline URI is not entirely clear. Here, we collected swabs from the conjunctiva and nasal cavity of cats diagnosed with feline infectious peritonitis (FIP), as well as from the conjunctiva, and nasal and oropharyngeal tissue of healthy cats and cats experiencing clinical signs associated with URI’s. FCoV was detected in the conjunctival and nasal cavity of cats displaying differing degrees of respiratory signs and detected within FIP cats upon necropsy. The nasal turbinate of an FIP cat was examined histologically and had multifocal regions of pyogranulomatous inflammation. Using immunohistochemistry, FCoV antigen was detected within macrophages in the areas of inflammation. These observations support respiratory tract infection due to FCoV and suggest that FCoV may be an under-recognized cause of feline URI.

Incidence of Urolithiasis in Dogs Naturally Infecte by Leishmania Infantum Treated with Allopurinol

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Allopurinol has been increasingly used for the treatment of canine visceral Leishmaniasis (CVL). The formation of urinary xanthine urolithiasis has been related as a possible complication of allopurinol therapy. The aim of this study is to evaluate the incidence of urolithiasis in dogs naturally infected by L. infantum. 193 dogs naturally infected by L. infantum. Were included in this study. The survey was conducted through a retrospective evaluation of medical records and laboratory tests from January 2015 to July 2017. The inclusion criteria of the dogs in the study were to have either positive Elisa, Rifi above 1:160 or positive reaction on Snap Leishmania Idexx. The medical record should contain complete anamnesis, detailing type of feeding, medications administered and complementary exams of abdominal ultrasound and routine urine analysis. Of the dogs, 74% (144/193) didn’t develop adverse urinary effects, 18% (34/193) developed xanthine urolithiasis and renal mineralization, 4% (8/193) struvite urolithiasis, 3% (6/193) calcium oxalate
Efficacy of Telmisartan for the Treatment of Persistent Canine Renal Proteinuria

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Information regarding the efficacy of the angiotensin II receptor blocker telmisartan for the treatment of proteinuria in dogs is limited. The objective of this prospective, randomized, double-masked clinical trial was to evaluate the antiproteinuric efficacy of orally administered telmisartan in dogs with persistent, pathologic, renal proteinuria, when compared to enalapril. Client-owned dogs with chronic kidney disease and urinary protein-to-creatinine ratio (UPC) ≥1.0 (if non-azotemic) or ≥ 0.5 (if azotemic) were eligible for enrollment. Dogs were block-randomized, according to presence or absence of azotemia and systemic arterial hypertension, to receive telmisartan (1.0 mg/kg PO) in the morning and an equal volume of placebo once daily in the evening, or enalapril (0.5 mg/kg PO) twice daily, for 30 days. Percentage decrease in UPC at day 30 compared to baseline was calculated. Data are presented as median (range).

A total of 39 (20 telmisartan-treated, 19 enalapril-treated) dogs were included. Baseline systolic blood pressure, serum creatinine concentration, and UPC were 157 mmHg (120-240), 0.9 mg/dL (0.7-5.0), and 4.7 (0.9-13.4), and 154 mmHg (126-210), 0.9 mg/dL (0.5-5.5), and 2.3 (0.8-15.5) for telmisartan- and enalapril-treated dogs, respectively. On day 30, percentage decrease in UPC was significantly greater for telmisartan-treated (0.8-15.5) vs enalapril-treated (4.70.9-13.4), and 154 mmHg (126-210), 0.9 mg/dL (0.5-5.5), and 2.3 (0.8-15.5) for telmisartan- and enalapril-treated dogs, respectively.

A subset of 10 HA and 10 controls were randomly selected out of the data set and their corresponding CBC and SC data were assessed by 30 veterinarians in a blinded fashion. Based on CBC and chemistry data, veterinarians correctly classified 78% of the cases vs 100% correctly classified by the MLA.

In conclusions, MLA can be implemented as an automatic screening test reported by laboratory, alerting the clinicians of the need to test for HA with confirmatory tests.

Dietary Phosphorus Studies in Cats: Does Calcium Affect Plasma Kinetics?

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Phosphorus (P) is an essential nutrient, present in pet foods in two forms: organic matter (meat and bone meal) and inorganic P (IP) salts. Recently, studies have confirmed that diets with a high IP content can negatively impact feline renal health. We hypothesize that calcium is a factor that may influence post-prandial plasma P levels, potentially providing insights on outcomes following long term exposure.

