# 2015 ACVIM Forum Research Reports Program

## Indianapolis, Indiana, June 3–6, 2015

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### THURSDAY, JUNE 4

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### FRIDAY, JUNE 5

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4:25 PM Teresa DeFrancesco  
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4:50 PM Rebecca Stepien  
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5:25 PM Simon Swift  
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5:50 PM Virginia Luis Fuentes  
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ONCOLOGY

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2:35 PM Douglas Thamm  
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3:10 PM Mario Dolera  
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3:35 PM Luca Malfassi  
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4:25 PM Jackie Wypij  
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4:50 PM Jackie Wypij  
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5:25 PM G.K. Ogilvie  
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SMALL ANIMAL INTERNAL MEDICINE

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8:25 AM Chen Gilor  
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9:25 AM Christian Leutenegger  
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4:25 PM Lisa Freeman  
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4:50 PM Eva Furrow  
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EQUINE

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8:25 AM Erica McKenzie  
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**FOOD ANIMAL**

| Time       | Presenting Author | Research Report Title                                                                 |
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**SATURDAY, JUNE 6**

### CARDIOLOGY

| Time       | Presenting Author | Research Report Title                                                                 |
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| 8:25 AM    | Lance Visser      | Diagnostic Value of Right Pulmonary Artery Distensibility Index in Dogs with Pulmonary Hypertension |

### NEUROLOGY

| Time       | Presenting Author | Research Report Title                                                                 |
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| 10:30 AM| Roger Clemmons| PLA Bead Treatment of Refractory Epilepsy in Dogs                     |
| 10:55 AM| G. Diane Shelton | The Spectrum of Inherited Myopathies in Young Labrador Retrievers  |
| 11:30 AM| Dennis O’Brien | Mutation in Laryngeal Paralysis/Polyneuropathy with Ocular Abnormalities and Spongiform Encephalopathy |
| 11:55 AM| Dennis O’Brien | Paroxysmal Non-kinesogenic Dyskinesia in Soft Coated Wheaton Terriers: Mutation and Response to Acetazolamide |
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| 4:50 PM | Stephanie Thomovsky | Serum Melatonin Levels in Normal Dogs and Dogs with Seizures     |

**ONCOLOGY**

| Time    | Speaker       | Title                                                                 |
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| 8:00 AM | Nicola Mason  | Combination Lm-LLO Immunotherapy Plus Radiation Delays Tumor Progression and Prolongs Survival in Osteosarcoma |
| 8:25 AM | Cailin Heinze | Association Between Body Condition Score and Survival in Dogs with Lymphoma and Osteosarcoma |

**SMALL ANIMAL INTERNAL MEDICINE**

| Time    | Speaker       | Title                                                                 |
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| 8:25 AM | Katie Tolbert | Feline *T. foetus* Cytotoxicity Can Be Inhibited by Selective, Small-molecule Cysteine Protease Inhibitors |
| 9:00 AM | Allyson Berent | Outcome Following Ureteral Stent Placement in Dogs for Benign Ureteral Obstructions: 44 Dogs (57 Ureters) 2009–2013 |
| 9:25 AM | Mahalakshmi Yerramilli | Prognostic Value of SDMA to Creatinine Ratio in Dogs and Cats with Chronic Kidney Disease |
| 10:30 AM| Jonathan Fogle | Epigenetics and CD8⁺ T Cell Dysfunction in an FIV Model               |
| 10:55 AM| Polina Vishkautsan | Pharmacokinetics of Voriconazole in Healthy Cats                   |
| 11:30 AM| Roschelle Heuberger | Pain Management and End of Life Care: Results of a National, Cross-Sectional, Survey of Small Animal Owners |
| 11:55 AM| Hiroshi Okawa  | Discovery and Clinical Effectiveness of a Composition That Promotes Hair Growth (Patent Pending) |

**EQUINE**

| Time    | Speaker       | Title                                                                 |
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| 9:00 AM | Nicola Pusterla | Use of Daily Diclazuril Pelleted Top Dress for the Prevention of *Sarcocystis neurona* Infection in Foals |
| 9:25 AM | Melody de Laat | The Effect of Oral and Intravenous Dextrose on C-Peptide Secretion in Ponies |
| 10:30 AM| Derek Knottenbelt | The Prevalence of Large Intestinal Mucosal Pathology in Horses |
| 10:55 AM| Jonathan Foreman | Medical Alternatives to Conventional Cyclooxygenase Inhibitors for Treatment of Acute Foot Pain in Horses |
| 11:30 AM| Julia Montgomery | Initial Characterization of the Tracheal Microbiomes in Healthy Horses and Horses with Heaves |
CARDIOLOGY

CANINE MITRAL VALVE INTERSTITIAL CELL GROWTH IS IMPROVED BY CANINE WHARTON’S JELLY MESCENCHYMAL STEM CELL CONDITIONED MEDIA. Vicky Yang, Dawn Meola, Sarah Crain, Kristen Thane, Airlie Davis, Andrew Hoffman, Tufts University Cummings School of Veterinary Medicine, North Grafton, Massachusetts, USA

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in dogs and the most common cause of congestive heart failure in dogs. Excessive valvular fibrosis will lead to valvular insufficiency, cardiac enlargement, and sometimes contractile dysfunction in the later stages of the disease. As the disease progresses, the amount of valvular regurgitation increases, eventually leading to volume overload, pulmonary venous congestion, and finally congestive heart failure with pulmonary edema. The common histologic findings seen in myxomatous valves include disarray of the collagen and elastin fibers as well as disruption of the microstructure within the valves. This disarray and disorganization is partly a result of phenotypic transdifferentiation of the valvular interstitial cells (VICs) from quiescent fibroblastic cells to myofibroblastic-like cells, which follows the classical paradigm of fibrogenesis. As the transformation takes place, the VIC density decreases, and these cells then express alpha smooth muscle actin (α-SMA) instead of vimentin. Similarly, TGF-β stimulation of VICs in vitro results in fibroblastic (vimentin<sup>high</sup>SMAlow+) to myofibroblastic (vimentin<sup>low</sup>SMAmid+) transition. Given that mesenchymal stem cells (MSC) can exert anti-fibrotic effects, we investigated the effects of conditioned media (CM) derived from canine Wharton’s Jelly MSCs (WJ-MSC) on the growth potential of canine VICs and its ability to counter the effects of TGFβ. VICs were isolated and cultured from normal and diseased canine valves. Immunohistochemistry and real-time (q-) PCR were used to evaluate the expression of α-SMA, vimentin, and collagen in VICs. CM was collected from WJ-MSC culture, and CM exosomes were isolated by either ultrafiltration or ultracentrifugation. Cell growth and replicative capacity were evaluated using MTT assay and colony forming units (CFU). Internalization of WJ-MSC exosomes by VICs was imaged with membrane or RNA staining. qPCR showed that diseased valve VICs had increased myofibroblastic phenotype and decreased cell growth by MTT and CFU. Culturing of VICs of either phenotype with WJ-MSC CM or exosomes alone resulted in increased number of viable cells, and depletion of exosomes had the opposite effect. Exosomal membrane and RNA labeling confirmed exosome uptake into VIC cytoplasm and nucleus, although RNA containing exosomes were internalized by <20% of VICs, implying selective endocytosis of RNA containing exosomes or they are less numerous. Increase in responsiveness to TGFβ stimulation when VICs were cultured in CM was lost in exosomes. WJ-MSC CM and exosomes improved cell growth for VICs isolated from both normal and diseased canine mitral valves and may exert protective effects on VICs to slow the progression of canine MMVD.

CARDIAC BIOMARKERS AND SEMI-DOMINANCE IN A GENETIC MODEL OF FELINE HYPERTROPHIC CARDIOMYOPATHY. Joshua Stern,1 Kun-Ho Song,1 Eric Ontiveros,1 Colin Schwarzwald, Iris Huesler. Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

HCM in Maine coon cats has the same A31P mutation in the cardiac regulatory protein myosin binding protein C as a disease with incomplete penetrance. Here we hypothesized that cardiac biomarkers will reveal a semi-dominant inheritance pattern in cats with the A31P mutation and may identify cats at risk for HCM development or complete penetrance. NTproBNP values were as follows: WT 15 (12.16.75); HET 20 (16.5,27.0); HO 49 (32,131) and were significantly different for each genotype.

In contrast to previous studies of A31P mutant cats, significant NTproBNP elevation was present in the absence of echocardiographic diagnosis of HCM. NTproBNP elevation follows a semi-dominance model where heterozygous cats are less severely elevated than homozygous cats. This matches previous observations in clinical characterization of disease. In context of incomplete penetrance, NTproBNP may represent a tool for early identification of HCM affected cats.

SAFETY AND BIOCOMPATIBILITY OF THE MITREX<sup>®</sup> EPICARDIAL ANNULOPLASTY DEVICE IN A CHRONIC MODEL. Jeffrey Solomon<sup>1</sup>, Thomas Fogarty<sup>2</sup>, Evan Anderson<sup>3</sup>, Pierluca Lombardi<sup>2</sup>. 1Infiniti Medical, Menlo Park, California, USA, 2Fogarty Institute for Innovation, Mountain View, California, USA, 3Maquet Cardiovascular, Wayne, New Jersey, USA

This study evaluated the safety of the myocardial compression required to perform epicardial annuloplasty and the biocompatibility of the Mitrex<sup>®</sup> device. Ten swine (seven test and three control) were used. The Mitrex<sup>®</sup> device was placed in all subjects such that the septo-lateral dimension of the mitral valve was reduced by 15–35%. Echocardiography and angiography were performed pre implant, post implant and at term. Test devices were secured in the test group and removed from the animals in the control group. Necropsy was performed at 180 days. Hearts were pressure fixed and analyzed. Test devices were placed without incident. Coronary flow, ejection fraction, left ventricular wall motion and mitral valve anteroposterior dimension were normal post implantation and at term. There were no remarkable postoperative events and all subjects survived to term with the exception of one test animal that was euthanized due to a non device related complication (refractory pleural effusion). Devices were well tolerated causing only minimal to mild fibrosis and chronic inflammation. No significant changes were observed in the myocardium except for muscle fiber atrophy near the tip of the anterior arm. There appeared to be ample tissue over the tip and no danger of perforation in all but one subject. No meaningful changes were noted in cardiac shape, ventricular wall thickness, chamber size, heart valves, and blood vessels. Myocardial compression necessary to perform epicardial annuloplasty was well tolerated. The Mitrex<sup>®</sup> device was safe and biocompatible.

ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT ATRIAL SIZE IN HORSES: DIAMETER OR AREA, DOES IT MATTER? Colin Schwarzwald, Iris Huesler. Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Echocardiographic assessment of left atrial (LA) size is routinely done in horses. A variety of conventional (linear) and novel (area-based) indices of LA size have been described, but their clinical use is not standardized and novel indices are poorly established. The goals of this study were to define reference intervals for indices of LA size in horses and to provide prove of concept for the use of area-based indices of LA size in this species. The agreement between conventional linear measurements and novel area-based indices of LA size was assessed in a population of healthy horses and horses with valvular regurgitation.

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Forty healthy horses (11 ± 4 years, 547 ± 84 kg) and 112 horses with mitral and/or aortic insufficiency (15 ± 6 years, 554 ± 76 kg) were included in this study. Echocardiographic examination was performed by a single operator (CCS) using a standardized protocol (GE Vivid 7 Dimension). The maximum LA diameter measured in a right-parasternal long axis view (LADmax), the maximum LA diameter measured in a left-parasternal long axis view (LADmaxL), the maximum LA area measured in a right-parasternal short axis view (LAareaAxLmax) were measured by the same operator. Measurements were allometrically scaled to a body weight of 500 kg. Reference intervals were calculated based on a subpopulation of 31 healthy Warmblood horses (Reference Value Advisor). Method agreement was investigated using linear regression, calculation of weighted Kappa (κ) and Bland-Altman analyses. The level of significance was 0.05.

For this study population of healthy and diseased horses, LADmax (reference interval 10.5–13.2 cm) was 0.9 (±0.9 to 2.7) cm [mean bias (limits of agreement)] smaller than LADmaxL (reference interval 11.8–14.0 cm); they were significantly related (P < 0.01, r² = 0.45) and in moderate agreement (κ = 0.59) for classification of LA dimensions in reduced, normal, or increased size. Of the horses with a LADmax within normal limits, 10% (12/118) had a LADmaxL above normal. Conversely, 6% (7/110) of horses with LADmaxL within normal limits had a LADmax above normal. The LAareaAxLmax (reference interval 82–103 cm²) was 18.9 (±9.0 to 27.0 cm²) smaller than LAareaAxLmax (reference interval 84–134 cm²); they were significantly related (P < 0.01, r² = 0.51). LADmax was significantly associated with LADmaxL (P < 0.01, r² = 0.80) and LAareaAxLmax (P < 0.01, r² = 0.50), respectively. However, out of all horses that had a LADmaxL within normal limits, 9% (11/121) were diagnosed with an enlarged LA based on LAareaAxLmax (k = 0.76) and 3% (4/121) had an enlarged LA based on LAareaAxLmax (k = 0.48). Of the horses with both LADmax and LADmaxL within normal limits, 7% (7/104) had an enlarged LA based on LAareaAxLmax and 3% (3/104) had an enlarged LA based on LAareaAxLmax, respectively. Of the horses with both LADmax and LAareaAxLmax within normal limits, 2% (2/94) had an enlarged LADmax and 6% (6/94) had an enlarged LADmaxL. Method agreement between LADmax and LAareaAxLmax was fair (κ = 0.29). Of the horses with a LAareaAxLmax within normal limits, 4% (4/110) had a LADmaxL above normal. Conversely, 17% (23/132) of horses with LAareaAxLmax within normal limits had a LADmaxL above normal.

In conclusion, linear measurements and area-based measurements of LA size are not always in good agreement. Therefore, LA enlargement should not be diagnosed based on a single unidimensional measurement. Instead, multiple measurements of LA size including both linear and area-based variables should be obtained to complement subjective assessment of LA dimensions in clinical patients.

**EVALUATION OF POINT-OF-CARE LUNG ULTRASOUND (VETBLUE PROTOCOL) FOR THE DIAGNOSIS OF CARDIOGENIC PULMONARY EDEMA IN DOGS AND CATS WITH ACUTE DYSPNEA.** Jessica Ward1, Greg Lisciandro2, Sandra Tou1, Bruce Keene1, Teresa DeFrancisco1, 1NC State University College of Veterinary Medicine, Raleigh, North Carolina, USA, 2Hill Country Veterinary Specialists & FASTV-eTM, San Antonio, Texas, USA

Point-of-care lung ultrasound (LUS) is an emerging imaging technique that can suggest the presence of cardiogenic pulmonary edema (CHF) by identifying ultrasound artifacts (B-lines) caused by interstitial or alveolar fluid. This study was designed to determine the accuracy of a protocolized LUS technique for diagnosing CHF in dyspneic dogs and cats. Seventy-six dogs and 24 cats were enrolled for evaluation of acute dyspnea. Exclusion criteria included trauma, pleural effusion, and the lack of a thoracic radiograph within 6 hours of LUS. Patients underwent LUS, quantifying the presence of B-lines at 4 sites on each hemithorax. An individual site was scored as positive if ≥3 B-lines were observed. LUS with ≥2 positive sites on each hemithorax was considered positive for CHF. Medical records were then evaluated for final diagnosis.

Patients with a higher number and different distribution of positive LUS sites compared to patients with noncardiac disease. Sensitivity and specificity of LUS for diagnosing CHF was 84% and 72%, respectively. When considering cats only, sensitivity and specificity of LUS was 87% and 89%, respectively. Diagnostic accuracy of LUS was similar to thoracic radiographs. LUS tended to misdiagnose CHF in cases of diffuse interstitial or alveolar disease, such as ARDS. Inter-observer variability for quantification of B-lines was low (kappa statistic: >0.85).

In conclusion, LUS was useful in predicting CHF as the cause of dyspnea, particularly in cats. Considering the utility and rapidity of this technique, point-of-care LUS should be considered as a diagnostic tool for dyspneic veterinary patients.

**USE OF NT-proBNP AND cTnI CONCENTRATIONS TO DETECT HEART DISEASE IN WHIPPETS,** Rebecca Stepien1, Virginia Luis Fuentes2, Heidi Kellihan1. 1University of Wisconsin School of Veterinary Medicine, Madison, Wisconsin, USA, 2Royal Veterinary College, London, UK

Systolic heart murmurs in whippets may be due to myxomatous mitral valve disease (MMVD) or may be unassociated with any structural heart disease (HD). Echocardiography (ECHO) may be required to differentiate MMVD from functional murmurs in screening programs but is not always available. In this pilot study, we compared the ability of NT-proBNP (BNP) and cTnI concentrations versus auscultation to predict ECHO-documented HD in a cohort of outwardly healthy whippets screened at a national show.

One hundred thirty five dogs underwent auscultation, ECHO and blood sampling for BNP and cTnI analysis. ECHO results were reviewed to identify the presence of HD based on presence of any of the following: mitral valve prolapse, mitral regurgitation, left atrial/ventricular dilation and presence of ventricular premature complexes during ECHO exam, and categorized as ECHO negative (ECHO−) or positive (ECHO+) for evidence of HD. Blood test results were analyzed (P < 0.05) for all dogs as a group (n=153), and for dogs with heart murmurs (n = 73). [BNP] is expressed as pmol/L; [cTnI] as nmol/mL. ECHO+ prevalence in this population was 47/135 (35%). 73/135 (54%) dogs had systolic murmurs (MUR), 41/73 (55%) left basilar (LB) and 32/73 (24%) left apical (LA). ECHO+ prevalence in MUR dogs was 55% (40/73). Median [BNP] was higher in dogs with LA (634 [range 169–2,303], P < 0.0001) versus no murmur (304 [112–1,048]) or LB (279 [164–804]). [BNP] was higher in ECHO+ dogs (574 [169–2,301], n = 47) versus ECHO− (297 [112–988], n = 88, P < 0.0001). In MUR dogs, [BNP] was significantly higher in ECHO+ dogs (578 [169–2,303], n = 40) versus ECHO− (364 [164–804], n = 33, P = 0.004).

Median [cTnI] was higher in LA dogs (0.07 [0.02–0.26], P < 0.0001) versus those with no murmur (0.03 [0.01–0.25]) or LB (0.03 [0.01–0.26], P < 0.0001). In MUR dogs, [cTnI] was higher in ECHO+ dogs (0.06 [0.01–0.26], n = 47) versus ECHO− (0.03 [0.01–0.25], n = 88, P = 0.0001). Test characteristics are reported below:

| Test | Sensitivity (Se) | Specificity (Sp) | Positive Predictive Value (PPV) | Negative Predictive Value (NPV) | Area Under the Curve (AUC) | P value |
|------|----------------|----------------|-----------------------------|-------------------------------|---------------------------|--------|
| BNP  | 0.70           | 0.83           | 0.80                        | 0.90                          | 0.70                      | <0.0001|
| cTnI | 0.065          | 0.83           | 0.90                        | 0.83                          | 0.065                     | <0.0001|

The results of this pilot study suggest that in the absence of auscultation, both [BNP] and [cTnI] may be useful to distinguish between whippets with HD from those without HD. If murmur status is known, the PPV of both tests is improved.
STENT ANGIOPLASTY FOR TREATMENT OF BALLOON RESISTANT CANINE VALVULAR PULMONIC STENOSIS. Simon Swift1, Ivan Sosa 1, Amara Estrada 1, Ashley Jones 1, Curt Fudge 1. College of Veterinary Medicine, Gainesville, Florida, USA. 2Congenital Heart Center, Gainesville, Florida, USA

Canine valvular pulmonic stenosis has been categorized based on the degree of leaflet thickening, leaflet fusion and pulmonary artery hypoplasia. Dogs with mild and moderate disease have normal life expectancies but those with severe disease die prematurely. Balloon dilation is often successful in severe cases with thin fused leaflets, resulting in decreased clinical signs and increased life expectancy. Dogs with thickened, dysplastic valves and pulmonary artery hypoplasia show limited response to balloon dilation. Two case reports describe the use of stents to treat obstruction to pulmonary blood flow in dogs. In one, 2 dogs with supravalvular stenosis were treated successfully with stents. In the second, 2 dogs with severe dysplastic pulmonary valve stenosis initially did well, but stenosis recurred within 6 months.

We describe the use of bare metal stents to treat severe dysplastic pulmonary valve stenosis in 3 dogs. All dogs initially had a single stent implanted with a good reduction in pressure gradients. This has been maintained long term in 2 dogs. One dog suffered a stent fracture resulting in stent embolization. That dog subsequently underwent repeat cardiac catheterization with four additional stents placed in the right ventricular outflow tract to treat severe, dynamic subvalvular pulmonic stenosis. All dogs continue to receive atenolol and clopidogrel.

Our findings suggest that in selected patients, stenting of severe dysplastic pulmonary valve stenosis is a viable option and can provide long term relief of the obstruction.