24 healthy neutered cats were fed a single exposure test meal, 50% daily energy requirement, in a 6-way crossover design trial lasting 12 weeks. Diets contained 0.75 or 1.5 g/1000 kcal P from sodium tripolyphosphate (STPP) and a calcium to phosphorus ratio (Ca:P) of 1, 1.5 or 2. Post-prandial plasma P, ionized calcium (iCa) and parathyroid hormone (PTH) were measured serially via cephalic catheter. Study protocol was reviewed and approved by the WALTHAM Animal Welfare and Ethical Review Body.

Compared to a diet with 1.5 g P from STPP/1000 kcal and Ca:P of 1, increasing Ca:P to 1.5 and 2 reduced the peak plasma P concentrations (1.28 and 1.25 mmoL/L v 1.51 mmoL/L, P ≤ 0.029) and the duration of this peak (plasma P returned to baseline at 240mins v 300mins, P < 0.001). In a diet with 0.75 g P from STPP/1000 kcal, increasing Ca:P prevented a peak in plasma P (P ≥ 0.269). PTH followed the same pattern as plasma P.

Increasing dietary Ca:P can reduce the peak and duration of plasma P and PTH elevation following a meal. Long term feeding trials should
confirm whether these measures reduce the risk of adverse kidney changes.

Clinical Efficacy of an Oral Long-Acting Analgesic with a Human Abuse Deterrent in Perioperative Dogs

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Currently pain control in the perioperative setting is difficult due to the need for frequent injections of opioid drugs in dogs. A novel long-acting oral methadone formulation containing the human abuse/misuse deterrent naltrexone was evaluated in 43 dogs undergoing an ovariohysterectomy (OHE).

Dogs were randomly assigned to one of three treatments, 1) methadone 0.5 mg/kg SC q4h (positive control, n = 13 dogs), 2) methadone 0.5 mg/kg with fluconazole 2.5 mg/kg and naltrexone 0.125 mg/kg PO q12h (n = 15 dogs) and 3) methadone 1 mg/kg with fluconazole 5 mg/kg and naltrexone 0.25 mg/kg PO q12h (n = 15 dogs). Injectable methadone was started 0.5-3 hr prior to IV catheter placement and oral methadone was started 14-17 hr prior to IV catheter placement. Acepromazine (0.05 mg/kg SC) was administered to all dogs 0.5-3 hr prior to catheter placement. Propofol induction doses and GCPS scores were not significantly different (P > 0.05) between treatments. No dogs required rescue analgesia (all GCPS <5). The most common adverse effect was perioperative vomiting, which was not significantly different between the treatments.

In conclusion, a novel twice daily oral opioid formulation containing a deterrent to human abuse/misuse was effective in providing preoperative sedation with acepromazine and for effectively controlling postoperative pain in dogs undergoing an OHE.

Incorporation of a Human Abuse Deterrent into a Long-lasting Oral Opioid Formulation in Dogs

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The purpose of this study was to assess the effects of adding naltrexone as a deterrent to human abuse/misuse of a novel long-acting oral methadone/fluconazole formulation in dogs. The hypotheses were: there would be no statistical differences in central opioid effects, and similar methadone exposure would occur between the groups.

Twelve healthy Beagle dogs were equally divided into two groups in a parallel study. The study was IACUC approved and used FDA approved drugs. Both groups were administered fluconazole, 5 mg/kg PO 12 hours prior to methadone/fluconazole administration. Groups 1 and 2 were administered a combination of methadone (1 mg/kg) and fluconazole (5 mg/kg) PO. Group 2 additionally received naltrexone (0.25 mg/kg) PO concurrently. Rectal temperature was monitored as a marker of central opioid effects. Blood was obtained at intervals for the determination of plasma methadone, naltrexone, β-naltrexol (active metabolite) and naltrexone-glucuronide (inactive metabolite). Rectal temperatures (previously correlated to analgesia in dogs) were decreased from baseline in both groups from 2-12 hours after drug administration and were not significantly different (P > 0.05) between the groups. Similar methadone plasma concentrations were measured in both groups. Naltrexone-glucuronide was not measured in any
that enteroids express P-gp in comparable amounts as reported/C6 (25.4 ± 3 time points and β P-gp gene expression was not significantly different (P = 0.06) used to compare the mean quantitation cycle obtained with qPCR, mean luminal intensity obtained with ImageJ. Kruskal-Wallis test was transfected enteroids. An un-paired breeds. Transfection efficiency was monitored by green fluorescent protein-1, MDR1), was achieved in canine enteroids by lipofectamine. Knockout of the gene coding for P-gp (Multi-drug resistance gp function was then quantitated by fluorescent microscopy and ImageJ. Fluorescence staining of canine enteroids in a transwell culture system was performed to assess the localization of P-gp receptors. Functional assays were performed using rhodamine123 (Rh123), a fluorescent permeate environment. The aim of this research was to assess whether enteroids could serve as a drug testing assay for canine P-gp-mediated intestinal transport. P-gp expression was assessed using qPCR of intestinal tissues and PLE (dog, and a dog infested with roundworms (RW). Immunofluorescence staining of canine enteroids in a transwell culture system was performed to assess the localization of P-gp receptors. Functional assays were performed using rhodamine123 (Rh123), a fluorescent dye substrate for P-gp, with or without verapamil (P-gp inhibitor). P-gp function was then quantitated by fluorescent microscopy and ImageJ. Knockout of the gene coding for P-gp (Multi-drug resistance protein-1, MDR1), was achieved in canine enteroids by lipofectamine transfection with a CRISPR/Cas9 all-in-one plasmid targeting the naturally occurring MDR-1 deletion mutation present in Collies and other breeds. Transfection efficiency was monitored by green fluorescent protein (GFP) expression in both control and CRISPR/Cas9 plasmid transfected enteroids. An un-paired t-tests was used to compare the mean luminal intensity obtained with ImageJ. Kruskal-Wallis test was used to compare the mean quantitation cycle obtained with qPCR, and P = 0.05 was considered as statistically significant. P-gp gene expression was not significantly different (P = 0.06) between enteroids and the epithelial layer of original intestinal tissues (quantitation cycle values expressed in Mean ± S.D): Organoid: PLE (24.5 ± 1.0); RW (24.7 ± 0.3); HC (25.7 ± 0.3); Tissue: PLE (25.4 ± 0.6); RW (26.3 ± 0.7); HC (24.1 ± 0.5). These results confirm that enteroids express P-pg in comparable amounts as reported in vivo. The culture of primary canine enteroids in a Transwell system showed apical expression of P-gp, which is consistent with in vivo observations. Co-incubation with verapamil significantly reduced Rh123 fluorescence in the lumen of enteroids to approximately 50% of that seen with Rh123 incubation alone (P = 0.05), indicating that P-gp-mediated transport function was successfully blocked. In addition, canine 3D enteroids were successfully transfected in a stable manner with CRISPR/Cas9 plasmid to knockout MDR1. In summary, P-gp gene expression, localization, and function in canine enteroids were similar to those of intestinal tissues they were originally derived from. The knockout of P-gp expression in canine enteroids mimics the dysfunctional P-gp present in certain dog breeds and contributing to adverse drug reactions. To conclude, our novel enteroid model can serve as a useful ex vivo system for pharmaceutical drug transport and safety studies in veterinary medicine. Efficacy of Robenacoxib for the Treatment of Chronic Musculoskeletal Disorder-Associated Pain and Inflammation in Cats Duncan Lascelles - North Carolina State University; Derek Adrian - North Carolina State University; Jonathan King - Elanco; Rudolph Parish - Elanco; Stephen King - Elanco; Steve Budsberg - University of Georgia; Margaret Gruen - North Carolina State University Chronic musculoskeletal disorder (CSMD) affects an estimated 30 million cats in the US, with no medications approved for this indication. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the mainstay of treatment of musculoskeletal pain and inflammation in other species. We hypothesized that the NSAID robenacoxib would increase objectively-measured physical activity and improve owner-assessed mobility, pain, quality of life, temperament and happiness in cats with CMSD. Otherwise healthy cats (n = 109) with CMSD and owner-rated impairment were enrolled in a blinded clinical trial. All cats received placebo (p) during a 2-week baseline period, before randomization to receive placebo (P) or robenacoxib (R) for each of two consecutive 3-week treatment periods. Treatment groups were pPP, pRR, and pRP. Activity and subjective owner assessment data were collected. Data were analyzed using mixed-effects and generalized linear models. Following three weeks of treatment, robenacoxib compared to placebo resulted in a 5.7% increase in overall activity (P = 0.24), a 6.6% increase in non-zero activity (P = 0.14), and a 10.0% increase in dusk-to-dawn activity (P = 0.078). Improvement persisted at six weeks. In within-cat analysis, the percentage of cats with higher activity while receiving robenacoxib than while receiving placebo was 17.2% for all counts (P = 0.058), 22.9% for non-zero counts (P = 0.021) and 20.0% for dusk-to-dawn activity (P = 0.035). Robenacoxib treatment was associated with a 50% decrease in owner-assessed disability, significantly more than with placebo (P ≤ 0.044), following six weeks of treatment. There were significant improvements in temperament (P = 0.0039) and happiness (P = 0.021) with robenacoxib. Adverse effects occurred with similar frequencies between groups.</>P>. These data demonstrate that robenacoxib is an efficacious treatment for CMSD in cats.
**Prognosis Following Medical Management Alone of Canine Tracheal Collapse Syndrome (CTCS): Results in 84 Cases**

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Objective to evaluate short, intermediate, and long-term outcomes following multimodality medical management alone for canine tracheal collapse syndrome (CTCS).