ONLINE SURVEY TO ASSESS INTER- AND INTRA-OBSERVER AGREEMENT ON ECHOCARDIOGRAPHIC CLASSIFICATION OF CARDIOMYOPATHY IN CATS. Lois Wilks1, Virginia Luis Fuentes 1, Mark Rishniw 1. The Royal Veterinary College, Hatfield, Hertfordshire, UK. 2Cornell University, Ithaca, New York, USA

Aim: to investigate inter- and intra-observer agreement on echocardiographic classification of cardiomyopathy in cats.

An online survey was devised with video loops and still images of echocardiographic recordings compiled from 21 cats with a range of cardiac phenotypes. Signalment and a brief clinical history were provided. The survey was distributed via the Veterinary Information Network (VIN) to members of a cardiology list serve. A list of 13 possible diagnoses was provided and participants were asked to indicate which described each case most accurately. Selection of multiple diagnoses was not allowed and respondents were asked to include a brief justification for their choice. Intra-observer agreement was assessed by repeating 4 of the cases. Fleiss kappa (κ) was calculated for all participants with complete responses to determine inter and intra-observer agreement. In order to assess whether agreement was greater among board-certified cardiologists, agreement was also calculated using only responses from ACVIM or ECVIM diplomates.

The survey was attempted by 86 participants routinely involved in echocardiographic assessment of cats with myocardial disease. Participants’ clinical experience in veterinary cardiology ranged from 6 months to 42 years; 49% were ACVIM diplomates and 25% were ECVIM diplomates. No case was scored with perfect inter or intra-observer agreement. Overall, inter-observer agreement ranged from x0.17 to 0.96 depending on the case, and intra-observer agreement x0.33 to 0.67. Among ACVIM/ECVIM diplomates, inter-observer agreement was x0.18 to 0.95 and intra-observer agreement x0.29 to 0.67.

The observed range of inter-observer Fleiss kappa values demonstrates that classification of cats with cardiomyopathy was inconsistent between participants, suggesting that different criteria may be in use. Intra-observer agreement was also poor, suggesting that irrespective of the diagnostic criteria used, they are inconsistently applied. Agreement might be improved by standardizing the echocardiographic criteria used to diagnose different cardiomyopathy phenotypes.

FELINE HYPSOMATOTROPISM IS A NATURALLY OCCURRING, REVERSIBLE CAUSE OF MYOCARDIAL REMODELING. Kieran Borgreat 1, Stijn Niessen 2, Christopher Scudder 1, Ruth Gostelow 1, Sophie Keyte 1, Julia Sargent 1, Patrick Kenny 1, Yaiza Forcada 1, David Church 1, Virginia Luis Fuentes 1, David Connolly 1. 1Royal Veterinary College, London, UK. 2Highcroft Veterinary Referrals, Bristol, UK

In humans, hypsomatotropism caused by a functional pituitary mass is recognized as a cause of increased left ventricular (LV) mass, and cardiovascular complications are a major cause of morbidity and mortality. We hypothesized that feline hypsomatotropism was associated with increased LV wall thickness and left atrial (LA) dilation, compared to control groups of non-hypsomatotropistic diabetic and age-matched healthy cats.

Cats with confirmed hypsomatotropism (IGF-1 >1,000 ng/mL and pituitary mass; n = 57) were prospectively recruited, as were two control groups: diabetic cats (IGF-1 <1,000 ng/mL; n = 27) and healthy cats with no history of diabetes or cardiovascular disease (n = 41). Cats with other endocrinopathies were excluded. Echocardiography was performed in all cases and studies were measured by one trained operator.

Normally distributed continuous variables were compared using a one-way ANOVA. Non-normally distributed variables were compared using a Kruskal-Wallis test. Categorical data were compared using Chi-square tests. Paired data were compared using Wilcoxon’s signed rank test. Significance was P < 0.05, with correction for pairwise comparisons.

There was no age difference between groups (P = 0.243). Cats with hypsomatotropism had a greater maximum LV wall thickness (6.7 mm, 4.1–10.1 mm) than diabetic (5.2 mm, 4.0–9.1 mm; P < 0.001) or normal cats (5.3 mm, 3.9–6.5 mm; P < 0.001). LA diameter was greater in cats with hypsomatotropism (17 mm, 14.1–29.5 mm) than in diabetic (15.3 mm, 10.1–21.3 mm; P < 0.001) and healthy cats (15.8 mm, 11.2–21.5 mm; P < 0.001). Aortic insufficiency was more common in both cats with hypsomatotropism and diabetes than in normal cats (P < 0.001). After hypophysectomy (n = 38), echocardiographic changes were mostly reversible (Figure 1). Hypsomatotropism is a naturally occurring, reversible cause of LV hypertrophy and LA dilation in cats.

DIAGNOSTIC VALUE OF RIGHT PULMONARY ARTERY DISTENSIBILITY INDEX IN DOGS WITH PULMONARY HYPERTENSION. Lance Visser, Minu Im, Joshua Stern. Department of Medicine & Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, California, USA

We sought to determine the value of right pulmonary artery distensibility index (RPADI) for the prediction of Doppler-derived estimates of systolic pulmonary artery pressure (sPAP) in dogs with pulmonary hypertension (PH) compared to other echocardiographic indices of PH.
Dogs with tricuspid regurgitation (TR) permitting Doppler-derived estimates of sPAP were prospectively recruited and grouped into control (n = 20; sPAP <30 mmHg) and dogs with mild (n = 12; sPAP 30–50 mmHg), moderate (n = 12; sPAP 50–
75 mmHg), and severe (n = 14; sPAP >75 mmHg) PH. Indices of 
PH quantified were RPADI (percent change in diameter of the 
PA from systole to diastole), pulmonary artery-to-aortic diameter 
( PA: Ao), acceleration time to peak PA flow velocity (AT), and 
AT-to-ejection time of PA flow (AT:ET). Associations between 
indices of PH, sPAP, right ventricular fractional area change 
(FAC), age, gender, heart rate, and body weight were performed 
using linear regression. Receiver operating characteristic analysis 
was performed to determine the optimal cutoff values for the 
indices of PH in the prediction of moderately increased sPAP (>50 mmHg).

RPADI (r = –0.89) showed the strongest correlation to sPAP 
followed by PA:Ao (r = 0.74), AT and AT:ET (both r = 0.69), 
and FAC (r = 0.52). AT weakly (r = 0.42) correlated with heart 
rate. No other significant correlations were identified. Cutoffs 
to predict moderate PH were defined for RPADI (29%; sensitivity [Sn] 
81%; specificity [Sp] 94%), AT:ET (0.30; Sn 65%; Sp 100%), AT 
(54 ms; Sn 81%; Sp 83%), and PA:Ao (1.04; Sn 100%; Sp 67%). 
RPADI may be useful and predictive of PH in dogs if TR is absent.

NEUROLOGY
EVALUATION OF SYSTEMIC MICRORNA ADMINISTRATION 
FOR IMMUNOMODULATION IN THE TREATMENT OF CANINE GLIOMA. C. Elizabeth Bouderau1, Nasser Yaghibi2, 
Padmanabha Chivukula1, Xiaoyang Ling2, Brian Porter3, Gwen- 
dolyn Levine1, Joseph Payne2, Amy Heimberger2, Jonathan 
Levine1, 1College of Veterinary Medicine and Biomedical Sciences, 
Texas A&M University, College Station, Texas, USA, 2University of Texas MD Anderson Cancer Center, Houston, Texas, USA, 
3Arcturus Therapeutics, San Diego, California, USA

The signal transducer and activator of transcription 3 (STAT3) 
pathway is a key regulator of tumorigenesis and tumor-mediated 
immune suppression. MicroRNA (miR)-124 has been shown to 
be an inhibitor of STAT3 signaling. In rodent models of glioma, 
systemic administration of nanoparticle-encapsulated miR-124 
(designated LUNAR-301) results in down-regulation of STAT3, 
up-regulation of anti-tumor immune effector responses, tumor 
regression, and enhanced survival. Additionally, miR-124 can 
reverse immune suppression and T-cell anergy in immune cells 
from human glioblastoma patients.

We investigated STAT3 and miR-124 expression levels in 
canine astrocytomas (n = 28) and normal brain (n = 6) to 
determine if these tumors share key biologic features with human 
and rodent gliomas. We found that miR-124 expression is absent and 
STAT3 is increased in canine gliomas relative to normal brain. 
The level of STAT3 expression and the number of glioma-infil-
trating CD3+ T cells directly correlated with astrocytic grade.

Next we investigated the safety and pharmacokinetics of 
LUNAR-301 in healthy dogs (n = 5). No significant adverse 
events or toxicity was noted during single-dose escalation or 
during sustained dosing. LUNAR-301 induced in vivo up-regulation 
of immune effector responses as detected by flow cytometry. 
Pharmacokinetic studies confirmed delivery of miR-124 to the 
immune compartment during intravenous administration of 
LUNAR-301. Cumulatively, these data indicate that STAT3 is 
an operational therapeutic target in canine high-grade gliomas 
and that systemic administration of LUNAR-301 is safe and fea-
sible. We are initiating a phase I/II clinical trial in client-owned 
canines with spontaneously arising high-grade gliomas to ascer-
tain if LUNAR-301 can induce radiographic regression and pro-
long survival.

ONSCREEN-GUIDED BRAIN TUMOR RESECTION 
THROUGH REGISTRATION OF A VARIABLE-SUCTION 
TISSUE RESECTION DEVICE WITH A NEURONAVEGA-
TION SYSTEM. Rebecca Packer, Stephanie Engel. College of 
Veterinary Medicine and Biomedical Sciences, Colorado State 
University, Fort Collins, Colorado, USA

This study describes a surgical technique in which a variable-suction 
tissue resection device is linked to a neuronavigation sys-
tem for direct on-screen guidance of brain tumor excision. This 
technique is considered the first step towards minimally-invasive, 
guided neurosurgery to excise otherwise inaccessible deep intrax-
traventricular tumors. Goals of this study were 1) improved access to deep 
and poorly accessible masses; 2) reduced surgical trauma by using 
targeted approaches and improved resection tools.

A retrospective evaluation of 7 dogs and 1 cat that underwent 
brain tumor excision using the NICO® Myriad (NICO Corpora-
tion) and Braini.nihg neuronavigation system (Rogue Research) was 
performed. The patient and neuronavigation instrument were registered 
to the neuronavigation system. Surgery was guided by real-time on-
screen visualization of the resection instrument position relative to 
the pre-operative MRI images, cross-referenced with direct visual-
ization where possible. In 7 of 8 cases degree of resection was evalu-
ated on post-operative MRI: gross-total resection (GTR, no 
residual tumor evident), near-total resection (NTR, <100% but 
>90% resection), or sub-total resection (STR, <90% resection). Of 
these 7 cases, 2 achieved GTR (1 equivocally), 3 achieved NTR 
(99%, 91%, 90%), 2 achieved STR (35%, 26%).

Neuronavigation-guided resection of cortical and subcortical 
canine brain tumors using the NICO® Myriad was feasible, and study 
goals were achieved in part. These refinements are required. Further studies will 
adapt this technique to minimally-invasive port-based surgical 
approaches for deep brain tumor resection (BrainPath™), to improve 
visibility and minimize effects of brain shift. The impact on clinical 
outcome for minimally-invasive approaches must also be determined.

A SIMPLIFIED METHOD OF WALKING TRACK ANALYSIS 
TO ASSESS LOCOMOTION IN DOGS WITH ACUTE SPINAL 
CORD INJURY. Rachel Song, Maureen O'Drach, Ronaldo da Costa, 
Sarah Moore. The Ohio State University, Columbus, Ohio, USA

The utility of a “finger painting” technique for walking track 
analysis was evaluated in 20 control dogs and 29 dogs with acute 
thoracolumbar spinal cord injury (SCI) caused by spontaneous 
intervertebral disc extrusion (IVDE). Stride length (SL), base-of-
support (BS) and the co-variance (COV) for both parameters 
were measured in all four limbs at three separate time points in 
normal dogs and on days 3, 10 and 30 following laminectomy in 
dogs with SCI. SL and BS were compared between control and 
SCI-affected dogs at each time point during recovery. P < 0.05 
was considered a statistically significant difference.

Mean SL (cm) for control dogs was 43.69, 43.68, 43.43, 43.54 for 
the left thoracic limb (TL), right TL, left pelvic limb (PL), and 
right PL respectively. COV-SL was 0.13 in each of four limbs in 
normal dogs and on days 3, 10 and 30 following laminectomy in 
dogs with SCI. SL and BS were compared between control and 
SCI-affected dogs at each time point during recovery. P < 0.05 
was considered a statistically significant difference.

Mean SL of all four limbs was significantly shorter in 
SCI-affected dogs at day 3, 10 and 30 compared to normal dogs. 
The mean difference in SL between normal and SCI-affected dogs 
decreased significantly over time with recovery from injury. BS-PL (cm) 
was significantly wider in SCI-affected dogs compared to con-
trols at days 3 and 30 after SCI surgery. BS-PL was significantly wider 
between groups at any time-point. These findings support the utility 
of this simplified method of walking track analysis to compare dif-
fences in pelvic limb SL between normal and SCI-affected dogs, 
and to assess changes in SL as a marker of recovery after SCI.

ATTEMPTS TO BREED OUT CHIARI-LIKE MALFORMATION: 
IS A CROSS THE ANSWER?. Susan Knowler1, Henny 
van den Berg2, Eric Noorman1, Roberto La Ragione1, Clare 
Rushbridge1. 1University of Surrey, Guildford, UK, 2Dierenklinik 
den heuvel, Best, The Netherlands

Chiari-like malformation (CM) and syringomyelia (SM) are 
complex inherited disorders observed most commonly in toy 
breed dogs and can cause a significant loss in quality of life through 
pain and disability. Branchycephalism is a risk factor and it has 
been suggested that crossbreeding with a different breed/s then 
backcrossing may produce individuals free of disease. This two 
and a half year project took advantage of a cross between a me-
phaslic normal Australian terrier and CM affected Griffon Brux-
lois (GB) with subsequent backcrossing to a GB to investigate the 
inheritance and phenotype of these conditions and a means of 
reducing the incidence of CM and risk of SM.

The study cohort comprised 2 control dogs (CM affected Aus-
tralian terrier and unrelated GB without CM) and a single of fam-
ily of 29 dogs, 12 of which were used in 8 different mating 
combinations resulting in 19 progeny. T1-weighted sagittal DICOM 
images were analyzed for traits (2 angles, 2 lines and a “best fit”
circle diameter) shown previously to have the greatest significance for CM in the GB. The quantitative findings in this study revealed these traits to be significant for CM-affectedness. Furthermore the external phenotypes showed that by outcrossing breed types and selecting for the conformation characteristics in the F1 generation, it is possible to regain the GB breed standard in the F2 generation and reduce the degree of CM. However this is dependent on careful selection of conformation and screening for CM and SM at 1 year of age. The 4 dogs affected with SM in the study all exhibited reduced caudal skull development compared to their relatives.

We showed that traits on MR images were useful to distinguish the phenotype and these exhibited segregation and may be additive towards the severity of CM. It suggests such traits might be useful to quantify the condition and the risk to SM. We propose that grading of CM takes account of quantitative traits that can be used in Estimated Breeding Values (EBV) to assist breeders with their mate selections. Such a system will have to be verified to ensure appropriateness for all breeds at risk.

CYTOGENETIC ALTERATIONS DEFINE TUMOR SUB-TYPES AND KEY PATHWAYS IN CANINE PRIMARY GLIOMAS. Peter Dickinson, Daniel York, Robert Higgins, Richard Loccouten, Daniela Bannasch, UC Davis School Of Veterinary Medicine, Davis, California, USA

Defining commonly occurring cytogenetic abnormalities in canine glioma is essential to an understanding of gliomagenesis, and the definition of appropriate therapeutic strategies. We used Illumina 173K SNP arrays to determine copy number alterations and allelic imbalances in 38 histologically confirmed spontaneous canine gliomas (11 astrocytomas, 23 oligodendrogliomas, 4 mixed oligoastrocytomas and the J3T cell line). Copy number calls and allelic events were determined by matched paired analysis using BioDiscovery Nexus Copy Number™ software.

Hierarchical clustering by aberration profile revealed two major groups with 5/6 glioblastomas and 2/3 grade II astrocytomas comprising one group. Comparative analysis showed loss of the INK4A locus (CDKN2A/CDKN2B tumor suppressor genes) was significantly associated with glioblastomas. Querying for aberrant events in specified genes showed common losses/loss of heterozygosity of tumor suppressor genes and amplification of oncogenes in the TP53, RB1 and PIK3/AKT/RAS pathways. Specifically, loss of TP53, CDKN2A/P14, CDKN1A/P21 and gain of MDM4 was present in the TP53 pathway; loss of RB1, CDKN2A/P16, CDKN2B/P15, and CDKN2C/P18 was present in the RB1 pathway and loss of PTEN and gain of PDGFRα and FGFR1 was present in the PI3K/AKT/RAS pathways. Evaluation of oligodendrogliomas for deletions syntenic to the commonly described 1p loss in human oligodendrogliomas revealed a syntenic loss in CFA 5 in 36% of canine tumors.

The preclinical data suggest that canine gliomas have pathway alterations in common with human tumor counterparts, however further definition of the pathways in greater detail is warranted to inform appropriate choice of targeted therapies and interpretation of future therapeutic trials.

METRONOMIC CHLORAMBUCIL CHEMOTHERAPY FOR CANINE GLIOMAS: A PHASE I/II CLINICAL TRIAL. R. Timothy Bentley*, Aaron Cohen-Gadol, David Jones, Deborah Knapp†, Pardee Veterinary Medicine, West Lafayette, Indiana, USA. 1Indiana University, Indianapolis, Indiana, USA

Gliomas in dogs are typically fatal and limited information is available to guide therapy. Spontaneous brain tumors in client-owned dogs have been suggested as a translational model for the development of human therapies. Chlorambucil is reported to display negligible penetration of the blood-brain tumor-barrier. Chlorambucil could not generally be detected in the cerebrospinal fluid. Metronomic chlorambucil chemotherapy is well tolerated in dogs with glioma and may penetrate the blood-brain tumor-barrier in individual cases. There is preliminary evidence of activity.

CANINE PERIPHERAL NERVE SHEATH TUMORS: CLINICAL ASPECTS, MAGNETIC RESONANCE IMAGING FINDINGS AND COMPARISON OF PALLIATION, SURGERY AND STEREOTACTIC RADIOTHERAPY. Mario Dolera, Luca Malfassi, Simone Pavel, Massimo Sani, Giovanni Maza, Silvia Marcarini, Nancy Carrara, Sara Finesco. La Cittadina Fondazione Studi e Ricerche Veterinarie, Romanengo, Italy

No updates for canine peripheral nerve sheaths tumor (PNST) appeared in recent literature. The aim of this study was to evaluate the correlation between clinical aspects and MRI findings of tumors involving a major peripheral nerve, plexus or root and to determine the survival time in dogs treated with palliation, surgery or stereotactic radiotherapy (SRT).

Records of dogs with PNST evaluated from 2000 to 2014 were reviewed to determine signalment, duration of clinical signs, neurological examination, MRI features, treatment option (palliation, surgery, stereotactic hypofractionated radiotherapy). Time to first event, survival times and statistical differences across categories were calculated by the Kaplan-Meier product limit method and log-rank test. Forty-seven dogs (median age 9 years, male:female ratio 1.76) were included, with Labrador retriever over-represented (17%). Roots lesions were the most frequent (46.8%), with C5-T1, V nerve and left side more involved (25.5%, 19.1% and 61.7%). Presenting signs were lameness, paresis and pain. Mean duration of clinical signs was 90 days. MRI findings comprises increased diameter, hyper intense and contrast enhancing nerve roots (57.1%), plexus or peripheral nerve (42.9%), focal hypointrometric and muscle hyper intensity (73%). The time to first event was 30 days after surgery and 240 days after SRT. Overall mean survival was 97, 144 and 371 days with palliation, surgery and SRT.

A predilection for Labrador retriever is observed. Comparing our results with published data, SRT seem to promise better results than palliation or surgery and warrant further evaluation.

PERIPHERAL GLYCAEMIA IN DOGS WITH LIMB THROMBOSIS: A PROSPECTIVE STUDY. Mario Dolera, Luca Malfassi, Roberto Vallati Facchini, Sara Finesco, Giovanni Maza. La Cittadina Fondazione Studi e Ricerche Veterinarie, Romanengo, Italy

The aim of this study was to document the peripheral glycaemia variations in hypoperfused limbs of patients affected by Metronomic Resonance Imaging (MRI)-confirmed arterial thrombosis.

Eleven dogs were recruited. Inclusion criteria were a clinical examination supportive of limb hypoperfusion and availability of blood cell count, biochemical profile and urine analyses. Two blood samples were sampled, one from the affected limb and one from a healthy limb. Plasmatic glycaemia was measured using an automated glucose analyser. All the patients underwent a total body MRI that provided the final diagnosis.

The thrombus was located: in the abdominal aorta (7/11), in the subclavian artery (1/11), in the axillary artery (1/11), in the iliac arteries (2/11). Of the total abdominal aortic thrombosis, 5/7 involved also the internal iliac arteries. 2/7 the external iliac arteries and 3/7 the subclavian artery. The extent of the thrombosis was classified as grade 1 when the greatest portion of the thrombus did not reach half of the vessel lumen (1/11); grade 2 when the greatest portion of the thrombus was between 1/2 and 2/3 of the vessel lumen (7/11); grade 3 when the thrombus exceeded 2/3 of the vessel lumen (3/11). A statistical decrease of peripheral glycaemia values was found in sampling arising from the affected limbs. Comparing affected limbs values with healthy limbs measurements from the same patient, the reduction was found from...
SURGICAL STABILIZATION OF CANINE LUMBAROSACRAL SPINE WITH STOP-SCREWS AND ILIAC WINGS SCREWS. Mario Dolera, Luca Mallfassi, Simone Pavesi, Maissimino Salu, Giovanni Mazza, Silvia Marcarini, Nancy Carrara. La Cittadina Fondazione Studio e Ricerche Veterinarie, Romanengo, Italy

Surgical stabilization of canine lumbarosacral spine can be challenging. The aim of this research was to evaluate two surgical techniques to achieve lumbarosacral stabilization in dogs either with normal or transitional vertebrae.

Lumbarosacral instability and degenerative stenosis were evaluated by dynamic Computed Tomography (CT) and Magnetic Resonance Imaging (MRI). In dogs with normal vertebrae two 4.5 mm screws were bicortically inserted in S1 with the heads behind the caudal articular process of L7 to prevent the extension of the lumbarosacral joint; if ventral listhesis of S1 was evident, the screws were augmented by methyl methacrylate. In dogs with transitional vertebrae, two 4.5 mm screws were inserted in the iliac wings, two 3.5 mm screws were inserted in the spinous process of L6 and L7; the emerging screws were embedded in methyl methacrylate after flexion of the lumbosacral spine. In cases of residual radicular compression, dorsal laminectomy and partial disectomy were accomplished. Serial clinical and imaging follow up examinations were performed.

Twenty-two large breed dogs were enrolled. In 14 dogs stop-screws (in 4 augmented) and in 8 dogs iliac wings screws were inserted. 2 dogs required additional decompression. During a mean follow up of 36 months, clinical examination and imaging revealed amelioration of presenting complaints and reduction of radicular compression, with no surgical complications.

Stop-screws and iliac wings technique are effective methods to obtain stabilization and indirect decompression of the lumbosacral joint. Comparing with other described surgical procedures, our obtained results are better but with lesser complications.

FRAMELESS STETEOXY FOR VERTEBRAL IMPLANT GUIDANCE IN DOGS. Fred Wininger1, Nicholas Archambault2, Stephen Frey3. 1Veterinary Specialty Services, St. Louis, Missouri, USA; 2University of Missouri, Columbia, Missouri, USA; 3Rogue Research, Montreal, Quebec, Canada

Multiple pathologies of the dog spine require stabilization with vertebral implants. Optimal implant corridors are described and vary between specific vertebrae throughout the spine. Current placement guidelines use general surface landmarks and proposed angles without opportunity to visualize vital structures at depth. Preferred corridors are often inaccessible because of soft tissue impediment. Potential complications of vertebral implant placement include neuro-vascular compromise or insufficient bone purchase for required biomechanical loads. Trans-articular implants present the added challenge of aligning a vertebral motion units and counteracting biomechanical stresses. Recent reports suggest the frequency of spinal canal violation by vertebral implants is high. Frameless stereotaxy for brain neuronavigation has been described in veterinary literature and validation studies show a high level of accuracy. These techniques utilize high resolution cross-sectional imaging to create data sets. Identification of fiducial markers visible on 3D models (image space) and on the subject intraoperatively (anatomic space) permits fusion for co-localizing guidance. A position sensor is used to track, in real-time, reflective instruments affixed to the skull and surgical tools within a virtual space. The surgeon can identify any point within that virtual space for tissue sampling or treatment. The purpose of this study was to develop a technique for implant placements in the dog spine using image-guided intervention. Unlike the brain, which is encased within the solid calvarium and once registered with the navigation software, all structures can be reliably identified relative to the origin of the coordinate system; There are two major obstacles when considering neuro-navigation of the vertebral column. First, multiple vertebral motion units require individual registration. The vertebral column is made of multiple articulating bones, making a single bone registration inapplicable for the remaining vertebrae. A novel subject tracker was created for attachment to single vertebra for individual registration. The second obstacle in adapting frameless neuro-navigation to the spine is the assignment of fiducial markers. Current brain protocols require pre-imaging procedures for placement of artificial fiducial markers rigidly affixed to the skull or teeth. Pre-imaging surgery and affixing fiducial arrays is logistically difficult in spinal applications. For this reason, identification of natural boney fiducial markers identifiable in both image and anatomic space is preferable. Evaluated landmarks included spinous processes, accessory processes and cranial costovertebral joints. Initially a cadaveric study validating the technique in the thoracolumbar spine was performed. Qualitative measures of spinous process and bone purchase in addition to quantitative measures of trajectory and depth were evaluated. Safety and efficacy findings of zero canal violations, statistically accurate placement, and mostly cross-body implants warranted clinical use. Completed clinical cases included three thoracolumbar fractures with vertebral body screws, two atlantoaxial stabilizations and three lumbarosacral stabilizations with transarticular screws. Stereotactic guidance enabled the surgeon to visualize cross-sectional implantation in real-time. All cases had post-operative imaging with adequate placement of implants and satisfactory clinical outcomes. The described adaptation provides a means of safe and effective placement of vertebral implants. Multiple trajectories could be created with the computerized guidance. Subjectively less soft tissue dissection was necessary and surgical time was minimally extended.

PLA BEAD TREATMENT OF REFRACTORY EPILEPSY IN DOGS. Roger Clemmons. University of Florida, Gainesville, Florida, USA

Gold-bead implants have been used to help treat epilepsy, but they interfere with modern imaging techniques like MRI examinations. Polylactic acid (PLA) is a biodegradable, implantable plastic that appears to offer similar effects to gold material, but degrades over time and is invisible to modern imaging. The purpose of this study was to investigate whether PLA beads (discs 1 mm by 0.5 mm) could be useful in assisting in the control of refractory epilepsy in dogs, when placed in specific acupuncture points thought to affect seizures.

This is a descriptive study of a case series which was done as a non-blinded, preliminary study. Ten patients presenting to the University of Florida Neurology Service who were experiencing frequent seizures that were refractory to more than 2 anticonvulsant medications were enrolled in the study. All patients had systemic evaluations to demonstrate no significant findings, MRI evaluations to demonstrate no significant changes in the central nervous system, and CSF analysis revealing no abnormalities. Many patients experienced cluster seizures requiring emergency interventions to control the seizures. No further adjustments to medications were made during the study, although some anticonvulsants could be reduced once the PLA bead treatment was given. A baseline EEG was performed in all in the anesthetized state (0.5% isoflurane, 15 µg/kg) before and after PLA bead placement and analyzed by a statistical package (Neurostat package of NeuroGuide by Applied Neuroscience, Inc., Seminole, FL). PLA beads were placed in transpositional and classical acupuncture points by a certified veterinary acupuncturist (Chi Institute, Reddick, FL). The injection sites were wiped with ethyl alcohol gel (Purell Advanced Hand Sanitizer, Gojo Industries, Akron, OH) and the PLA beads inserted using a modified 16 ga needle and syringe. Points used were GV20, Long hui, Nao shu, Tian shu, HJ7, LI4, LI9, Bai hui, CV13 and the auricular point, Shen men. Owners were asked to keep calendars of seizure events for comparison of pre- and post-treatment seizure frequency data. Data was compared by pair-t test and ANOVA using a statistical program (SPSS ver 14.0).

Of the ten patients enrolled in the study, 9 were deemed to have satisfactory results by the owners. These patients showed a reduction of seizures by >50%, moreover, the seizure characteristics changed so that seizures were more likely to be singular rather than multiple. (<0.5) The patient who did not respond was euthanized by the owner because of seizures that occurred 2 weeks after bead placement. We later recognized that this is a period of time when patients are most likely to seizure and it was speculated that this was a consequence of the healing from the implantation procedure before the bead took over the protection. One patient who had severe clusters every 13 days prior to bead implantation remained seizure free for 4 years (using re-implantation of beads every 6 months). From preliminary investigations, we found that the bead implantation lasted about 8 months on
average, so patients were re-implemented every 6 months, based upon owner’s desires. EEG examinations of patients prior to implantation showed slow-wave, high-amplitude activity with occasional epileptiform discharges and spike activity. The most frequent wave form was an enhanced alpha-alpha waves pattern. Following bead implantation there was a significant reduction (48 ± 13%) in amplitude of EEG activity. (P < 0.05)

PLA bead placement in specific points around the head and in other acupuncture points which have been found to reduce seizure propensity and significantly reduce seizure activity in myotonic dystrophy patients. There is also evidence of an acute reduction in the electrical activity of the cortex based upon changes in the EEG following bead implantation. This may represent a promising new method to help control refractory seizures in dogs.

THE SPECTRUM OF INHERITED MYOPATHIES IN YOUNG LABRADOR RETRIEVERS, G. Diane Shelton, University of California San Diego, La Jolla, California, USA

Over the past 10 years, several inherited myopathies have been reported in young Labrador Retrievers. While some have distinct clinical phenotypes and can be easily recognized, others require in depth examinations including state of the art evaluation of muscle biopsies and DNA testing. This report describes the current status of inherited myopathies in the Labrador Retriever breed with a guide to clinical recognition.

An early onset of severe and generalized muscle weakness occurring in the first several weeks of life has been described in the congenital myasthenic syndrome associated with an autosomal recessive mutation in COLQ (2014), which encodes the collagenous tail of acetylcholinesterase. Weakness in this disorder is fatigable and anticholinesterase drugs exacerbate the weakness. Severe generalized and progressive weakness has also been described in the X-linked myotubular myopathy associated with a mutation in the lipid phosphatase MTM1 (2010). In both of these disorders, creatine kinase (CK) activity is normal or only minimally elevated. Another, is not as rapidly progressive. Affected dogs can live well into adulthood. The CK activity is normal or only mildly elevated and a muscle biopsy shows a myopathic phenotype with numerous central nuclei. A very mild form of XLMD has recently been identified in several related male Labrador Retrievers (2010). Both affected CK activity and muscle biopsy showed a dystrophic phenotype in muscle biopsies, helps to distinguish this severe myopathy from other congenital myopathies.

Other less severe myopathies can occur at several months of age including autosomal recessive centronuclear myopathy associated with a mutation in PTPRA (2005). The clinical presentation can appear similar to that of x-linked myotubular myopathy, however, is not as rapidly progressive. Affected dogs can live well into adulthood. The CK activity is normal or only mildly elevated and a muscle biopsy shows a myopathic phenotype with numerous central nuclei. A very mild form of XLMD has recently been identified in several related male Labrador Retrievers (2010). Both affected CK activity and muscle biopsy showed a dystrophic phenotype in muscle biopsies, helps to distinguish this severe myopathy from other congenital myopathies.

PAROXYSMAL NON-KINESOGENIC DYSKINESIA IN SOFT COATED WHEATEN TERRIERS IS ASSOCIATED WITH A MISENSE MUTATION IN PIGN AND RESPONDS TO ACETAZOLAMIDE THERAPY, Dennis O’Brien,1 Ana Klicheshki,2 Rebecca Packer,3 Stephanie Thomovsky,4 Jeremy Taylor5 1University of Missouri, Columbia, Missouri, USA, 2Colorado State University, Fort Collins, Colorado, USA, 3University of Missouri, Columbia, Missouri, USA, 4Colorado State University, Fort Collins, Colorado, USA, 5Gulf Coast Veterinary Neurology & Neurosurgery, Houston, Texas, USA

The purpose of this study was to characterize a hereditary, paroxysmal, non-kinesogenic dyskinesia in Soft Coated Wheaten Terriers (SCWT) and identify the responsible mutation.

Medical records and videos were reviewed for 20 SCWT or SCWT/poodle crosses that presented for a movement disorder. Whole genome sequences (WGS) were performed on 2 affected SCWT/poodle crosses that presented for a movement disorder. The produced frame shift predicts a premature stop codon and a truncated gene product missing 730 C-terminal amino acids. DNA was available from 8 other affected BRT which were all homozygous for the variant, while 48 normal BRT were either heterozygous or homozygous for the wild-type allele. Mutations in PIGN cause a similar phenotype in humans, Warburg micro syndrome.
EVALUATION OF SECONDARY NEUROAL INJURY AFTER INTRACEREBRAL HEMORRHAGE IN DOGS BY PERFUSSION-WEIGHTED MRI, PROTON MR SPECTROSCOPY AND HISTOPATHOLOGY, Sun-Hyung Cho1, Dae-Gi An1, Junyoung Park2, Chulhyun Lee1, Dong-In Jung1, Dongwoo Chang1, Ji-Hoon Kang1, Mhan-Pro Yang1, Byoung-Tek Kang1, Cheung Buk National University, Cheongju, Korea, 2Korea Basic Science Institute, Ochang, Korea, 3Gyeongsang National University, Jinju, Korea

Intracerebral hemorrhage (ICH) is a subtype of stroke with high mortality and disability. The effects of ICH upon brain tissue are biphasic, including primary and secondary injury. Although hematoma volume is the main factor that influences ICH outcome, secondary brain injury itself results in severe neurological deficits. Therefore, the purpose of this study was to investigate the mechanisms of secondary neuronal injury by evaluating cerebral blood flow (CBF), metabolites and pathological changes in and around the intracerebral hematoma.

ICH was induced in 5 healthy laboratory beagle dogs by injecting 500 U of bacterial collagenase from Clostridium histolyticum, which was delivered into the parietal lobe over 5 minutes with a microinfusion pump. Perfusion-weighted magnetic resonance imaging (PW-MRI) and proton MR spectroscopy (MRS) were performed serially at 6 different time points using a 3T MR system: before and at 12 hours, 2.5, 4.5, 9.5 and 23.5 days after ICH. Prussian blue and immunohistochemical staining were performed to demonstrate iron, glial fibrillary acidic protein (GFAP), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-10 in and around the intracerebral hematoma.

In hematoma, cerebral blood volume (CBV) was significantly decreased at 2.5 and 4.5 days after ICH. Additionally, significant decrease of CBF was observed at 4.5 days after ICH. In perihematoma, there were no significant changes of CBV and CBF except for the decrease of CBV at 4.5 days after ICH. Proton MRS revealed no abnormality of the levels of N-acetyl-asparate and lactate in perihematoma. A significant loss of GFAP immunoreactivity was noted in hematoma, whereas iron deposition and expressions of TNF-α, IL-6 and IL-10 were increased at 2.5 and 4.5 days after ICH.

In conclusion, decreased CBF, iron overload and inflammation may contribute to ICH-induced injury and could provide therapeutic targets in ICH of dogs.

ONCOLOGY

ANTINEOPLASTIC EFFECTS OF AURANOIN IN CANINE LYMPHOMA CELLS. Douglas Thamm1, Barbara Rose, Travis Lefever, Alex Pyaen. Colorado State University, Fort Collins, Colorado, USA

Lymphoma is a serious condition for which there remain unmet medical needs in humans and dogs. The gold complex auranoin has been utilized as a human therapeutic, primarily as an antitumor agent, and some limited information about its use in dogs has been reported. Antiproliferative and pro-apoptotic activity has been observed in a variety of human tumor-derived cell lines, including carcinomas of the breast, head and neck, ovary, lung, and a variety of hematopoietic tumors, including lymphoma. Putative antitumor mechanisms include inhibition of NF-κB and STAT3 signaling, and induction of reactive oxygen species via thioredoxin reductase (TrxR1) inhibition. The dog is a well-established model for spontaneous lymphoma in humans, owing to striking similarity in biology and gene expression. The goal of the current study was to investigate the antineoplastic effects of auranoin in a panel of canine lymphoma-derived cell lines, and to identify potential biomarkers of drug activity for future translational studies.

Four canine lymphoma -derived cell lines were incubated in varying concentrations of auranoin +/- bortezomib, elesclomol, or the conventional antineoplastic agents doxorubicin (DOX), CCNU and vincristine (VCR) for 24–72 hours. Relative viable cell number was assessed using MTS. Cell cycle distribution and apoptosis induction were evaluated using flow cytometry with propidium iodide staining and Annexin V / PI staining respectively. Induction of ROS was assessed using the redox-sensitive fluorescent dye CM-H2DCFDA. TrxR activity was assessed using a commercial colorimetric kit (Cayman). Changes in STAT3 and NF-κB phosphorylation status were assessed via western analysis.

Auranoin induced dose-dependent antiproliferative effects in all canine lymphoma cell lines, with 50% inhibitions (IC50s) between 0.1 and 1 μM. These are similar to IC50s observed in human hematopoietic tumor cells, and well within clinically achievable serum concentrations. Dose- and time-dependent apoptosis induction was observed, and additive to synergistic antiproliferative/proapoptotic activity was observed with bortezomib and elesclomol, but not DOX, CCNU or VCR. Dose- and time-dependent induction of ROS and inhibition of TrxR activity was observed following auranoin treatment, and antiproliferative activity could be partially blocked with the free-radical scavenger N-acetylcysteine. There was no change in STAT3 or p65 phosphorylation following auranoin treatment.

In conclusion, antitumor activity is observed with auranoin treatment in vitro in canine lymphoma cells, at pharmacologically achievable concentrations. This is similar to activity observed in human lymphoma and leukemia. Cooperative activity was observed with the targeted agents bortezomib and elesclomol. These data support clinical evaluation of auranoin in canine lymphoma. Measurement of ROS accumulation and/or TrxR activity may be useful pharmacodynamic markers of drug activity.

RABACFASODINE AND PREDNISONE: EFFICACY OF A Q21 DAY ADMINISTRATION SCHEDULE IN CANINE LYMPHOMA. Douglas Thamm1, Michelle Morges1, Craig Cliftord1; Kristine Burgess1, Corey Saba2, David Vail2, Cheryl London3. 1Colorado State University, Fort Collins, Colorado, USA, 2Hope Veterinary Specialists, Malvern, Pennsylvania, USA, 3Tufts University, North Grafton, Massachusetts, USA, 4University of Georgia, Athens, Georgia, USA, 5University of Wisconsin - Madison, Madison, Wisconsin, USA, 6The Ohio State University, Columbus, Ohio, USA

Lymphoma is one of the most common canine cancers. While current therapies induce remission in most naive dogs with lymphoma, drug-resistant relapse is common and there is a distinct need for novel agents. The acyclic nucleotide phosphonate 9-(2-phosphonylmethoxymethyl)guanine (PMEG) forms an active phosphorylated metabolite, PMEGpp, in cells and causes cytotoxicity in dividing cells due to inhibition of DNA polymerases α, δ, and ε; however, PMEG’s use as an anticancer agent is limited by poor cellular permeability and nonspecific toxicity. Rabacfosadine (VDC-110; GS-9219/TANOVA®), a novel double produg of PMEG,
was designed to preferentially target lymphoid cells with significantly reduced systemic toxicity. Rabacfosadine has been administered on a variety of dosing schedules to dogs with lymphoma. Objective responses were noted in 100% of chemotherapy-naive dogs and 60% of refractory dogs, with a median remission duration of 128 days. Given the ease of administration and equivalent activity with a q21-day administration schedule, we sought to generate additional data regarding efficacy of this regimen through completion of a prospective clinical trial. Additionally, we explored the potential of concomitant low-dose prednisone to mitigate previously observed cutaneous and pulmonary adverse effects.

Dogs with cytologically or histologically confirmed lymphoma were treated with rabacfosadine (0.82 mg/kg free base, as a 30-minute IV infusion once every 21 days). Dogs experiencing a complete response (CR) received 5 doses of rabacfosadine, followed by monthly rechecks. Complete clinicopathological assessment and clinical assessment of remission and adverse effects (AEs) were performed every 21 days. Response assessment was performed according to published VCOG criteria and AEs according to the VCOG-CTCAE v1.1.

44 dogs were prospectively enrolled. 63 were evaluable for response assessment and 73 were evaluable for progression free interval (PFI) assessment. While 13% of evaluable dogs were treatment-naive and 29% had received a single line of previous treatment, the majority of dogs (59%) had received 2 or more lines of previous therapy; 50 evaluable dogs had B cell lymphoma and 14 had T cell lymphoma. The overall response rate (ORR) was 57% (25% CR, 32% PR). The ORR was 64% and 23% for B cell and T cell respectively. Degree of pre-treatment impacted response rate: the ORR was 88% in naive dogs, 56% in dogs treated 2nd-line, and 51% in 3rd-line and beyond. The median PFI was 112 days for dogs experiencing a CR and 42 days for a PR (overall median PFI 41 days). Degree of pre-treatment significantly impacted PFI (164, 84 and 32 day for naive, 2nd-line and 3rd-line respectively).

The majority of AEs were mild and self-limiting: gastrointestinal (GI) and hematologic AEs were most common. Grade 3 AEs included liver enzyme elevation (4), lethargy (4), GI (2) and urinary (1). 3 dogs experienced grade 4 hematologic toxicity, and 2 developed severe hemorrhagic gastroenteritis leading to euthanasia, several weeks after the first rabacfosadine treatment and therefore of uncertain attribution. 4 dogs experienced grade 1 dermatologic AEs, and 1 dog developed grade 1 pulmonary fibrosis.