Design is a retrospective case series.

Animals are 84 dogs.

Materials and methods are medical records of all CTCS dogs prescribed a multimodality medical regimen were included. Patients receiving endoluminal stents or extraluminal ring prostheses were excluded from further analysis. Medical records were reviewed for pertinent patient data, clinical scores, and survival outcomes. Clinical scores were divided into mild (1-3), moderate (4-7), and severe (8-10) categories.

Results are total of 84 medically managed dogs with CTCS were included and followed for a median of 1130 days (range 107-3134 days). In the study population, 70 dogs had traditional-type tracheal collapse (TTT), 5 dogs had malformation-type tracheal collapse (MTC), and 9 dogs had CTCS of indeterminable type. At the last examination by phone or AMC visit, worsening of clinical signs for honking/raspy breathing, coughing, and dyspnea clinical scores was reported in 36.9%, 34.5%, and 47.6% of dogs despite medical management, respectively. Median survival time (MST) was 1330 days (range 970-1528 days) and older dogs (>8.8 years old) had a shorter MST of 893 days compared to younger dogs (P < 0.01). Dogs with a severe clinical score in at least one category at last examination had a MST between 733 and 953 days.

Conclusions and clinical relevance is multimodality medical management of CTCS alone, in the absence of endoluminal or surgical treatment, was associated with a median survival time of under 4 years in this population of dogs.

**Differentiation of Feline Inflammatory Bowel Disease and Small Cell Lymphoma using Histology Guided Mass Spectrometry**

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Chronic enteropathy in cats mostly comprises inflammatory bowel disease (IBD) and small cell lymphoma (SCL). Differentiation remains challenging despite various diagnostic measures using histopathology, immunohistochemistry, and clonality testing (PARR). Histology guided mass spectrometry (HGMS) is a technology that allows for the analysis of endogenous molecules directly in formalin-fixed paraffin embedded (FFPE) tissue sections with the assistance of histopathology annotation for the targeted analysis of cell subpopulations. HGMS was used to generate in situ molecular fingerprinting from FFPE tissue sections from cats with IBD (n = 19) and SCL (n = 25).

Cases were classified as either IBD or SCL based on histopathology and, where indicated, additionally by immunohistochemistry and clonality testing. FFPE tissue sections were deparaffinized, underwent antigen retrieval, and were subjected to on-tissue trypsin digestion. Mass spectra were collected from histopathologically preselected areas of monomorphic or pleomorphic lymphocytic populations (50-μm target regions).

A linear discriminant analysis classification algorithm was created using the acquired mass spectral data, resulting in 94.3% accuracy when performing a leave-10%-out internal cross validation assessment. A total of 211 peaks were used to build the model, of which, 194 were found to be statistically significant between the two groups after applying a Bonferroni correction. Two significant peaks within the mass spectra underwent further analysis using tandem mass spectrometry and were tentatively identified as beta actin and histone H2A. While expression of beta actin was higher in cats with IBD, histone H2A expression was higher in cats with SCL.

Results indicate that mass spectrometry may be a powerful tool for accurate differentiation of feline SCL and IBD. Further studies are currently in progress to validate these results using an independent larger cohort of samples.

**Ursodeoxycholic Acid (Ursodiol) Significantly Alters the Gut Microbiota and Bile Acid Metabolome in Conventional Mice**

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Ursodeoxycholic acid (Ursodiol) is a naturally occurring bile acid that is used to treat a variety of hepatic and gastrointestinal diseases. Ursodiol has a multitude of beneficial therapeutic effects and can modulate bile acid pools. Bile acids can alter the gut microbiota community structure through various mechanisms. In turn, the gut microbial community can modulate bile acid pools, thus highlighting the interconnectedness of the gut microbiota-bile acid-host axis. Despite these interactions, it remains unclear if and how exogenously administered Ursodiol shapes the indigenous gut microbial community structure and intestinal bile acid metabolome. This study aimed to provide a comprehensive analysis of how Ursodiol alters the intestinal ecosystem in conventional mice.