In conclusion, rabacfosadine was generally well tolerated and had substantial antitumor activity in dogs with both treatment-naive and refractory lymphoma when administered on a q21-day schedule. Response rates and PFI observed in this study are comparable to historical data with rabacfosadine when degree of pre-treatment is accounted for. There was a reduction in both the frequency and severity of AEs relative to historical data; however, it is not clear whether this is a result of less frequent dosing, concurrent prednisone, or lower cumulative rabacfosadine exposures in this heavily pre-treated population. Further studies are warranted to explore rabacfosadine at higher doses.

DEFINITIVE HIGH-DOSE HYPO-FRACTIONATED STE-REOTACTIC BRAIN-SPARING IRRADIATION OF STAGE IV CANINE NASAL TUMORS: A FEASIBILITY STUDY AND FIRST CLINICAL EXPERIENCES. Luca Malfassi, Mario Dolera, Simone Pavesi, Massimo Sala, Giovanni Mazza, Silvia Marcarini, Nancy Carrara, Sara Finesso. La Cittadina Fondazione Studi e Ricerche Veterinaria, Romanengo, Italy

The prognosis for canine nasal tumours with intracranial extension is poor with an expected survival of 1 month with palliation and 6.7 months with irradiation. However, studies regarding stage IV nasal tumours treated with brain-sparing irradiation techniques are lacking. The aim of this prospective study was to evaluate feasibility and efficacy of definitive intent stereotactic radiotherapy in dogs with nasal tumours with massive intracranial extension.

Seven dogs with stage IV nasal tumours were treated with high-dose hypo-fractionated stereotactic radiotherapy with VMAT technique. Dose prescriptions were 32-36 Gy in four consecutive-day fractions to the gross tumour and 30 Gy to lymphatics. Adjuvant treatment included carboplatin. Serial clinical and CT/MRI examination were performed. Disease control and toxicity effects were evaluated according to RECIST and VRTOG criteria. Median survival time (MST) was evaluated using Kaplan-Meier curves.

Six carcinoma and 1 sarcoma were treated. Prescription goals were obtained in four cases with V95% >95% and V107% >2%, whereas in 3 dogs V95% = 86-90% was accepted to limit maximum brain punctual dose <27 Gy. Two partial response and 5 complete responses were obtained. MST was 9 months. One grade II brain radioxicity and two brain ascending infections were observed. Relapse pathways involves diffuse meningeal and sphenoid invasion.

The initial experiences with the RT regimen adopted indicate a feasibility and effectiveness in modified stage IV nasal tumours. The relapse pathways observed suggest to evaluate alternative adjuvant treatment in dogs treated with stereotactic radiotherapy.

INVESTIGATION OF THE FOXM1 TRANSCRIPTIONAL PATHWAY IN CANINE LYMPHOMA. Jackie Wypij, Sara Goldschmidt, Holly Pondenius. University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Forkhead box M1 (FOXM1) is a transcription factor regulating cell cycle progression, angiogenesis, and apoptosis. Increased FOXM1 is observed in mammalian lymphomas and is recognized as both a prognostic factor and a therapeutic target. Thiostrepton, an antibiotic used in commercial veterinary topical products, is a putative FOXM1 inhibitor. The purpose of this study was to 1) evaluate the expression of FOXM1 pathway proteins in canine lymphoma cell lines and 2) evaluate the anti-proliferative effects of the FOXM1 inhibitor thiostrepton on canine lymphoma in vitro.

Four canine lymphoma cell lines (17-71, GL-1, CL-1, and OT-9) were used. Western blots were performed to evaluate protein expression of FOXM1 and FOXM1 pathway proteins cyclin B, survivin, and Cdc25. 17-71 canine lymphoma cells were treated with thiostrepton (0-100 μM) for 24 hours. Cell viability (cell proliferation and cell death) were assessed with a commercial MTT assay and Trypan Blue exclusion.

Canine lymphoma cell lines express FOXM1 and FOXM1 pathway proteins. Thiostrepton decreases cell viability in a dose-dependent manner (P < 0.05).
In vitro results support the FOXM1 pathway as a putative target in canine lymphoma. The FOXM1 inhibitor thiostrepton has anti-cancer activity in vitro. These results warrant further evaluation of thiostrepton in vitro for canine lymphoma. This research may lead to the repurposing of thiostrepton-containing topical compounds for treatment of canine lymphoma, which may be particularly relevant in cutaneous lymphoma.

**TUBULIN-MODULATING EFFECTS OF THE ANTHELMITIC MEBENDAZOLE IN FELINE CANCER.** Jackie Wu, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Limited effective treatment options are available for feline cancer patients. One mechanism for new drug development is drug repositioning of low-toxicity pharmaceuticals with inherent anti-cancer activity. Benzimidazole anti-parasitics such as mebendazole (MBZ) are putative novel mitotic spindle inhibitors. The purpose of this study was to 1) evaluate the potential synergism between MBZ and a traditional mitotic spindle inhibitor in vitro. 2) evaluate MBZ effect in vitro on tubulin polymerization and 3) evaluate serum tubulin polymerization activity in healthy cats treated with MBZ.

Two feline cancer cell lines (SCCF1, oral squamous cell carcinoma and K12, feline mammary carcinoma) were incubated with MBZ at 0–100 µM and vinorelbine at various concentrations for 24 hours and cell proliferation was assessed with a commercial MTS assay. Synergism was computed utilizing a combination index. Two healthy adult cats were treated with 22 mg/kg MBZ daily. A commercial assay was used to assess direct tubulin polymerization activity in serum of the treated cats. Given the known low toxicity, MBZ may be a potential candidate for future clinical trials in pet cats with cancer.

**TREATMENT OF CANINE B-CELL LYMPHOMA WITH CHEMOTHERAPY AND A CANINE ANTI-CD20 MONOCLONAL ANTIBODY: A PROSPECTIVE DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED STUDY.** G.K. Quinn1, D.R. Proulx1, L. VanHorn2, G. Archer3, B. Monreal1, E. Moore4, L. Pope1, G. Hansen2, B. Alkuzweny3, W.M. FitzPatrick1, 1Angel Care Cancer Center, California Veterinary Specialists, Carlsbad, California, USA, 2Aratana Therapeutics, Inc., Carlsbad, California, USA, 3Colorado State University, College of Veterinary Medicine and Surgery, Fort Collins, Colorado, USA, 4Veterinary Biosciences, The Ohio State University, Columbus, Ohio, USA, 5School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA, 6Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri-Columbia, Columbia, Missouri, USA, 7Department of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University, North Grafton, Massachusetts, USA, 8Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, Maryland, USA, 9Laboratory of Human Toxicology and Pharmacology, Applied/Developmental Research Support Directorate, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA

Indenoisoquinolones act as topoisoerase 1 inhibitors and are known to be active anticancer agents in both humans and dogs. A clinical trial in tumor-bearing dogs with lymphoma was conducted through the NCI Comparative Oncology Trials Consortium to define the safety, pharmacokinetics (PK) and pharmacodynamic modulation of three related novel indenoisoquinolones.

Sixty-eight dogs with lymphoma were enrolled in the dose escalation phase. Dogs were randomized to receive 3 consecutive intravenous daily doses of one agent. Each agent was escalated independently with 3 dogs/cohort. Serial pre- and post-treatment tumor biopsies, tumor aspirates, and bone marrow aspirates were collected. Two 24-hours PK curves were completed after the first and last dose of drug. Tumor and plasma PK, TOP1 levels, and gH2AX induction data were collected along with clinical toxicity and response data.

The maximum tolerated dose (MTD) was defined for two of these indenoisoquinolines (NSC725776 and NSC734400). The overall response rates for evaluable dogs were 30% (7/23), 39% (3/23), and 78% (14/18) for NSC725776 and NSC734400. The overall response rates for evaluable dogs were 30% (7/23), 39% (3/23), and 78% (14/18) for NSC725776, NSC734400, and NSC706744, respectively. Correlative relationships between clinical response, pharmacokinetic profiles and pharmacodynamic markers were established for all 3 agents.

All 3 novel agents studied herein are both tolerable and effective in tumor-bearing dogs. Study of NSC706744 at 100 mg/m² in dogs with lymphoma and measurable soft tissue sarcoma is ongoing.

**COMBINATION AT-014 (ADXS31-164) LIV-L-ULO IMMUNOTHERAPY PLUS PALLIATIVE RADIATION DELAYS TUMOR PROGRESSION AND PROLONGS OVERALL SURVIVAL IN CANINE OSTEOSARCOMA.** Nicola Mason1, Josephine Gnanandarajah1, Ana Caceres2, Lilian Diada3, Kim Angello4, Cara Blake1, Falon Grey1, Julie Engiles1, Anu Wallacha1, Yvonne Paterson1, 1University of Pennsylvania, Philadelphia, Pennsylvania, USA, 2Advaxis Inc, Princeton, New Jersey, USA

Radiation therapy (RT) induces immunogenic tumor cell death and promotes anti-tumor immunity that can result in regression of metastatic lesions, a phenomenon known as the abscopal effect. The canine Mab was well-tolerated and, in conjunction with L-CHOP, caused significant increases in survival in dogs with DLBCL.
effect. We hypothesized that combining RT with a recombinant HER2/neu expressing Loo-LLO immunotherapy (AT-014, ADXS31-164) could promote anti-tumor immunity, delay primary tumor progression and prevent metastatic disease in dogs with osteosarcoma.

Ten dogs with histopathologically confirmed, treatment naive, appendicular osteosarcoma were enrolled to this pilot study. All dogs received 16 Gy of RT in 2 fractions on consecutive days, followed by AT-014 intravenously every 3 weeks for 8 administrations. Radiographs were performed at weeks 0, 10, 22 and every 2 months thereafter to assess primary tumor progression and development of pulmonary metastases. At these times dogs were evaluated for toxicity, lameness and quality of life (QOL) and PBMCs were collected to evaluate HER2/neu specific T cell responses. The primary endpoint was time to progression (TTP); secondary end points were safety and overall survival.

Four of 8 AT-014 administrations were well tolerated. Lameness and QOL improved in 7 dogs. At present, 6 dogs are still alive. After 8 doses of AT-014, 5 dogs showed minimal radiographic evidence of primary tumor progression. 4 dogs developed pulmonary metastatic disease and 4 suffered pathological fractures. Median TTP is 204 days and median survival time is 285 days. Immunological assessment is pending. Results compare favorably to historical reports of RT alone and warrant performance of a randomized, placebo controlled clinical trial.

CHARACTERIZATION OF THE RELATIONSHIP BETWEEN BODY CONDITION SCORING AND SURVIVAL IN DOGS WITH LYMPHOMA AND OSTEOSARCOMA. Frank Romano1, Cailin Heinz2, Lisa Barber1, Joel Mason1, Lisa Freeman1, Cummings School of Veterinary Medicine, North Grafton, Massachusetts, USA.

In humans, obesity exacerbates many types of cancer by increasing the risk of poorer development, tumor aggressiveness, recurrence, and the risk of cancer death. However, this relationship in dogs has not been thoroughly evaluated. The aim of this study was to determine whether body condition score (BCS) at the time of lymphoma (LSA) or osteosarcoma (OSA) diagnosis in dogs is predictive of survival time (ST). We hypothesized that an overweight BCS at the time of cancer diagnosis would be associated with a shorter ST. Medical records of dogs diagnosed and treated for LSA or OSA between 2000 and 2010 were reviewed. Data on signalment, body weight, BCS, treatment, and survival were collected. Dogs were grouped by BCS (underweight, normal, and overweight) and STs were compared using Kaplan-Meier survival analysis. A total of 325 dogs (LSA = 270 and OSA = 55) were included in the study. Overall, 5.5% of dogs were underweight, 54.2% were normal weight, and 40.3% were overweight. On univariate analysis, ST was significantly shorter for underweight dogs with LSA (P = 0.017), but not for OSA (P = 0.518). Dogs with LSA that gained >10% body weight after diagnosis had a longer ST (P = 0.003). On multivariate analysis of all dogs, change in body weight (P < 0.001) and anemia (P = 0.026), but not BCS group, were significantly associated with ST. In conclusion, although no relationship between BCS and ST was detected, changes in body weight after diagnosis were associated with ST.

SMALL ANIMAL INTERNAL MEDICINE

TREATMENT OF FELINE HYPERSOMATOTROPISM – EFFICACY, MORBIDITY AND MORTALITY OF HYPOPHYSECTOMY.  Patrick Kenny, Christopher Scudder, Sophie Keyte, James Swann, Robert Fotwes, David Church, Yaiza Forcada, Stijn Niesen. Royal Veterinary College, London, UK.

Hypersomatotropism (HS) in cats is an important cause of diabetes mellitus (DM) in cats. Though excision of the somatotrophinoma has so far been infrequently reported in cats, we aim to describe the efficacy of hypophysectomy as a treatment for HS and the morbidity and mortality encountered in a cohort of cats.

Hypophysectomy has been offered to owners who presented diabetic cats with confirmed HS (insulin-like growth factor-1 [IGF-1] >1,000 ng/mL, pituitary mass) to the Royal Veterinary College since 2012. All cats were operated on by one neurosurgeon. Hypophysectomy was performed by manual extirpation using fine surgical tools via a trans-oral trans-sphenoidal approach. All cats were rigidly positioned in a commercially available surgical head frame, and the approach to the pituitary fossa guided by referencing bony landmarks to computed tomographic scans. Cats received perioperative monitoring of electrolytes, glucose and blood pressure, and were initially administered conjunctival desmopressin (DDAVP), intravenous infusions of insulin and hydrocortisone, before being transitioned to conjunctival DDAVP, oral hydrocortisone and levothyroxine and subcutaneous glargine insulin.

In total, 21 diabetic cats with HS underwent hypophysectomy from April 2012 – Oct 2014 (median, range; age: 10.3 years, 5.4 to 14.8; pituitary height: 6.0 mm, 4.0 to 10.6; IGF-1: 1,833 ng/mL, 1,138 to >2,000, fructosamine: 574 μmol/L, 339 to 1,076). Other than mild pelvic limb weakness, no cat displayed overt neurological deficits prior to surgery.

Three (14%) cats died post-operatively. Two cats did not recover from anaesthesia and were euthanized within 24 hours; one cat developed septic meningitis and was euthanized 17 days post-operatively.

All surviving cats (n = 18) saw a reduction of serum IGF-1, 16 cats (89%) showed IGF-1 normalization (Median post-op serum IGF-1: 38 ng/mL, 15-1495; Wilcoxon Signed Rank Test, P < 0.001).

Fourteen of the 18 surviving cats (78%) achieved diabetic remission; the remaining 4 achieved superior glycaemic control with lower insulin dosages (median fructosamine pre- and post-operatively: 692 and 547 μmol/L respectively; median insulin dose pre- and post-operatively: 20.5 and 3.5 units/kg/day respectively).

Congestive heart failure was encountered as transient problem in 4/19 cats that recovered from the surgery, all 4 cases occurred prior to implementing a reduction in volume of intravenous fluid delivered as part of the post-operative protocol. Two cats developed paresis of the left orbicularis oculi muscle, which resolved in the surviving cat. Cardiac arrest occurred in 1 cat post-operatively at time of jugular catheter placement, which was successfully revived and made an uneventful recovery. One cat developed a left pelvic limb monoparesis, which improved but did not resolve. Palatal wound breakdown was not encountered.

Hypophysectomy as a treatment for feline HS/DM resulted in a high incidence of diabetic remission and resolution of HS.

DURATION OF FASTING BUT NOT DIURNAL VARIATION AFFECTS THE RESPONSE TO GLUCAGON IN HEALTHY CATS. Chen Gilor, Rebecca Glock, Shir Gilor. The Ohio State University, Columbus, Ohio, USA.

The role of glucagon disturbances in diabetes is increasingly recognized. Glucagon stimulation tests have been described in healthy and diabetic cats previously but information is lacking on the response of healthy cats to glucagon under specific conditions. The aim of this study was to assess the effect of diurnal variation and duration of fasting on the response to glucagon in healthy cats.

Five healthy cats were used in this repeated-measures study. Cats were free-fed regularly at 0730 and 1730 hours for 30 minutes. Glucagon stimulation tests (20 μg/kg, IM) were performed on each cat 3 times, 2 weeks apart: At 0900 hours after a 25 hours fast (PM25), at 0900 hours after a 25 hours fast (AM25), and at 0900 hours after a 15 hours fast (AM15). Glucose and insulin concentrations were measured at 15, 0, 15, 25, 35, 45 and 60 minutes post stimulation. Baseline (mean of 15 and zero minutes) and peak concentrations were compared using the Friedman test (P < 0.05 considered significant).

Baseline glucose and insulin did not differ significantly between treatment groups. Peak glucose concentrations occurred at 15 minutes and were significantly higher (P = 0.0085) at AM15 (mean ± SD = 185.2 ± 43.0 mg/dL) but did not differ between AM25 (144.4 ± 10.5 mg/dL) or PM25 (128.0 ± 18.4 mg/dL).
Similarly, peak insulin concentrations occurred at 15 minutes and were significantly higher ($P=0.04$) at AM15 $(1,911 \pm 1,153$ ng/L) but did not differ between AM25 $(739 \pm 452$ ng/L) or PM25 $(549 \pm 366$ ng/L).

In conclusion, prolonged fasting (25 hours) significantly blunted the glycemic response to glucose compared to shorter fasting (15 hours) but diurnal variation had no significant effect on glucose or insulin responses.

**A FELINE-SPECIFIC ANTI-NERVE GROWTH FACTOR ANTIBODY IS SAFE AND EFFECTIVE FOR THE ALLEVIATION OF INFLAMMATORY PAIN IN CATS.** David Gear- ing1, Patrick Gearinger1, Elena Virtue1, Duncan Lascelles2, Alexander Drew1. Nexvet Biopharma, Melbourne, Victoria, Australia, 2North Carolina State University, Raleigh, North Carolina, USA

Options for the management of chronic pain in cats are extremely limited - in particular, the use of NSAIDs is limited by the potential for significant side effects. A safe and effective analgesic for cats is highly desirable.

Neutralizing monoclonal antibodies (mAb) against Nerve Growth Factor (NGF) are analgesic in rodent models and in humans with osteoarthritis. Using a novel technique for interspecies conversion of antibodies based on expressed cDNA sequence analysis (PETizing) we have recently described a canine-specific anti-NGF mAb (NV-01) that alleviates pain due to osteoarthritis in dogs. Using the same approach, we now describe the design and development of a novel therapeutic feline-specific anti-NGF mAb (NV-02) for the alleviation of pain in cats. The PETized amino acid sequences of the NV-02 mAb heavy and light chains were converted to cDNA sequences by complete oligonucleotide-based chemical synthesis, cloned into a mammalian cell expression vector and expressed in Chinese Hamster Ovary (CHO) cells in chemically-defined animal-component free media. The NV-02 mAb was purified to homogeneity by affinity chromatography and sterile filtration. Purified NV-02 mAb demonstrated very high affinity and potency for neutralizing NGF and did not bind complement.

In pilot studies, no adverse events were observed following injection of NV-02 mAb i.v. or s.c. in four cats. The NV-02 mAb had an elimination half-life of approximately 1 week and did not induce neutralizing antibodies following repeated injection. Further studies, using the kaolin model of inflammatory pain in the cat, demonstrated that NV-02 mAb was effective at reducing signs of lameness due to inflammation in a dose-dependent manner ($P<0.05$).

Together, these data suggest that NV-02 mAb has the potential to be a safe and effective therapeutic analgesic in the cat. Studies are now in progress in cats with degenerative joint disease.

**SPIKE GENE MUTATIONS IN FELINE CORONAVIRUS AND THEIR CORRELATION TO FELINE INFECTIOUS PERITONITIS.** Christian Leutenegger1, Nancy Sanders1, Jane Robertson1, Peter Rottier1. IDEXX Laboratories, Inc, West Sacramento, California, USA, 1Utrecht University, Utrecht, The Netherlands

The feline coronaviruses (FCoV) occur as 2 pathotypes with a set of 203 European clinical samples acquired from either FIP confirmed cats or from healthy cats that previously tested FCoV positive. Of these archived samples, 187 FCoV positive samples were included into the validation. From these, 13 samples did not pass quality control and 17 had virus levels that were below the limit of detection of the PCR assay. Of the remaining 157 samples, 156 were typed correctly with an accuracy of 99.4%. One FIP characterized sample was typed FECV (diagnostic sensitivity 98.6%) while all of the healthy cats were typed FECV (100% diagnostic specificity).

To confirm that these spike gene mutations are not unique to European cats with FIP, additional validation studies from US and Japanese samples were conducted. The US clinical study included 68 cases, 46 from FIP suspicious cases and 22 with non-FIP compatible disease. The FIPV RealPCR biotyping assay was able to accurately differentiate between the FIP or non-FIP (FCoV) etiologies ($P<0.0001$) and did not biotype cats with confirmed non-FIP disease as FIPV, confirming the high diagnostic specificity of the molecular test.