C57BL/6J wildtype mice were orally gavaged with one of three doses of Ursodiol (50, 150, or 450 mg/kg/day; represented as U50, U150, and U450 respectively) for 21 days. Alterations in the gut microbiota and bile acid pools were examined with 16S rRNA gene Illumina sequencing and targeted bile acid metabolomics in stool, ileal, and cecal content. Bile acids were also measured in serum. Alterations in the microbial community structures were seen in ileal and cecal content compared to baseline, and longitudinally in feces following the 21-day Ursodiol treatment. Specifically, in ileal content, the microbial community structure of the U150 and U450 treatments were significantly different from baseline. In cecal content, the microbial community structure of the U450 treatment was significantly different from baseline. In both ileal and cecal content, members of the Lachnospiraceae family significantly contributed to the alterations observed. Using Random Forest analysis, a significant increase in
serum tauroursodeoxycholic acid (TUDCA), ursodeoxycholic acid (UDCA), and lithocholic acid (LCA) were identified in mice treated with Ursodiol, in a dose dependent manner. In ileal content, UDCA and TUDCA significantly increased in a dose dependent manner. In cecal content, TUDCA and TCDCA were significantly increased.

In conclusion, Ursodiol administration in conventional mice resulted in significant alterations in the indigenous gut microbial community structure and bile acid metabolome. This study is the first to provide a comprehensive profile of how exogenously administered Ursodiol shapes the intestinal ecosystem. Further studies to investigate how these changes in turn modify the host physiologic response are ongoing. In veterinary medicine, Ursodiol is routinely administered, however the ramifications of how this drug impacts the canine and feline intestinal ecosystem is unknown. Additional studies to elucidate how Ursodiol modifies the gut microbiota-bile acid-host axis in these species is necessary.

Indoleamine-Pyrrole-2,3-Dioxygenase-1 is Over-Expressed and Negatively Correlated with Serum Tryptophan Concentrations in Dogs with Protein-Losing Enteropathy

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Indoleamine-pyrrole 2,3-dioxygenase (IDO-1) is the initial rate-limiting step in the pathway for the oxidation of tryptophan to kynurenine. IDO-1 expression is increased in the gastrointestinal tract of humans with inflammatory bowel disease (IBD) due to over-expression of proinflammatory cytokines and over activation of toll-like receptors. This increased IDO-1 expression is associated with lower serum tryptophan concentrations in human IBD due to increased tryptophan catabolism. Dogs with protein-losing enteropathy (PLE) have decreased serum tryptophan concentrations compared to apparently healthy dogs. However, IDO-1 expression has not been previously assessed in the intestinal mucosa of dogs with PLE. Therefore, this study aimed to determine if there was a significant difference in mRNA expression of IDO-1 in the duodenal mucosa of dogs with PLE compared to healthy dogs and whether this expression correlated with serum tryptophan concentrations in dogs with PLE.

Our study was a retrospective study using archived paraffin-embedded duodenal biopsies from 8 client-owned dogs diagnosed with PLE at the Bristol Veterinary School that previously had serum tryptophan concentrations measured as part of a separate study and 8 healthy Beagle dogs from the Iowa State University Service Colony. A novel RNA in situ hybridization (ISH) technology, RNAscope was used to hybridize IDO-1 mRNA probe onto at least 10 duodenal biopsy tissue sections from each dog. RNAscope signals (H-score) were visualized using a microscope and a single operator performed all quantitative assessment.

Dogs with PLE had significantly higher mRNA expression of IDO-1 in the duodenal mucosa compared to healthy control beagle dogs:

(i) average IDO-1 copies per cell: \( P = 0.04 \), mean (±S.D): PLE 3.6 (±1.6), Control 2.1 (±1.1); (ii) average IDO-1 area per cell: \( P = 0.04 \), PLE 3.7 (±1.6), Control 2.1 (±1.1); (iii) percentage IDO-1 positive: \( P = 0.01 \), PLE 31.0 (±8.7), Control 19.4 (±7.1); (iv) IDO-1 H-score: \( P = 0.02 \), PLE 75.7 (±26.0), Control 45.3 (±19.3). mRNA expression of IDO-1 in the duodenal mucosa was negatively correlated with serum tryptophan concentrations in dogs with PLE:

(i) average IDO-1 copies per cell: Spearman’s rank correlation coefficient (SRCC) = −0.74, \( P = 0.043 \); (ii) average IDO-1 area per cell: SRCC = −0.71, \( P = 0.047 \); (iii) percentage IDO-1 positive: SRCC = −0.81, \( P = 0.015 \); (iv) IDO-1 H-score: SRCC = −0.81, \( P = 0.015 \).

Our study suggests that decreased serum tryptophan concentrations in dogs with PLE might be due to increased intestinal IDO-1 expression. Further studies are needed to determine the inflammatory pathways responsible for the increased expression of IDO-1 in the intestinal tract of dogs with PLE.