**EVALUATION OF WEIGHT LOSS OVER TIME IN CATS WITH CHRONIC KIDNEY DISEASE.** Lisa Freeman1, Marie-Paul Lachaude2, Sean Matthews3, Linda Rhodes4, Bill Zollers5. 1Tufts Cummings School of Veterinary Medicine, North Grafton, Massachusetts, USA, 2Aratana Therapeutics, Inc, Paris, France, 3Statistical Consultant, Dublin, Ireland, 4Aratana Therapeutics, Inc, Kansas City, Kansas, USA

Thin body condition, weight loss, and muscle loss are common in cats with chronic kidney disease (CKD). However, the time course and progression of weight loss before and after diagnosis have not been thoroughly evaluated. Therefore, the purpose of this retrospective analysis was to describe the weight loss experienced by cats with CKD before and after diagnosis. Cats with CKD from 6 US veterinary practices for which International Renal Interest Group (IRIS) Stage was available were eligible. Only those with age, date of CKD diagnosis, and weight measurements available in the 3 years before and after diagnosis were included in the analysis. A total of 569 cats, with a mean age at diagnosis of 14.5 ± 2.8 years, were evaluated (55.5% spayed females and 44.5% castrated males). Cats were categorized at diagnosis as IRIS Stage 1 [$n=54$ (6%)], Stage 2 [$n=345$ (61%)], Stage 3 [$n=141$ (25%)], and Stage 4 [$n=49$ (9%)]. Median body weight at diagnosis was 4.2 kg (range, 1.6–9.9 kg). Cats lost a median of 8.9% of body weight in the 12 months before diagnosis, but weight loss began as early as 3 years before diagnosis and accelerated after diagnosis of CKD. Cats below median body weight (4.2 kg) at diagnosis had a significantly shorter survival time compared to cats ≥4.2 kg at diagnosis ($P<0.0001$). Weight loss can be detected in cats before diagnosis of CKD, accelerates after diagnosis, and is associated with survival so careful monitoring could benefit feline health.

**PROTEINURIA IN MINIATURE SCHNAUZER DOGS WITH AND WITHOUT HYPERTRIGLYCERIDEMIA,** Eva Furrow1, Valerie Parker2, Jordan Jaeger2, Susan Murdoch2, John Bruzelli1. 1University of Minnesota, College of Veterinary Medicine, Saint Paul, Minnesota, USA, 2The Ohio State University, College of Veterinary Medicine, Columbus, Ohio, USA, 3University of Washington, School of Medicine, Seattle, Washington, USA, 4Carolina Veterinary Specialists, Charlotte, North Carolina, USA

Laboratory rodents with spontaneous hyperlipidemia develop glomerular injury and proteinuria. Idiopathic hypertriglyceridemia is common in Miniature Schnauzers, and a previous report demonstrated a possible link to proteinuria in the breed. The objective of this study was to evaluate the relationship between hypertriglyceridemia and proteinuria in Miniature Schnauzers of an allele-specific real-time PCR typing test which can identify each mutation separately will be described.

The diagnostic sensitivity and specificity will be reported from a set of 203 European clinical samples acquired from either FIP confirmed cats or from healthy cats that previously tested FCoV.
linear regression as well as multiple regression including age, sex, and body condition score as covariates.

Nine dogs had normal serum triglyceride concentrations (median = 68 mg/dL, range = 14–81 mg/dL), and 18 dogs had hypertriglyceridemia (median = 303 mg/dL, range = 87–2,089 mg/dL). There was a strong positive correlation between triglyceride concentration and UPC (r = 0.75, P < 0.001). Ten of 18 dogs (56%) with hypertriglyceridemia had proteinuria (UPC ≥0.5) compared to none of the dogs with normal triglyceride concentrations (P = 0.009). Proteinuria was most severe in dogs with triglyceride concentrations >400 mg/dL; 5/6 dogs (83%) with triglyceride concentrations above this level had a UPC ≥2.0. None of the dogs were azotemic or hypoalbuminemic. Endocrine disease was not diagnosed in any dog.

In conclusion, significant proteinuria is present in dogs with idiopathic hypertriglyceridemia and may be due to lipid-induced glomerular injury. Longitudinal studies and evaluation of renal pathology are warranted to further investigate these findings.

NEUTROPHIL EXTRACELLULAR TRAP FORMATION IS INCREASED IN DOGS WITH IMMUNE MEDIATED HEMOLYTIC ANEMIA. Stephanie Smith, Maureen McMicheal, Ron Achiel, Katrina Jung. University of Illinois, Urbana, Illinois, USA

It has recently been discovered that stimulated neutrophils can release chromatin fibers decorated with enzymes to form extracellular traps (NETs). Formation of NETs (NETosis) is associated with histone hypercitrullination. NETs are part of the innate immune response, but also associated with thrombosis, organ damage, and autoimmunity.

In this prospective observational study, EDTA plasma was collected from 30 apparently healthy dogs, and 18 dogs with IMHA. Plasma DNA concentration was measured by diluting samples 10–160 fold in phosphate-buffered saline, 0.1% albumin, and adding 1 mM SytoxGreen. Fluorescence was recorded (Ex485/Em538) and corrected for autofluorescence using diluted samples without SytoxGreen. DNA concentrations were calculated based on a standard curve. Nucleosomes (DNA-histone complexes) were quantified by ELISA and normalized to canine pooled normal plasma (defined as 1). The presence of citrullinated histone H3 in plasma was confirmed using western blot. Results were compared between healthy and IMHA dogs by Wilcoxon rank sum test.

Plasma from dogs with IMHA (median 3.1 µg/mL, range 0–27.1) contained significantly (P < 0.001) higher concentrations of free DNA than normal plasma (median 0.7, range 0–1.2). IMHA plasma also contained significantly (P < 0.001) higher concentrations of nucleosomes (median 6.8, range 1.4–103) as compared to normal plasma (median 0.9, range 0.4–3.1). Plasma from 16/18 (89%) dogs with IMHA contained citrullinated histone H3, but this marker was identified in only 4 of 30 (13%) of healthy dogs.

Excessive NETosis appears to be a feature of canine IMHA, and may contribute to the prothrombotic state in this disease.

FELINE T. FOETUS CYTOTOXICITY CAN BE INHIBITED BY SELECTIVE, SMALL-MOLECULE CYSTEINE PROTEASE INHIBITORS. Katie Tolbert, Emily Gould, Mabre Brand. University of Tennessee, Knoxville, Tennessee, USA

Trichomonas foetus (Tf) is a mucosal protozoan parasite that infects the feline distal ileum and proximal colon resulting in chronic diarrhea. Tf has a worldwide distribution with no consistently effective drugs to treat the infection. Cysteine proteases (Cp) have recently been demonstrated to promote adhesion-dependent cytotoxicity of feline Tf to the intestinal epithelium. These results support further investigation of Cp inhibitors as potential therapeutic targets for ameliorating the pathological effects of feline trichomoniasis. To reduce host toxicity, anti-protease drugs must be tailored to inhibit only those proteases produced by pathogens that are responsible for cytotoxic effects and that have redundant mechanisms of production for fund with other host proteases. The aim of this study was to investigate currently available small-molecule Cp inhibitors for their feasibility in targeting specific regions of feline Tf Cp activity and ameliorating Tf-induced cytopathogenicity.

The effect of small molecule Cp inhibitors on specific Cp activity was measured for proteins extracted from 4 feline Tf isolates. A region of histone H3 was identified by means of SDS-PAGE in gel zymography in the presence or absence of 0.1–1.0 mM calpain inhibitor, antipain, cystatin, leupeptin, chymostatin, WRR, and K11777. The effect of inhibition of specific Cp activity on Tf cytopathogenicity was determined using feline Tf that were allowed to adhere to monolayers of porcine intestinal epithelial cells (IPEC-J2) in co-culture. The cytopathogenic effect of Tf following inhibition of specific Cp activity was evaluated by light microscopy, crystal violet spectrophotometric analysis and immuno-staining Tf-infected IPEC-J2 cells for the M30 antigen of cleaved keratin 18 as a marker of apoptosis. A minimum of 8 replicates were performed for each cytotoxicity experiment. Data were analyzed using Systat software (P < 0.05).

Patterns of gel zymography, demonstrated by 4 different feline isolates of Tf, were similar and revealed the ability of the vinyl sulfone Cp inhibitors (K11777, WRR) and cystatin to target specific zones of feline Tf Cp activity. These inhibitors had no effect on Tf growth and significantly inhibited cytotoxicity towards the intestinal epithelium in all 4 feline Tf isolates tested (P < 0.001).

These studies establish that currently available small-molecule inhibitors of cysteine proteases are capable of inhibiting specific regions of feline Tf cysteine protease activity and ameliorating Tf-induced cytopathogenicity to the intestinal epithelium in vitro. The results of these studies provide strong evidence-based justification for identification of the specific cysteine proteases inhibited. Moreover, examination of the effect of specific cysteine protease inhibitors on amelioration of clinical signs in cats naturally infected with Tf is warranted.

OUTCOME FOLLOWING URETERAL STENT PLACEMENT IN DOGS FOR BENIGN URETERAL OBSTRUCTION: A PROSPECTIVE STUDY. 44 DOGS (57 URETERS) 2009–2013. Stephanie Pavia, Allyson Berent1, Chick Weisse1, Demetrius Bagley2. 1The Animal Medical Center, New York, New York, USA, 2Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Ureteral obstructions are a serious clinical problem in dogs and traditional surgery is associated with a high complication rate. Ureteral stenting provides a minimally-invasive option for immediate decompression, and is considered the standard-of-care in human medicine.

To describe the use of double-pigtail ureteral stents in dogs for benign ureteral obstructions hypothesizing it is a safe and effective treatment option.

The diagnosis of benign ureteral obstruction was made via ultrasonography, radiography, and ureteropyelography. Ureteral stents were placed endoscopically and/or surgically, with fluoroscopic-guidance. The medical records were reviewed for pre-, intra, and postoperative data and outcome.

44 dogs (57 ureters) underwent stent placement for ureterolithiasis (48/57 [84%]), stricture (5/57 [9%]), or both (4 [7%]). Endoscopic/fluoroscopic or surgical/fluoroscopic techniques were successful in 45/55 (82%) and 12/12 ureters, respectively. Preoperative azotemia was present in 52/23 (44%) (median 2 mg/dL) and 37/46 (81%) (median 1.3) after decompression. Urinary tract infections were present in 59/26 (44%) pre-operatively, and 58/25 (43%) post-operatively, 76/19 (25) clearing with treatment. The perioperative mortality rate was 2% (1/44), and was not procedure-related. Three major complications occurred during stent placement. Major peri-operative, short-term, and long-term complications requiring further intervention occurred in 0%, 4%, and 14%, respectively. Median follow-up time was 1,158 days (range, 30–1,555), with 30/44 alive at last follow-up.

Findings suggest that ureteral stents are a safe and effective minimally invasive short and long-term treatment option for benign ureteral obstructions in dogs. Complications were typically minor, but may necessitate stent exchange or use of an alternative device.
PROGNOSTIC VALUE OF SYMMETRIC DIMETHYLGARGINE (SDMA) TO CREATININE RATIO IN DOGS AND CATS WITH CHRONIC KIDNEY DISEASE (CKD). Mahalakshmi Yerramilli1, Murthy Yerramilli1, Edward Obare3, Dennis Jewell1, Jean Hall1. 1IDEXX Laboratories Inc, Westbrook, Maine, USA, 2Hill’s Pet Nutrition, Inc, Topeka, Kansas, USA, 3Oregon State University, Corvallis, Oregon, USA

SDMA is one of the dimethylated derivatives of arginine and is released into cytoplasm after proteolysis. As circulating SDMA is mostly eliminated by the kidneys, plasma concentrations are affected by changes in GFR. We have previously shown that increased serum SDMA concentrations were observed in cats and dogs with reduced renal function. We have also demonstrated that serum SDMA allows for earlier detection of chronic kidney disease (CKD) in cats and dogs when compared with serum creatinine. In general, serum SDMA and creatinine concentrations correlate with each other (R^2 ~ 0.7–0.9). However in some CKD patients, SDMA concentrations increased disproportionately compared with serum creatinine. This discordance is seen in both cats and dogs, but the prevalence is much higher in cats. This observed discordance in CKD patients can be quantified in terms of a SDMA to creatinine ratio; the higher the ratio the greater the discordance. Although the typical SDMA to creatinine ratio is <10 in the majority of animals with CKD, we have observed that the larger the SDMA to creatinine ratio (>10) in CKD patients, the greater the chance of mortality. It should be noted that the ratio only applies to CKD animals; dogs and cats with creatinine and/or SDMA concentrations within normal limits can have ratios >10. We have previously reported that the upper normal reference limit for SDMA in dogs and cats is <14 µg/dL.

For example, in a retrospective study consisting of 21 CKD cats, 15/16 that had a SDMA to creatinine ratio of >10 were deceased within 1 year, whereas 5/5 CKD cats that had a normal ratio of SDMA to creatinine (<10) were still alive at that time. Similar trends were observed in another study with dogs. Kaplan-Meier survival curves were generated for both these cats and dogs based on SDMA concentrations greater than and less than 14 µg/dL. Cats with SDMA <14 µg/dL survived approximately 1.6 times longer than cats with SDMA >14 µg/dL. Dogs with SDMA <14 µg/dL survived approximately 2.6 times longer compared with dogs with SDMA >14 µg/dL. All cats and dogs were maintained with high quality care, including optimal nutrition and veterinary health care with opportunities for socialization and play time with caretakers and with daily opportunities to exercise, and play with toys. Elevated SDMA concentrations in general, and SDMA to creatinine ratios in particular, for feline and canine CKD patients could potentially provide prognostic value. These results warrant further study.

THE CONVERGENCE OF EPIGENETICS AND CD8+ T CELL DYSFUNCTION IN THE FELINE IMMUNODEFICIENCY VIRUS MODEL OF VIRAL PERSISTENCE. Jonathan Fogle, Yan Wang. North Carolina State University College of Veterinary Medicine, Raleigh, North California, USA

Generation of mature, fully-functional CD8+ lymphocytes is dependent upon epigenetic changes such as DNA demethylation and histone acetylation at the promoter regions of cytokines essential for CD8+ T cell function and differentiation. Conversely, dysfunctional CD8+ T cells exhibit altered patterns of methylation and acetylation, and failure of complete differentiation, suggesting that there are early transcriptional events contributing to the induction of CD8+ T cell dysfunction. For many persistent viral infections such as Herpes Simplex Virus (HSV), Hepatitis C Virus (HCV), Rotavirus (RV), Human Immunodeficiency Virus (HIV), and Feline Immunodeficiency Virus (FIV), virus specific CD8+ T cell activation is followed by the induction of CD8+ T cell dysfunction, which is characterized by lack of interleukin-2 (IL2) production and poor proliferation following T cell receptor (TCR) stimulation. Loss of IL2 production signals the onset of a progressive decline in cytokine production and antiviral function. Using the feline immunodeficiency model (FIV) for lentiviral persistence, we have clearly demonstrated that virus-activated CD4+CD25+ T regulatory (Treg) cells provide a strong inhibitory signal and induce cell cycle arrest and down-regulation of IL2 in activated CD8+ lymphocyte targets early and progressively during the course of infection. Further, we have shown that Treg cells are activated by FIV infection and induce the expression of the repressive transcription factor Foxp3 in CD8+ T cell targets following Treg cell / CD8+ co-culture. Finally, we have recently demonstrated that Foxp3 can bind the IL2 promoter in “bulk” (virus non-specific) CD8+ T cells and that blocking epigenetic rearrangements prevents Foxp3 binding to the IL2 promoter in virus non-specific CD8+ T cells.

Recent findings from our laboratory suggest that early epigenetic changes (histone acetylation and DNA demethylation), while essential for antiviral function, render the CD8+ T cell highly permissive to Treg-induced, Foxp3-mediated repression of IL2 transcription. Our hypothesis is that activated Treg cells exploit virus-induced epigenetic changes in CD8+ T cells to cause dysfunction. The purpose of these investigations was to demonstrate that Foxp3 binds the IL2 promoter in virus specific CD8+ T cells. Using chromatin immunoprecipitation (ChIP) and bisulfite reduction of DNA, we have assessed histone acetylation and DNA demethylation at the IL2 promoter region in virus specific CD8+ T cells from FIV+ cats. Briefly, virus specific T cells were identified by ex-vivo proliferation in response to inactivated virus. These cells were then purified and co-cultured with autologous Treg cells. In concert with our previous work in virus non-specific CD8+ T cells, our data suggest that following coculture with autologous Treg cells, virus specific CD8+ T cells exhibit increased Foxp3 mRNA and decreased IL2 mRNA by RT-qPCR (n = 2). More importantly, our most recent ChIP studies demonstrate that Foxp3 binds the IL2 promoter in virus specific CD8+ T cells from FIV+ cats following Treg cell coculture (n = 2). Collectively, these results suggest that it will be possible to block epigenetic rearrangements in virus specific CD8+ T cells thereby blocking Foxp3 binding to the IL2 promoter. These mechanistic studies will provide a new avenue of investigation for restoring antiviral CD8+ T cell function during the course of persistent viral infection.
PHARMACOKINETICS OF VORICONAZOLE IN HEALTHY CATS. Polina Vishkautsan1, George Thompson2, Mark Papiè1, Jane Sykes1, UC Davis VMTH, Davis, California, USA, 2UC Davis School of Medicine, Davis, California, USA, 3North Carolina University, Raleigh, North Carolina, USA

Voriconazole is a potent azole antifungal drug but is contraindicated in cats because of life-threatening adverse effects. We sought to determine the pharmacokinetics of voriconazole in healthy cats after oral and IV administration so that a safe dose might be established. Six cats were administered 1 mg/kg of voriconazole IV. Plasma voriconazole concentrations were measured at multiple time points for 24 hours after administration, using high performance liquid chromatography. Subsequently, voriconazole suspension was administered to 3 groups of 2 cats at 4, 5 and 6 mg/kg PO. Plasma concentrations were measured at multiple time points for 24 hours after administration. The pharmacokinetics of tablet and suspension preparations were also compared using a range of doses from 4.0 to 4.7 mg/kg. Plasma measurements were performed over a 72-hours period after administration.

Voriconazole half-life after IV administration was approximately 20 hours. After oral administration, maximal plasma concentrations were reached in 30-60 minutes. A dose of 4 mg/kg resulted in optimal plasma drug concentrations (1–4 µg/mL). The predicted half-life after oral administration was 80-90 hours. Adverse effects included hypersalivation coincident with oral suspension administration (not tablets) and miosis. Miosis was associated with peak plasma voriconazole levels >2.5 µg/mL and persisted up to 48 hours.

Voriconazole has excellent oral bioavailability in cats. An oral dose of 4 mg/kg q48h may maintain adequate antifungal plasma concentrations, but multi-dose administration studies are required. Miosis was a significant adverse effect and occurred at plasma concentrations at the high end of the target range recommended for humans.

PAIN MANAGEMENT AND END OF LIFE CARE: RESULTS OF A NATIONAL, CROSS-SECTIONAL, SURVEY OF SMALL ANIMAL OWNERS. Roschelle Heuberger1, Michael Petty2, Page Burnia1, Jill Prior1, 1Central Michigan University, Mt. Pleasant, Michigan, USA, 2Arbor Pointe Veterinary Hospital, Canton, Michigan, USA

The objective of this study was to describe knowledge, attitudes and beliefs (KAB) of a national sample of owners with regard to End of Life (EOL) care for their pets. The hypothesis included: paradigms for EOL decision-making are few and dependent on factors such as owner education. EOL decisions are influenced by many variables, requiring large samples (n = 945) for data segmentation. Additional objectives included: describing EOL and Quality of Life (QOL) awareness, beliefs regarding Pain Management (PM), and recognition of terms such as “in home care” for pets by owners.

This study was “exempt category #2 anonymous survey” by Federal Statute on 2/12/14 by Institutional Review Board. Computerized links were opened with informed consent. Instrumentation was developed through modifications of validated surveys of KAB in EOL. Veterinarians and specialty associations with known involvement in EOL and QOL were consulted, followed by owner focus groups and piloting. Designed in Survey Monkey1, the data were password protected. Recruitment through social media was performed (2014-2015) and data were processed in SPSS v.21.

Respondent age was μ = 45.4 ± 13 years, μ = 2 ± 1.2 pets/household, pet age μ = 3 ± 1.7 years and pet losses/lifetime μ = 1 ± 1.2. Only 4% were first time pet owners, and only 14% had pet health insurance. Respondents were primarily: Female (92%) Caucasian (94%) Married (55%) Religiously Unaffiliated (41%), Education >HS (75%), Non-Healthcare Occupation (75%). Familiarity with “ hospice” or “in home EOL care for pets” was <33%, QOL rating scales was 20%, but willingness to use QOL scales in the future was >70%. In terms of PM in EOL care, owners were likely or very likely to use oral medication in pill form (80%), liquid (82%), injection (70%), acupuncture (55%), accupressure/massage (54%), physical therapy (67%), hydrotherapy (61%) surgery (62%) nerve block (41%) corticoste-roid injections (52%), opt for euthanasia (15%). Other (5%) included Chiropactic, transdermal patch, laser, herbal, naturopathy, aromatherapy, prayer etc. Thirty-five percent of owners reported fiscal concerns as influential in continued EOL care. If pets could not be controlled almost 80% of owners said they would euthanize.

Further data segmentation is warranted to discern differences among owners with regard to KAB in EOL, PM, QOL and euthanasia. These findings will provide a basis for educational outreach and protocol development for owners of ill or geriatric animals.

DISCOVERY AND CLINICAL EFFECTIVENESS OF A COMPOSITION THAT PROMOTES HAIR GROWTH (PATENT PENDING). Gen-Ichiro Soma1, Hiroshi Okawa2, 1BioMedical Research Group Inc., Tokyo, Japan, 2Scarecrow Inc., Tokyo, Japan

To overcome alopecia related to a variety of diseases in pets, we have identified materials that can promote hair growth, particularly in bald spots, leading to the development of products that can be helpful in the field of food and medicine. We developed a composition that promotes hair growth in animals, which can be used in numerous formulations, including drugs, quasi-drugs, dressings, adhesive bandages, supplements, foods, snacks, water, hair care supplies, dental supplies, clothes, shoes, and socks. This formulation contains lipopolysaccharide (LPS) as an active ingredient. LPS tablets and pine bark polyphenol tablets were orally administered to dogs, cats, rabbits, and hamsters with alopecia in a study conducted in Japan, with the cooperation of practitioners. The study involved no restriction on food intake and medication, and prohibited the concomitant use of other supplements. The animals were divided into 2 groups, those that received LPS tablets alone and those that received LPS tablets and pine bark polyphenol tablets. The duration of the study was approximately 30 days. Hair growth was noted in 10 of 24 animals (41.6%) administered LPS tablets alone and all 13 animals (100%) administered LPS tablets and pine bark polyphenol tablets.

PREVALENCE OF CANINE INFECTIOUS RESPIRATORY DISEASE IN DOGS IN CHICAGO OUTBREAK (MARCH-APRIL 2015). Jill A. Richardson1, Amy Glaser2, Nyssa Reine-Sal1, Edward J. Dubovi2, 1Merck Animal Health, Madison, New Jersey, USA, 2New York State Veterinary Diagnostic Laboratory, Cornell University, Ithaca, New York, USA

Canine infectious respiratory disease complex (CIRDC) is a common disease complex caused by many different viruses and bacteria, including Bordetella bronchiseptica, Mycoplasma cynos, adenovirus type 2, distemper, influenza A virus, parainfluenza virus, pneumovirus and respiratory coronavirus. In March 2015, veterinarians in the Chicago area noted an increase in incidence of signs of canine infectious respiratory disease in dogs. Nasal and pharyngeal swabs from dogs showing clinical signs were submitted to the Cornell University Animal Health Diagnostic Center (AHDC). A canine respiratory polymerase chain reaction (PCR) screening panel was utilized which allows identification of the following CIRDC pathogens: B. bronchiseptica, Mycoplasma cynos, adenovirus type 2, distemper, influenza A, parainfluenza virus, pneumovirus and respiratory coronavirus.

On March 16, 2015, a diagnostic sampling program was initiated which tested over 350 samples from dogs with respiratory signs using this screening panel as of April 23, 2015. Samples were collected by the reporting clinics and shipped based on the Cornell AHDC specifications.

Of the over 350 screened sick dogs, 198 dogs tested positive for canine influenza using a PCR assay. None of the tested dogs were confirmed to have the H3N8 influenza strain. Further testing by Cornell and the University of Wisconsin identified the strain as Canine Influenza H3N2. The H3N2 influenza virus is of avian origin and was first isolated from clinically ill dogs in China in 2006 and South Korea in 2007. Canine H3N2 influenza virus has been associated with severe respiratory signs and other clinical signs such as fever, reduced body weight, and interstitial pneumonia. In addition, 24 dogs tested positive for parainfluenza...
virus, nine dogs tested positive for *B. bronchiseptica*, three dogs tested positive for adenovirus type 2, 27 dogs tested positive for pneumovirus and 29 dogs tested positive for respiratory coronavirus. Records were reviewed to determine the vaccination status of the positive dogs. None of the 198 dogs that tested positive for the H3N2 influenza virus were vaccinated against the H3N8 influenza virus. Twenty-two of the 24 dogs that tested positive for parainfluenza virus had received an injectable distemper, adenovirus type 2, pneumovirus and parainfluenza virus combination vaccine, 13 had received a monovalent *B. bronchiseptica* vaccine and five had been vaccinated with an intranasal *B. bronchiseptica* and parainfluenza combination product. Of the five dogs that received the *B. bronchiseptica* combination vaccine, timing of vaccination suggests that the virus sampled may have been of vaccine origin.

The information gathered from this testing program represents the first time that the H3N2 influenza virus has been isolated from dogs in the United States. A point of origin could not be determined. In addition, data collected supports the role of parainfluenza virus as a major preventable pathogen in CIRDC and route of vaccination should be considered in vaccination protocols.

**EQUINE**

**A CLINICAL AND NECROPSY EVALUATION OF ACUTE LUNG INJURY (NALI) AND ACUTE RESPIRATORY DYS- TRESS SYNDROME (NARDS) IN NEONATAL FOALS.** Daniela Bedenice, Samuel Jennings, Mauricio Solano, Mary Rose Paradis. Cummings School of Veterinary Medicine at Tufts University, North Grafton, Massachusetts, USA

The study purpose was to evaluate the clinical and post-mortem characteristics of neonatal foals fulfilling the criteria of neonatal ALI (nALI) and ARDS (nARDS). A retrospective cohort analysis of 786 foals (1990-2014) admitted to a university hospital under 4-weeks old was performed. Diagnostic criteria for nALI and nARDS were applied to a preexistent database, including all patients with lateral thoracic radiographs and arterial blood gas results obtained on room air within 24-hours of arrival. Neonates born following cesarean section, induction or with a diagnosis of prematurity were excluded. The diagnostic PaO2/FiO2 threshold was adjusted for age as previously described (Wilkins-2007). Thoracic radiographs were evaluated using a pre-existent scoring system. Histopathologic abnormalities were graded according to the distribution and frequency (0-3) of edema/congestion/hypercellularity, fibrin exudation, hyaline membranes, type-2 pneumocyte hyperplasia, and interstitial fibrosis to establish a diagnosis of diffuse alveolar damage (DAD). All data were presented descriptively. Thirty-four of 786 (4.3%) admitted foals fulfilled the clinical and PaO2/FiO2 threshold criteria for nALI (786) and nARDS (786). Of these, 10/24 (42%) and 4/10 (40%) survived, respectively. Histopathologic review of lung tissue was available for 10 affected foals, and showed severe, diffuse edema or congestion in all patients. Hyaline membranes, and thus DAD, were observed in 7/10 (70%), being rare in 4/7 (57%). Interstitial fibrosis was not identified. Associated pulmonary diagnoses in foals with DAD included meconium aspiration (n = 2), EHV-1 infection (n = 2), and suppurative bronchopneumonia (n = 1).

In conclusion, histopathology showed exudative rather than proliferative or fibrotic DAD in foals with nALI or nARDS.

**METABOLIC RESPONSES TO A STANDARDIZED FIELD EXERCISE TEST IN ARABIAN ENDURANCE HORSES WITH A HISTORY OF EXERTIONAL RHABDOMYOLYSIS.** Erica McKenzie1, Lauren Eyrich1, Mark Payton1, Stephanie Valberg2, Erica McKenzie2, Raffaele Teixeira, James Mickelson, Stephanie Valberg, Molly McCue. University of Minnesota, St Paul, Minnesota, USA

At least 4% of Arabian endurance horses suffer from exertional rhabdomyolysis (ER) of unknown etiology. This study compared muscle histopathology and metabolic responses to a field exercise test between endurance Arabians with and without previous ER (ER: n = 10, age 15 ± 6 years; control: n = 9, 13 ± 6 years). No horses possessed the GYS1 mutation. After 24-48 hours stall rest, paired ER and control horses, fitted with a telemetric ECG, performed 47 minutes of standardized intervals of walk and trot, and one interval of canter. Blood samples were obtained before, immediately after and 3 hours post-exercise, and plasma was frozen immediately in liquid nitrogen. Percutaneous glutens medius muscle samples frozen in liquid nitrogen were obtained before and 3 hours post-exercise for glycogen analysis. Pre-exercise muscle samples were also fixed in formalin for histopathologic analysis. Fisher’s exact test, t-test, and ANOVA were performed (P < 0.05). No horses displayed clinical signs of ER and log-transformed serum creatine kinase activity 3 hours post-exercise was not significantly different between ER and control Arabians. Muscle glycogen, heart rate, PCV, and plasma total protein, glucose, lactate and electrolyte concentrations did not differ between exercising ER and control horses. ER horses had more central nuclei in mature myofibers, and higher myopathic scores compared with control horses.

Arabian endurance horses with a history of ER have similar metabolic responses to a submaximal exercise test as healthy Arabians, in spite of histopathologic indicators of chronic ER. Thus, Arabian ER does not appear to be associated with a consistent metabolic myopathy.

**LONGITUDINAL EFFECT OF A MULTI-STRAIN PROBIOTIC ON THE MICROBIOTA OF NEONATAL FOALS.** Angelica Schöster1, Henry Staempfl2, Miranda Abrahim3, Mohammad Jalali1, Luca Guardabassi2, Scott Weese3. 1University of Zurich, Vetsuisse faculty, Clinic for Equine Internal Medicine, Zurich, Switzerland, 2University of Guelph, Ontario Veterinary College, Department of Clinical Studies, Guelph, Ontario, Canada, 3University of Guelph, Ontario Veterinary College, Department of Pathobiology, Guelph, Ontario, Canada.

It is suggested that probiotics modify the intestinal microbiota but scientific proof is lacking. The objective of this study was to evaluate the effect of a probiotic containing strains selected for anti-clostridial activity, on the intestinal microbiota of neonatal foals.

14 healthy foals were randomly allocated to receive a probiotic or placebo formulation for 3 weeks. The probiotic formulation contained two *Lactobacillus rhamnosus* strains, two *L. plantarum* strains and *B. animalis lactis*. Fecal samples were collected at 2, 4 and 6 weeks of age. Mthor analysis of next generation sequencing data was used to compare data between treatment groups.

There was no effect of treatment group on alpha diversity indices, Chao richness, Simpson’s diversity and Shannon evenness (all P > 0.37). There were no significant differences in the relative abundances of any phyla, classes, or predominant genera, including *Bifidobacterium* (P = 0.37, 0.18 and 0.90 at 2, 4 and 6 weeks of age) and *Lactobacillus* (*P* = 0.61, 0.16 and 0.52 at 2, 4 and 6 weeks of age). Several differences between treatment groups were noted using LEfSe. Most notably at week 6, an unclassified Lactobacillaceae (LDA score 2.1, P = 0.016) was enriched in the probiotic group. There was no significant difference in beta diversity between groups, as assessed by unifrac applied to Jaccard and Yue&Clayton trees (all P > 0.30).

Probiotic treatment had limited effects on the composition of the microbiome. Further studies including larger numbers of foals have to be performed to assess whether this difference is significant and can be used to therapeutically modify the microbiome of foals.

**SKELETAL MUSCLE GENE EXPRESSION PROFILE IN TYPE 1 POLYSACCHARIDE STORAGE MYOPATHY.** Raffaele Teixeira, James Mickelson, Stephanie Valberg, Molly McCue. University of Minnesota, St Paul, Minnesota, USA

Type 1 Polysaccharide Storage Myopathy (PSSM1) is an inheritable neuromuscular disorder caused by a gain of function mutation in skeletal muscle glycogen synthase that leads to excessive glycogen and abnormal polysaccharide accumulation in muscle fibers.
**MYELOPATHY.**

Motor evoked potential latencies and axon relationship between transcranial magnetic stimulation and spinal cord damage.

PSSM horses utilize glycogen, but develop exercise intolerance and rhabdomyolysis during sub-maximal exercise. The link between excessive glycogen, abnormal polysaccharide and rhabdomyolysis is unknown. Controlled regular exercise and a low starch/high fat diet improve PSSM clinical signs. We hypothesized that 1) excess glycogen and/or improper regulation of glycogen synthase results in altered gene expression in skeletal muscle energy metabolism pathways leading to rhabdomyolysis in PSSM; and 2) that clinical improvement with daily exercise is due to normalization of gene expression in PSSM1 horses. Gluteal muscle biopsies were collected from PSSM1 cases and controls on a high starch diet prior to training (t1), before (t2) and immediately after exercise (t3) following a standardized 3-week training protocol. RNA was isolated and sequenced at 40 million reads/sample; reads were mapped to EquCab2 and de novo assembly was performed to identify novel transcripts. Differential gene expression was measured using EdgeR. 195, 294 and 739 genes were differentially expressed between cases and controls at t1, t2 and t3, respectively. The largest differences in expression were in cases between t1-t3 (4,822 genes) and t2-t3 (2,474 genes). Genes involved in glycogen metabolism, glycolysis and mitochondrial electron transport were differentially expressed between cases and controls. Understanding altered energy regulation in PSSM1 should lead to new therapies and new insights in metabolic myopathies and muscle glycogen regulation.

**BLOOD AND CEREBROSPINAL FLUID ALPHATOCOPHELIN LEVELS IN TETANUS FOALS WITH NEUROAXONAL DYSTrophy.**

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Equine neuroaxonal dystrophy/equine degenerative myeloneuropathy (NAD/EDM) is a neurodegenerative disorder affecting genetically predisposed foals maintained on an α-tocopherol (α-T) deficient diet during the first year of life. We hypothesized that (1) the administration of E-Se at 4-days of age would have no significant effect on serum or cerebrospinal fluid (CSF) [α-T] in healthy foals and (2) serum and CSF [α-T], but not [Se], would be significantly decreased in NAD/EDM-affected foals during the first year of life. Fourteen Quarter horse foals were included in the study; ten healthy foals supplemented with 0.02 mL/kg E-Se (n = 5) or saline (n = 5) at 4 day of age and four unsupplemented NAD/EDM-affected foals. Complete neurologic examinations, blood and CSF collections were performed before (4 day of age) and after supplementation at 10, 30, 60, 120, 180, 240 and 360 day of age. At 540 days, NAD/EDM affected foals and one unsupplemented healthy foal were euthanized and full necropsy performed. A significant decrease in blood and CSF [α-T] and [Se] was found during the first year of life in all foals, with the most significant changes in serum [α-T] occurring from 4-150 day. Dam [α-T] and [Se] significantly impacted foal concentrations through 10 day of age. An injection of E-Se did not significantly increase CSF [Se], blood or CSF [α-T] in healthy foals. NAD/EDM-affected foals had significantly lower CSF [α-T]. Despite all 14 foals having deficient [α-T] only the four genetically predisposed foals developed NAD/EDM.

**RELATIONSHIP BETWEEN TRANSCRANIAL MAGNETIC MOTOR EVOKED POTENTIAL LATENCIES AND AXON LOSS IN EQUINE CERVICAL VERTEBRAL STENOTIC MYELOPATHY.**

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Current diagnostic strategies for equine cervical vertebral stenotic myelopathy (CVSM) are limited to identification of structural changes within the vertebral canal and inferring spinal cord dysfunction. Transcranial magnetic motor evoked potentials (TMMEPs) have been associated with spinal cord dysfunction in horses, although histopathologic confirmation and correlation with axon loss is lacking. The purpose of this study was to investigate the relationship between TMMEP latency and axonal loss in horses diagnosed with CVSM. The hypothesis is that cervical spinal cord axonal loss will correlate with TMMEP latency.

20 horses were recruited randomly from the extensor carpi radialis and cranialis tibialis muscles. Ten horses had clinical signs consistent with CVSM (CVSM group) while the ten remaining horses presented for non-neurologic disorders (control group). All horses had lateral cervical radiographs performed prior to humane euthanasia. Cerebrospinal fluid from all horses tested negative for Sarcocystis neurona (SAZG2/3 ELISA). Postmortem harvesting of cervical spinal cords in all horses was via dorsal laminectomy (C1-T2), in-situ formalin fixation, transverse sectioning (cranial, dorsal and caudal to each intervertebral disc space). Tissue blocks were stained with luxol fast blue and an H&E counterstain. Digital images of the spinal cord transverse sections were acquired and pixel density counts were determined (Adobe Photoshope®) to determine axon density at each of the following locations within each section; dorsal lateral funiculus (DLF), dorsal funiculus (DF), ventral funiculus (VF) and lateral funiculus (LF). TMMEP latencies of the extensor carpi radialis and cranialis tibialis were compared between control and affected horses (unpaired t test). The ratio of axon densities at each site between CVSM and control horses (termed the CCPR) was plotted against the TMMEP latency of the cranialis tibialis muscle and examined for correlation. Individual funiculus axon density from the最 severely affected transverse section were plotted against TMMEP latency of the cranialis tibialis muscles to investigate the effect of location within the spinal cord.

TMMEP latency showed a significant difference between groups for the cranialis tibialis muscle exclusively. Cranialis tibialis TMMEP latency linearly correlated with CCPR. Loss of axons was most severe in the ventromedial funiculi of CVSM horses. These data confirm that horses affected by CVSM have prolonged TMMEP latencies and that TMMEP latency correlates with severity of axon loss. Both of these findings indicate that TMMEP might be useful to identify and quantify severity of spinal cord damage in clinical CVSM. The severity of ventral funiculal changes is a unique finding and might suggest that this area is more vulnerable to injury in horses with CVSM than previously recognized.

**NOVEL DETERMINANT CONFRoNS MARCODILE, LINCO-SAMIDES, AND STREPTOGRAMIN B RESISTANCE IN RHODOCOCCUS EQUi.**

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The incidence of macrolide and rifampin resistance in *R. equi* isolated from foals has increased considerably in recent years. The objective of this study was to identify the molecular mechanism of emerging macrolide resistance in *R. equi* and to determine its transferability. Macrolide-resistant (n = 62) and macrolide susceptible (n = 62) clinical isolates of *R. equi* from foals in the USA were studied. Whole genome sequencing of a sample of 18 macrolide-resistant and 6 macrolide-susceptible *R. equi* was performed. PCR was used to screen for the presence of the resistance determinant in the other isolates. Mating experiments were performed to document transfer of the determinant. The genomes of resistant isolates were virtually identical whereas there was marked chromosomal variability among susceptible isolates, suggesting expansion of a resistant clone. A novel *erm* gene, *erm(46)* was identified only in resistant isolates. There was perfect association between macrolide resistance and presence of *erm(46)* as detected by PCR in 124 isolates of *R. equi*. Expression of *erm(46)* in a macrolide-susceptible strain of *R. equi* induced high level resistance to macrolides, lincosamides, and streptogramins B, but not to other classes of antimicrobial agents. Transfer of *erm(46)* from resistant to susceptible strains of *R. equi* was confirmed and occurred at a transfer frequency of up to 2 × 10⁻³.
This is the first molecular characterization of macrolide, line- 
samides and streptogramins B resistance in *R. equi*. Resistance is 
caused by a novel *erm* gene, *erm(46)*, which is transferrable likely 
by conjugation.

**Determining Optimal Sampling Site for *Strep-

tococcus Equi* Subj Equi CARRIERS Using a 
*Loop-Mediated Isothermal PCR Assay*. Ashley Boyle-

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We hypothesized that samples obtained from the guttural 

pouch would be more sensitive than samples obtained from the 
nasopharynx to identify carriers of *Streptococcus equi* (S. equi) 

and that a Loop-mediated isothermal (LAMP) PCR assay that 
targeted the *eqbE* gene would be more sensitive than a realtime 

PCR assay that targeted the *seel* gene.

Three samples were collected from each horse: nasopharyngeal 
flocked nylon swab (NPFS), nasopharyngeal wash (NPW), and 
endoscopically-guided guttural pouch lavage (GPL). *eqbE* LAMP 

assay was performed on NPFS, NPW and GPL samples. The 

GPL sample was split into 3 aliquots; S. equi culture, *eqbE* 

LAMP and *seel* realtime PCR. Logistic regression and area 

under the receiver-operator curve (ROC) were performed using 

STATA 13. P-values ≤0.05 were considered significant.

A total of 123 samples were obtained from 40 horses (41 

NPFS, 38 NPW, 44 GPL). 1/41 NPFS, 6/38 NPW and 24/44 

GPL sample was positive by *eqbE* LAMP. 18/26 GPL samples 

were positive with *seel* PCR. *S. equi* was isolated from 44/44 

GPL samples. GPL was the best sample to detect carriers compared to 

NPFS (OR 48, *P* < 0.001) and NPW (OR 6.4, *P* = 0.001). When 

*eqbE* LAMP results were compared to an endoscopically abnor-

mal guttural pouch, sensitivity was 74.07% and specificity was 

76.47%. LAMP ROC = 0.75. *Seel* PCR ROC = 0.78. Sensitivity 

and specificity of *eqbE* LAMP was 83.3% and 65.4% compared to 

the *seel* PCR: ROC = 0.70.

Our study demonstrates that GPL should be used to detect *S. 

equi* carriers and that *eqbE* LAMP assay was comparable to *seel* 

PCR.

**Pro-Resolution Mechanisms of Inflammation in 

Equine Recurrent Airway Obstruction: 

Tamoxifen as a New Therapeutic Option.** Benjamin 

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Henriquez, Jose Sarmiento, Gabriel Morán. *University Austral 

De Chile, Valdivia, Región De Los Ríos, Chile*

Equine recurrent airway obstruction (RAO) can severely limit 

athletic function and quality of life of adult horses. Inhala-

tion of a variety of environmental allergens induces chronic non-infec-

tious respiratory inflammation, leading to hyper-responsiveness 

of the lower airways, increased production of mucus and airway 

remodeling. Swelling and narrowing of the airways leads to 
decreased lung function and limited gas exchange. There is evi-

dence that many cells and structures of the lung play an impor-
tant role in the pathophysiology of the disease: the contribution 
of bronchial epithelial cells, vascular endothelium, lymphoid and 

myeloid cells has been well documented. The interaction of an 

allergen with resident innate immune cells induces the release of 

more inflammatory cells, via cytokines and chemokines. Once the 

inflammatory reaction has fulfilled its task, pro-resolution mecha-
nisms take the stage: the shut-down of chemokine signaling leads to 

the abrogation of inflammatory cell influx (mainly neutroph-

ils). Neutrophils initiate their apoptotic program and release mol-

ecules that attract macrophages, which in turn perform 

neutrophil clearance. The phagocytosis of apoptotic neutrophils 
eventually reprograms macrophages towards an anti-inflammato-

ry phenotype. The successful completion of the pro-resolution 

mechanism leads to the restoration of tissue homeostasis. Our 

preliminary results demonstrate that tamoxifen, a selective estro-
gen receptor modulator used as treatment in all stages of estro-
gen, positive human breast cancer, has a marked effect on equine 

neutrophil function and apoptosis.

In vitro results showed that tamoxifen significantly inhibited 

neutrophil chemotaxis in horses and humans in response to IL-8, 

from 1 μM onwards (*P* < 0.05, one-way ANOVA). Additionally, 
tamoxifen significantly inhibited production of reactive oxygen 

species from 2.5 μM onwards. Early neutrophil apoptosis was 

induced in approximately 90% of cells after 30 minutes of incu-
bation with tamoxifen at 5 μM (Sarmiento, P. et al., 2013). This 

induction of apoptosis coincided with in vivo results, in 

which neutrophils obtained from tamoxifen-treated animals 

showed a marked increase of early apoptosis.

IV and PO pharmacokinetic analysis of tamoxifen in horses is 
currently underway. A proof-of-concept trial was performed 
recently, in which 4 stalled horses with acute exacerbation of 

RAO were treated with tamoxifen (100 mg PO q 24 hours for 7 
days). This dose was extrapolated from human and murine 

pharmacokinetic data. Horses received no other treatment and 

underwent no management changes. There was a marked 

decrease in bronchoalveolar lavage neutrophil count after treat-
mament (day 0 = 28% ± 6; day 8 = 4% ± 7). Clinical parameters 

(spirometry, respiratory rate and pattern) improved from 3 days 
of treatment onwards. Tracheal mucus content did not change 

significantly over the course of treatment. These results suggest 

that tamoxifen has an effect on neutrophil survival in the pulmo-
nary environment, and warrant further investigation. Recruitment 
of additional subjects with acute exacerbation of RAO is 

ongoing.

**The Impact of Prolonged Hyperinsulinemia on 

the Downstream Insulin Signaling Pathway 

in Equine Striated Muscle and Lamellar 

Lamellae.** Allison Campolo1, Lauren Keith1, Melody de 

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Equine metabolic syndrome, an increasingly recognized dis-

case, is characterized by obesity and insulin resistance. Although 

hyperinsulinemia predisposes horses to developing laminitis, the 

exact relationship between these conditions has not been deter-

mined. We hypothesized that gene expression of proteins 

involved in the downstream insulin signaling and glucose trans-

port pathways will be altered during prolonged hyperinsulinemia-

induced laminitis.

Standardbred horses were treated with a prolonged euglyce-

mic, hyperinsulinemic clamp (p-EHC) for 48 hours or a bal-

anced electrolyte solution. Gene expression of key proteins 

involved in the insulin signaling pathway was evaluated in 

archived striated muscle and lamellar samples using real-time 

reverse transcription PCR with primers selected for insulin recep-

tor substrate-1 (IRS-1), AKT-2, and glycogen synthase kinase 

beta (GSK-3b). Gene expression of the basal glucose transporter 

1 (GLUT-1) and the insulin-sensitive GLUT-4 were evaluated 

using RT-PCR.

The p-EHC group became markedly hyperinsulinemic while 

maintaining normoglycemia and developed clinical laminitis. 

There was no significant difference in gene expression of IRS-

1, AKT-2, GSK-3b, GLUT-1 and GLUT-4 in skeletal muscle and 

lamellar tissue between groups. In contrast, there was a signifi-

cant upregulation of AKT-2, GSK-3β, GLUT-1 and GLUT-4 in 

cardiac tissue of the p-EHC group compared to controls (*P* < 0.05).

These data suggested that the prolonged, euglycaemic hyper-

insulinemic clamp induced an increase in insulin sensitivity in 

the heart, as well as a transcriptional activation of glucose trans-

port. In addition, the lack of downregulation of the insulin sig-

naling pathway in both the skeletal muscle and digital lamellae 

supports that insulin resistance is not required for the onset of 

laminitis.

**Tissue Microdialysis Studies of Equine Lamel-

lar Energy Metabolism and Microvascular 

Blood Flow.** Carlos E. Medina-Torres1, Christopher C. Pol-
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Disruption of cellular energy pathways may play a role in the different forms of laminitis. The aim of this work was to characterize the normal lamellar bioenergetic profile and establish if changes in metabolite patterns and microvascular perfusion occur as a result of modifications in limb load cycling activity and during the development of experimentally induced sepsis-associated laminitis.

First, a microdialysis technique for serial measurement of lamellar tissue energy metabolites (glucose, lactate, pyruvate, urea and glycerol) over a 24 hours period was developed, and the lamellar energy profile of 14 clinically normal horses characterized. Subsequently, 9 horses instrumented with lamellar and skin microdialysis probes were subjected to sequential interventions designed to modify tissue perfusion (4 interventions) and limb load cycling activity and weight bearing (4 interventions). Urea (20 mmol/L) was added to the perfusion fluid to investigate if urea clearance (UC; a microdialysis-based method used to assess local blood flow) could identify lamellar perfusion changes. Energy metabolite concentrations, UC and UC total ratios and energetic metabolism lactate to glucose (L:G) and lactate to pyruvate (L:P) ratios were determined during each intervention. Then, changes in lamellar bioenergetic composition and perfusion during the development of experimentally induced, sepsis-associated laminitis were investigated in 12 horses. Treatment (n = 6) and control (n = 6) horses received oligofructose (OF) and water via nasogastric tube. Lamellar metabolite composition, L-G, L-P and UC were determined bihourly and compared. Lastly, using lamellar and skin dialysate and tissue sections from healthy horses (n = 7), and lamellar dialysate from OFT (n = 4) and CON (n = 4) horses, targeted metabolomic analysis (i.e. 44 central carbon metabolites: CCM) was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The differences between lamellae and skin in healthy horses and the differences between OFT and CON horses were investigated.

In the intervention studies, UC alone and in combination with fluctuations in energy metabolite patterns detected profound and mild changes in lamellar perfusion, respectively. Significant changes in lamellar energy metabolism composition and UC were observed when limb load cycling activity was modified: increases in glucose and UC, compatible with increased lamellar perfusion, were observed when limb activity increased. In the OF study, glucose decreased significantly in OFT lamellar dialysate (OFT-L), and was consistently lower in OFT than in CON horses. Lactate was higher in OFT skin (OFT-S) compared with OFT-L. Pyruvate decreased significantly in OFT-L, and was significantly less than CON-L. L-G and L-P increased significantly in OFT-L and OFT-S, but not CON-L. Urea decreased significantly (increased UC) in OFT-L and remained stable in CON-L. Hyperaemia rather than ischaemia together with some bioenergetic disturbances occurred during the developmental phase of OF-induced laminitis. Though perfusion increased, lamellar glucose was markedly reduced, which could have compromised lamellar basal epithelial cell metabolism despite the lack of definitive evidence of lamellar bioenergetic failure. Metabolomic analysis of lamellar and skin dialysate showed no difference in metabolism composition in these two tissues in healthy horses, and the metabolome of dialysate and tissue samples was also found to be similar. A difference in CCM between OFT and CON horses was observed. Metabolomic analysis of lamellar CCM was capable of differentiating OFT from CON horses. Lamellar malate, pyruvate, aconitate and glycolate, were identified as the source of differentiation between OFT and CON groups.

These studies demonstrated that lamellar microdialysis and UC can be used to assess lamellar bioenergetic and perfusion changes. Microdialysis UC suggests an increase in lamellar perfusion during the development of sepsis-associated laminitis. The findings also suggest that lamellar perfusion and energy balance may be related to limb load cycling and are particularly affected by ambulation, supporting the hypothesis that reduced limb load cycling, but not increased weight bearing, may be associated with reduced lamellar perfusion.

**INCRETINS POTENTIATE THE INSULIN RESPONSE TO ORAL GLUCOSE IN INSULIN-RESISTANT PONIES.** Melody de Laat, Jessica van Haeften, Martin Silrence. Queensland University of Technology, Brisbane, Queensland, Australia

Equine insulin secretion varies between individuals and is affected by diet and metabolic status, with dysregulation resulting in hyperinsulinaemia, a significant laminitis risk. Gastrointestinal hormones may exacerbate insulin responses to feeding. This study investigated whether the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), augment insulin secretion following oral glucose, compared with intravenous glucose infusion.

Ponies (n = 9) received D-glucose (0.75 mg/kg) in a low glycaemic ratio (0.8%BW). Assays were validated for insulin, glucose and incretin analysis of blood samples collected before and every 30 minutes for 6 hours after feeding. Seven days later the experiment was replicated however the D-glucose was administered intravenously at a variable rate matched to individual meal consumption (~75 minutes).

Ponies were stratified for insulin sensitivity status using basal values/indices. Although intravenous glucose elicited a larger area under the curve for insulin (AUC<sub>insulin</sub>), the insulin response to oral glucose was larger (P < 0.05) and bimodal in insulin-resistant (compared to insulin-sensitive) ponies and correlated well with AUC<sub>glucose</sub>. Importantly, insulin resistance did not affect insulin responses to intravenous glucose, whereas with oral glucose the AUC<sub>glucose</sub> was higher (P < 0.05) in insulin-resistant ponies. Secretion of GLP<sub>1</sub>-active and GIP, but not GLP<sub>1</sub>-total, was higher (P < 0.05) following oral glucose (compared to intravenous) and GLP<sub>1</sub>-active was positively correlated (P < 0.05) with insulin. Further, oral glucose stimulated a larger (P < 0.05) AUC<sub>GLP<sub>1</sub>-active</sub> in insulin-resistant ponies.

Thus, the larger insulin response to oral glucose in insulin-resistant ponies is likely due in part to incretin action. In particular, GLP<sub>1</sub>-active may be a key factor in equine insulin dysregulation.

**USE OF DAILY DICLAZURIL PELLETED TOP DRESS FOR THE PREVENTION OF SARCOCYSTIS NEURONA INFECTION IN FOALS.** Nicola Pusterla, Andrea Packham, Sarah Mackie, Philip Kass, Laslo Hunyadi, Patricia Conrad. SVM, UC Davis, Davis, California, USA

Therapeutic treatment strategies for the prevention of Sarcozystis neurona infection in horses have been empirical. A pelleted top dress 1.56% diclazuril anti-protozoal drug recently introduced to the equine market and labelled for the treatment of equine protozoal myeloencephalitis (EPM) has the potential to be used for the prevention of EPM due to its convenient formulation. A low-dose of diclazuril given at 0.5 mg/kg body weight has been shown to reach plasma and cerebrospinal fluid concentrations at steady-state in excess of the minimal concentration known to be inhibitory to S. neurona merozoite production in cell culture. The purpose of this study was to evaluate the temporal serological response against S. neurona in foals receiving daily diclazuril and in untreated herdmates.

Thirty-three foals from a farm with a high exposure rate to S. neurona were randomly assigned to either an untreated control group or a diclazuril treated group. Treatment consisted of the administration of 0.5 mg/kg body weight of diclazuril pelleted top dress starting at 4 weeks of age until the foals were 12 months of age. Whole blood was collected from every day and foal 24 hours post-foaling and monthly thereafter from every study foal for the duration of the study. The blood was tested for IGG against S. neurona using the indirect fluorescent antibody test.

Following ingestion of collostral antibodies to S. neurona, there was a steady and continuous decline in seroprevalence and antibody titers to S. neurona until the foals from both groups reached weaning age. Thereafter, untreated foals showed an increase in monthly seroprevalence ranging from 53 to 88%. Diclazuril treated foals showed significant lower monthly seroprevalences ranging from 6 to 29%. All but one of the study; 88 and 6% of untreated and treated foals, respectively, tested seropositive to S. neurona.

In conclusion, daily supplementation of diclazuril pelleted top dress at 0.5 mg/kg body weight demonstrated a significant reduction in seroconversion to S. neurona between diclazuril treated and control foals up to 12 months of age. To the author’s knowl-
edge this is the first report determining dose and duration of treatment for the prevention of seconvarion in foals originat- ing from a farm with high infection rate to \textit{S. neurona}. From an economic standpoint, supplementation of diclazuril at a low-dose during early exposure and high-risk periods may benefit the horse industry by reducing costs associated with EPM.

**THE EFFECT OF ORAL AND INTRAVENOUS DEXTROSE ON C-PEPTIDE SECRETION IN PONIES.** Melody de Laat, Jessica van Haften, Martin Silence. Queensland University of Technology, Brisbane, Queensland, Australia

Managing equine hyperinsulinemia is crucial for preventing laminitis. C-peptide is secreted from the pancreas proportionally with insulin and can be used to study insulin dysregulation, but no C-peptide assays have been validated thoroughly for horses. This study aimed to identify a suitable, non-radioactive immunoassay for equine C-peptide and compare C-peptide secretion following oral and IV dextrose administration in ponies. Seven assays were assessed for precision, accuracy and specificity. Only one assay was deemed acceptable and was used to measure the response of nine ponies to oral and IV dextrose (0.75 mg/kg). The ponies were designated as insulin-resistant (IR) or insulin-sensitive (IS) based on fasted glucose-to-insulin ratios and oral glucose tests. C-peptide concentrations increased rapidly from fasted levels following both oral (\(P < 0.01\)) and IV (\(P < 0.001\)) dextrose, with similar AUC for both tests. C-peptide and insulin concentrations were correlated (\(P < 0.05\)), when AUC were com- pared. The AUC\(_{C-peptide}\) for oral dextrose was 7-fold higher (\(P < 0.05\)) in IR, compared to IS ponies. Insulin clearance (fractional difference to C-peptide) was greater for the oral test, and in IS ponies 60 minutes after dextrose administration, for both tests. Whereas C-peptide and insulin responses to IV dextro- se indicated that pancreatic capacity was similar for both groups, only IR ponies maintained this magnitude of response to oral dextrose. Increased insulin secretion is a major component of hyperinsulinemia, although reduced clearance also appears to contribute, with increased capacity for insulin response to oral carbohydrate in IR ponies.

**THE PREVALENCE OF LARGE INTESTINAL MUCOSAL PATHOLOGY IN HORSES BEING EUTHANIZED FOR NON-GASTROINTESTINAL REASONS.** Derek Knottenbelt, Nicola Kerbyson, Timothy Parkin. University of Glasgow, Glasgow, UK

The prevalence of colonic ulceration has previously been stated as being 63% in a large study of 545 horses (1) although these lesions were not defined grossly or histopathologically. Detailed gross and histological examination of the gastrointestinal tract of 36 horses euthanized for reasons unrelated to the gastrointestinal tract revealed that 97% had grossly obvious colonic mucosal pathology; of these 24 cases (67%) were considered to be of likely clinical significance. These included sand enteropathy, active cya-thostominosis, right dorsal colitis and focal and diffuse colonic ulceration. Focal congestion of the caecum (15/36), ventral colon (13/36), right dorsal colon (14/36) horses and small colon (1/36) was obvious visually but the clinical significance is equivocal. This study demonstrates that a range of large intestinal mucosal pathology may be present even in the absence of overt clinical signs. Subclinical large colon / caecal disease should be considered in all cases with signs of abdominal disease although the specific diagnosis in most cases would require significant invasive investiga- tion. Rectal biopsy is considered to be poorly correlated with most of the conditions we identified and this concurs with other recent research [2]. Minimally invasive diagnostic tests such as fecal occult blood, fecal pH and detailed microbiota panels need to be established to allow effective and accurate ante-mortem diagnosis and this needs to be correlated closely with clearer definition of the range of clinical diseases identified pathologically in this study. References:

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**MEDICAL ALTERNATIVES TO CONVENTIONAL CYCLO-OXYGENASE INHIBITORS FOR TREATMENT OF ACUTE FOOT PAIN IN A REVERSIBLE LAMENESS MODEL IN HORSES.** Jonathan Foreman, Catherine Foreman, Benjamin Bergstrom. University of Illinois, Urbana, Illinois, USA

In the treatment of acute equine foot pain such as laminitis, conventional treatments include the pan-cyclooxygenase (COX) inhibitors phenylbutazone (PBZ) and flunixin meglumine (FM). These pan-COX inhibitors (conventional NSAIDs) are known to cause side effects such as gastrointestinal ulceration and renal disease. Veterinarians treating laminitis often search for efficacious alternna- tives to conventional NSAIDs. The hypothesis in this series of 4 experiments was that non-pan-COX-inhibitor therapies would be shown to be more efficacious than conventional NSAIDs for the treatment of acute equine foot pain in a reversible model of lameness in horses.

8 horses were shod with an adjustable heart bar shoe on one foot. Horses were monitored by a blinded investigator for 12- or 24-hours post-medication. Variables included HR and Lameness Score with results compared by RM ANOVA and Tukey’s test with \(P < 0.05\).

Firocoxib (0.09 mg/kg IV) was no different than saline and both were less effective than PBZ (4.4 mg/kg IV) (\(P < 0.05\)). A firocoxib loading dose (0.27 mg/kg IV) was similar in efficacy to PBZ (4.4 mg/kg IV). Acetaminophen (20 mg/kg PO) and oral FM (1.1 mg/kg PO) administered separately were comparable to one another and both were better than saline (\(P < 0.05\). Acetaminophen (20 mg/kg PO BID) combined with firocoxib (0.27 mg/kg SID) was similar to PBZ (4.4 mg/kg IV BID) and both regimes were better than saline (\(P < 0.05\)) in a 24-hours study.

Intravenous firocoxib (loading dose 0.27 mg/kg IV SID) and oral acetaminophen showed promise in alleviating acute equine foot pain.

**INITIAL CHARACTERIZATION OF THE TRACHEAL MICROBIOSES IN HEALTHY HORSES AND HORSES WITH HEAVES.** Julia Montgomery, Katharina Lohmann, Bonnie Chab- han, Lisa Johnson, Scott Dos Santos, Jordan Steedman, Janet E. Hill. Western College of Veterinary Medicine, University of Saskatchewon, Saskatchewan, Saskatoon, Saskatchewan, Canada

Microbiomal communities colonizing the airways may play a role in the development of chronic inflammatory airway diseases. To investigate whether this may be true for equine heaves, we char- acterized the tracheal microbiomes of three healthy horses and five horses with historical, clinical and/or bronchoalveolar lavage cytolological findings consistent with heaves. On two occasions, approximately 2 weeks apart, tracheal aspirate (TA) samples were collected transendoscopically using a two-stage sampling catheter. Total genomic DNA was extracted from one aliquot and DNA integrity verified by PCR amplification of the horse mitochondrial \textit{cox1} gene. Total bacterial load was estimated by quantitative PCR targeting the 16S rRNA gene, and PCR ampli- con libraries based on the universal 60 kDa chaperonin (cpn60) gene were prepared using established protocols and sequenced using the 454 GS Junior pyrosequencing platform. Sequences were assembled into operational taxonomic units (OTU) and compared to the cpnDB reference database (www.cpndb.ca) to identify the closest reference sequence (nearest neighbor) for each OTU. Phylum assignment of OTU sequences was based on the taxonomic lineage of the nearest cpnDB reference sequence. Alpha and beta diversity were calculated using QIIME, and hier- archical clustering of microbiome profiles was performed in R. A second aliquot of each sample underwent routine microbiologic analysis using aerobic culture on blood and McConkey agar plates, followed by biochemical testing and identification. Up to 12 colonies per microbiologic culture plate were amplified using cpn60 universal primer PCR, sequenced and compared to the micro- biome library from the corresponding horse.

A total of 15 TA samples were available for analysis. Of the five horses with heaves, two had a light disease at the time of sampling and three were considered to be in remission. Total bacterial load (number of 16S rRNA copies per ml of TA) appeared higher in horses with heaves although considerable variation existed within groups and between samplings. A total of
94,800 sequence reads were analyzed (median 1,488 per sample; range 137-35,926). Consistent with the 16S rRNA copy number, microbiomes of horses with heaves appeared to have higher species richness than those of healthy horses. Microbiome profiles of horses in both clinical categories were dominated by Firmicutes, Actinobacteria and Proteobacteria. OTU sequences corresponds to 329 nearest neighbor reference sequences. OTU with similarity to *Pseudomonas fluorescens*, *Rothia sp.*, *Pantoea agglomerans*, and *Streptococcus spp.*, were the most prevalent among all samples and were frequently the most abundant in individual microbiomes. Both phylum level profiles and hierarchical clustering of OTU level profiles based on Bray-Curtis dissimilarity showed that microbiome profiles of individual horses were largely similar between the two samplings, and that microbiomes did not cluster by clinical category. Sequence data were obtained from 51 bacterial colonies, which represented 35 unique sequences. Only 8/35 of these sequences were >99% identical to OTU in the relevant microbiome libraries. Species identified by both culture and microbiome sequencing included only Firmicutes and represented only 6.7% of the sequence data generated from all horses, consistent with the more comprehensive view of the microbiome provided by the culture-independent approach.

In conclusion, this pilot study suggests that horses with heaves may have a higher bacterial load with greater species richness, but their microbiome profiles are not distinguishable from those of healthy horses at the phylum or OTU level. For most of the horses, tracheal microbiomes appeared stable during the timeframe of the study. Microbiome analysis at the genetic level enhanced the assessment of microbial communities compared to conventional culture methods. Work is ongoing to confirm these initial findings in a larger group of horses.

FOOD ANIMAL

CHARACTERIZATION OF THE PATHOGENICITY AND SHEDDING OF BOVINE PARAINFLUENZA 3 GENOTYPES, Benjamin Newcomer1, John Neill2, Binu Velayudhan3, Paul Waj2, Anubhav University, Auburn, Alabama, USA, 2National Animal Disease Center, Ames, Iowa, USA, 3Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, Texas, USA

Bovine parainfluenza-3 virus (PI3) is a widespread respiratory pathogen of cattle capable of causing disease as a unique pathogen but more commonly predisposing cattle to secondary bacterial pneumonia. The development of pneumonia. Non-A genotypes in the United States (US) have only recently been reported but little is known of their pathogenicity or shedding compared to PI3-A. Therefore, the purpose of this pilot study was to compare the pathogenicity and shedding of clinical US isolates of all three genotypes (A, B, C). Sixteen weaned Jersey bull calves seronegative to PI3 were randomly assigned to one of four groups; calves were challenged intranasally with approximately 10^7 CCID50 of one of the PI3 genotypes or served as negative controls. The calves were maintained in isolation rooms and daily nasal swab collection and observation for signs of disease by a blinded investigator were performed throughout the 14-day study. Mild clinical signs were observed similarly in all challenged groups and included serous nasal discharge and coughing. Onset of nasal shedding differed between groups with all PI3-A challenged calves shedding beginning on Day 4, 3 of 4 PI3-B challenged calves on Day 5, and PI3-C challenged calves on Day 6. Shedding of PI3-C lasted 5 days; shedding of the other viruses extended 7–8 days. This study demonstrates a difference in shedding dynamics between PI3 genotypes.

PERCUTANEOUS TUBE CYSTOSTOMY USING RUTNER SUPRAPUBIC CATHETER FOR TREATMENT OF OBSTRUCTIVE UROLITHIASIS IN FIVE GOATS, Katharine Simpson1, Robert Stroock2, Sarah Fajmonski1, Joseph Lozier1. 1The Ohio State University, Columbus, Ohio, USA, 2Oklahoma State University, Stillwater, Oklahoma, USA

Obstructive urolithiasis in male small ruminants is a common metabolic disease that often requires surgical intervention. Temporal or permanent urinary diversion is indicated if urethral process amputation and/or retrograde urethral hydropulsion are unsuccessful in restoring urethral patency. Surgical cystostomy tube placement is often considered the treatment of choice to allow for urinary diversion and ultimately the reestablishment of urethral patency. However, the costs associated with general anesthesia, surgery and post-operative care can be substantial. Advantages of percutaneous tube cystostomy rather than surgical tube cystostomy placement include shortened procedure time, cost-effectiveness, and provision of rapid urinary diversion in systemically compromised patients. Complications of general anesthesia are avoided as the procedure can be performed utilizing local anesthesia with or without sedation. We describe use of a commercially available suprapubic catheter1 for percutaneous tube cystostomy in conjunction with urinary acidification as a primary treatment modality in cases of obstructive urolithiasis in goats with suspected or confirmed struvite urolithiasis.

Five goats (four Pygmy, one French-Alpine) were diagnosed with obstructive urolithiasis. Retrograde urethral catheterization was unsuccessful in relieving the obstructions in four of the goats. The other goat was considered to be emergent and a poor anesthetic candidate. A Rutner suprapubic catheter2 was percutaneously inserted into the urinary bladder utilizing ultrasound guidance in all five goats to provide urinary diversion. Patency of the urethra was reestablished in 4 of the 5 goats. Mean and median cost associated with percutaneous cystostomy was $677.86 and $701.54 respectively, compared to $1406.05 (mean) and $1393.14 (median) associated with surgical tube cystostomy during the same year. Complications noted post-cystostomy included obstruction of the catheter (n = 1), transient uroabdomen (1), and urethral rupture (1).

Based on these outcomes, we suggest that percutaneous tube cystostomy using a commercially available Rutner suprapubic catheter2 (16 Fr, 4 mL balloon) in conjunction with urinary acidification may be a useful non-invasive and cost-effective alternative to surgical interventions for treatment of obstructive urolithiasis; particularly in cases with uroliths amenable to dissolution. Additional research is indicated to fully elucidate post-procedural complications and long-term prognosis.

1Rutner Suprapubic Balloon Catheter Set, Cook® Medical, 16 French, 4 mL balloon.

PHARMACOKINETICS OF AN EXTENDED RELEASE FORMULATION OF EPRINOMECTIN IN HEALTHY ADULT ALPACAS AND ITS USE IN ALPACAS CONFIRMED WITH MANE:G, John Pollock1, Daniela Bednarek1, Mark G. Parch2. 1Cummings School of Veterinary Medicine, Tufts University, Grafton, Massachusetts, USA, 2North Carolina State University, Raleigh, North California, USA

The study purpose was to determine the pharmacokinetics (PK) and clinical effects of extended release 5% eprinomectin (Longrange®, Merial) following subcutaneous (SC) injection in healthy (n = 6) and mange-infected (n = 4) adult alpacas. High-performance liquid chromatography was performed on plasma samples obtained at regular intervals for 161-days following a single 5 mg/kg injection SC in healthy alpacas, and for 5-days following each dose (3 treatments, 2 months apart) in mange affected animals. Clinical monitoring included biweekly hematology and physical examination; monthly weight and fecal egg counts (FEC-double centrifugation). Skin scrapings and biopsies were performed pre- and post-treatment at two comparable sites in alpacas with mange. Four alpacas served as healthy controls. Pharmacokinetic analysis utilized a non-compartmental model (WinNonlin®6.0). Results were compared between time-points using repeated-measures ANOVA and paired samples t-test. Eprinomectin plasma concentrations showed a biphasic peak (Cmax-1 and 2) in all animals:

| Parameter | Cmax-1 | T1/2 (days) | Cmax-2 | T1/2 (days) | AUC (ng/mL) | T1/2 (day*ng/mL) |
|-----------|-------|-------------|-------|-------------|------------|-----------------|
| Mean      | 5.72  | (3.25)      | 3.88  | (5.2)       | 6.06       | (2.47)          |
| (SD)      |       |             |       |             | 77         | (12.52)         |
|           |       |             |       |             | 456.6      | (154.5)         |
|           |       |             |       |             | 15.53      | (3.16)          |


Epinormectin plasma concentrations remained above 1.27 ± 0.96 ng/mL for up to 120 days. Hematocrit (35.8 versus 31.3%, *P < 0.003) and albumin (3.5 versus 2.8 g/dL, *P < 0.006) reduced significantly over 6 months in multi-dose animals, while FEI did not differ between groups. Self-limiting jejunal lesions and reactions occurred in 9/10 animals. Pre- and post-treatment skin biopsies showed reduced hyperkeratosis, but increased fibrosis, with 1/4 alpacas remaining positive on skin scraping for mange.

In conclusion, alpacas require a higher epinormectin dose (5 mg/kg SC) than cattle, to reach comparable plasma concentrations.

DEVELOPMENT OF A ZINC IMPLANT-BASED MODEL FOR UROLITHIASIS IN GOATS. Meredith Jones, Allen Roussel, W. Shawn Ramsey. Texas A&M University, College Station, Texas, USA

The study of urolithiasis in small ruminants is hampered by the lack of a convenient and reliable model that would allow the quantitative assessment of the calculogenic potential of rations. The objective of this study was to develop a nonsurgical experimental model of urolithiasis in goats.

Ten, 1-year old Boer-cross doelings were fed a calculogenic diet designed to promote struvite urolithiasis. Zinc washers, 6–13 mm in diameter, were used with some washers triple wrapped with chromic catgut or polyester fiber suture. Goats were anesthetized and the implants were placed into the lumen of the urinary bladder retrograde through the urethra. Different combinations of washer size and suture material and pattern were used in 4 separate experiments. At the end of each experiment, 28–118 days after placement, the implants were retrieved via the urethra. Two implants required removal via cystotomy due to the large amount of crystalline precipitation.

Across experiments, 25–67% of implants were spontaneously passed. All implants that remained in the bladders until the end of the experiment had grossly visible calculus accumulation. Discs with suture material accumulated 1.5–46 mg/day compared to 0.03–1.9 mg/day for those without suture material. Urolith analysis revealed that the predominant material accumulated was either struvite or amorphous magnesium calcium phosphate. This study demonstrates that uroliths can be created consistently in a nonsurgical model in goats. This model should be useful in the study of rations and to evaluate interventions that may reduce or prevent the formation of urinary calculi in small ruminants.

EFFICACY OF DIFFERENT MULTIVALENT MLV VACCINES ADMINISTERED TO CALVES POSSESSING MATERNALLY-DERIVED IMMUNITY AND SUBSEQUENTLY CHALLENGED WITH BVDV OR BVDV AND BOHV-1. Manuel F. Chamorro1, Paul H. Walz2, Thomas Passler2, Kay Ridell2, Benjamin Newcomer1, Roberto Palomares1. 1Auburn University, Auburn, Alabama, USA, 2University of Georgia, Athens, Georgia, USA

The objective of these studies was to evaluate efficacy of different commercially-available multivalent MLV vaccines to prevent clinical disease, viremia, and virus shedding in early weaned beef calves challenged with BVDV or BVDV and BOHV-1. In the first study, 48–male beef calves were early weaned at a median age of 72.2 days and assigned to 1 of 4 treatment groups: control (A), vaccine B, Vaccine C, and Vaccine D. Forty-five days after vaccination, calves were challenged with virulent BVDV 2. In the second study, 54–male beef calves were early weaned (median age 93.5 days) and assigned to 1 of 5 treatment groups: control (A), vaccine B, vaccine C, vaccine D, and vaccine E. Forty-five days after vaccination calves were simultaneously exposed to 6 cattle PL with BVDV and 8 calves acutely infected with BoHV-1. Samples were collected in all calves for virus isolation and virus neutralization analysis before vaccination and before and after virus challenge. Calves vaccinated with vaccines B and C in the first study and calves vaccinated with vaccines B, C, and E in the second study had greater BVDV-antibody responses and a lower proportion of viremia and BVDV shedding compared with controls. Vaccination of early weaned beef calves resulted in increased BVDV-antibody responses and reduced viremia and BVDV shedding. Differences in vaccine efficacy to prevent BVDV viremia and shedding were observed.

COMPARISON OF JOHNE’S DISEASE PREVALENCE ON ORGANIC AND CONVENTIONAL DAIRY FARMS IN PENNSYLVANIA. Marie-Eve Fecteau, Helen Aceto, Terry Fock, Raymond Sweeney. School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pennsylvania, USA

Johne’s disease (JD) affects approximately 70% of all US dairies, and can be a cause of great economic loss to dairy producers. To qualify for the label “organic”, certain restrictions on management practices may predispose for the transmission of JD on the farm. The objectives were: 1- to compare JD prevalence between Pennsylvania organic and conventional dairy farms, and 2- to identify risk factors associated with differences in JD prevalence between groups. A JD milk ELISA was performed on individual milk samples from each lactating cows in the study herds. Information regarding management practices was collected during a farm visit. Overall herd prevalence, and within-herd prevalence were compared between groups. Logistic regression was used to identify risk factors associated with differences in JD prevalence between groups. A total of 2,739 cows from 50 herds (25 organic and 25 conventional) were included in the study. Median herd size was 58 (range 20–114 cows) for conventional farms, and 39 (range 20–211 cows) for organic farms. Of all the farms included in the study, 27/50 (54%) were positive, with 15/25 (60%) positive conventional farms and 12/25 (48%) positive organic farms. From the conventional farms, 25/1,506 (2%) cows were positive, compared with 28/1,233 (2%) cows from organic herds. After adjusting for herd size, there was no significant difference in herd prevalence (*P = 0.56), or within-herd prevalence (*P = 0.41) between conventional and organic farms. Risk factors are currently being analyzed. Preliminary results indicate that there is no difference in JD prevalence between Pennsylvania organic and conventional dairy farms.

A RANDOMIZED CLINICAL TRIAL EVALUATING METABOLISM OF COLOSTRAL AND PLASMA DERIVED IMMUNOGLOBULIN G IN JERSEY BULL CALVES. Kelly Pipkin, Jill Hagey, Maire Rayburn, Munashe Chigerwe. UC Davis, Davis, California, USA

The objective of this study was to determine the rate of catabolism of colostral derived IgG administered by oroesophageal tubing compared to IV administered plasma IgG.

A randomized clinical trial was performed. Thirty newborn Jersey calves were enrolled. Fifteen were fed colostrum (CL group) and 15 were given bovine plasma IV (PL group). Calves in the CL group were fed 3 L of colostrum once, by oroesophageal tubing compared to IV administered plasma IgG. Across experiments, 25–67% of implants were spontaneously passed. All implants that remained in the bladders until the end of the experiment had grossly visible calculus accumulation. Discs with suture material accumulated 1.5–46 mg/day compared to 0.03–1.9 mg/day for those without suture material. Urolith analysis revealed that the predominant material accumulated was either struvite or amorphous magnesium calcium phosphate. This study demonstrates that uroliths can be created consistently in a nonsurgical model in goats. This model should be useful in the study of rations and to evaluate interventions that may reduce or prevent the formation of urinary calculi in small ruminants.

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PLASMA LIPIDOMIC PROFILE IN COWS DURING THE TRANSITION PERIOD AND IN COWS WITH FATTY LIVER. Christian Gerspach1, Hanspeter Naegeli1, Maude Guibler1, Claudine Bieli1, Endre Lackzo2, Maja Rutter1, Sandro Imhaisl2. 1Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, 2Functional Genomics Center Zurich, Zurich, Switzerland

The transition time around parturition and early lactation involves critical physiologic changes in dairy cows. An excessive demand for nutrients due to the increased performance required for milk production results in a negative energy balance. One major adjustment consists in the rapid mobilization of energy sources from tissue depots in the form of non-esterified fatty acids.
acids. Cows poorly adapting to NEB are at high risk of developing disease. Many transition period diseases, including fatty liver, occur in a subclinical form, affecting milk production and reproductive performance of dairy cows. Fatty liver is interrelated with other production diseases. A non-invasive and accurate test would be helpful to diagnose fatty liver in cattle.

The purpose of a large-scale screening approach was to provide a hypothesis for the development of a novel diagnostic test based on lipidome profiles that is less invasive and more accurate.

We compared the plasma lipidome of diseased dairy cows using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. A study on 63 cows revealed 20 masses, which could distinguish between healthy cows and cows with different stages of disease.

In a study on 12 clinical healthy calves, during their transition period, 30 masses could be analyzed by MS/MS as potential biomarkers for determination of changes within the lipidome relative to calving. The main lipid groups detected, were triacylglycerides, phosphatidylethanolamines, and lysophosphatidylethanolamines.

**Bayesian Estimation of the Accuracy of Clinical Examination and Systematic Thoracic Ultrasonography for the Diagnosis of Bovine Respiratory Disease in Pre-Weaned Dairy Calves**, Sébastien Bucziński,1 Teresa Ollivetti,2 Nandini Dendukuri,2 1Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Quebec, Canada, 2Department of Medical Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA, 3Department of medicine, Department of epidemiology, Biostatistics and Occupational Health, McGill University, Montréal, Quebec, Canada

There is no gold standard method for the diagnosis of bovine respiratory disease (BRD) complex in Holstein pre-weaned dairy calves. Systematic thoracic ultrasonography (TUS) has been used as a proxy for BRD that can be performed in the field by veterinarians, but cannot be directly used by producers. The TUS examination focuses on lung consolidation, a common finding in bronchopneumonia. The Wisconsin calf respiratory scoring chart (CRSC) is a simpler alternative that systematically assesses rectal temperature, cough, ear position, nasal and ocular discharge and attributes a score to each of these items as well as the decision based on the total score obtained (i.e. do nothing, monitor, or treat). However, the accuracy of CRSC is still unknown.

Our objective was to estimate the accuracy of CRSC in two different populations, while adjusting for the lack of a gold-standard to define BRD status.

Two cross sectional study populations with a high BRD prevalence (n = 106 pre-weaned Holstein calves) and an average BRD prevalence (n = 85 pre-weaned Holstein calves) from North America were studied. All calves were simultaneously assessed using CRSC (cutoff ≥5) and TUS (cutoff ≥1 cm of lung consolidation). When using TUS as a gold standard CRSC Se were 20.0% and 55.4% and Sp were 58.0% and 100.0% respectively. Bayesian latent class models with conditional dependence were used with informative priors for BRD prevalence in both settings and TUS accuracy (Se_{TUS} and specificity (Sp_{TUS})) and non-informative priors for CRSC accuracy (Se_{CRSC}/Sp_{CRSC}).

The Se_{CRSC} (95% credible interval (CI) and Sp_{CRSC} were 62.4% (47.9–75.8) and 74.1% (64.9–82.8). The Se_{TUS} was 79.4% (66.4–90.9) and Sp_{TUS} 93.9% (88.0–97.6). Sensitivity analysis revealed the results were robust to prior specification.

Despite their imperfect accuracy both tools are helpful for BRD management. Improvement of the accuracy of BRD detection is a key step to decrease its negative impact as well as over-use of antimicrobials for false positive cases.

**Impact of Milk Feeding Levels and Housing on the Incidence of Respiratory Disease and Subsequent Productivity of Young Dairy Calves**, Maria Prado, John Wilkerson, Peter Krawczel, Chris Boyer, Arnold Saxton. University of Tennessee, Knoxville, Tennessee, USA

Dairy calf pneumonia (DCP) continues to be a highly prevalent condition affecting calves during the pre-weaning and/or post-weaning periods. However, its impact extends beyond the actual disease episode, having a negative effect on subsequent productivity and survivability of replacement stock. The ability to identify calves during the initial stages of respiratory disease development by monitoring activity and feeding behavior would allow for earlier disease intervention and thus potentially decrease the effects that DCP has later on the calf’s life. Therefore, the objective of this study was to determine the impact of different milk feeding levels (standard or high) and housing (individual or group) on respiratory disease development and subsequent productivity of young dairy calves.

Female Holstein calves (n = 215) from a commercial dairy farm were used in this study. Calves were randomly assigned to one of three treatment groups: (1) individual hutch with standard milk (4 L/day), (2) individual hutch with high milk (8 L/day), and (3) paired hutch (2 calves) with standard milk in a randomized block design. Social interaction/activity levels as well as feeding behavior were measured by fitting calves with collars containing sensors during the pre-weaning period. Calves were screened daily for clinical signs of disease. DCP was confirmed by PCR or serology. Body weights were collected from pre-weaned and weaned calves. Data collected were analyzed to identify behavior changes in social interaction and/ or feeding associated with the development of respiratory disease. Serum samples were collected for determining passive transfer at 48 hours after birth. Farm records are being used to evaluate the effects of management during the pre-weaning phase on the long-term health and productivity of these calves by a retrospective analysis.

The automated sensor system successfully recorded activity levels of calves in the different treatment groups. Total activity levels were correlated with milk consumption showing significant increases during the time preceding the feedings. In addition, we established a unique time series signature from the acceleration data (activity levels) that corresponds with normal specific behaviors such as sleeping, standing, walking and/or bottle feeding. We used these data to identify any deviations from the norm. Overall, respiratory disease levels at this farm were low. All calves developed diarrhea when moved to the hutches. Calves in the high milk group gained an average of 1.28 lbs/day versus 1.06 lbs/day in the control group. Average daily gain was comparable between calves in the high milk group and the paired group (1.25 Lbs.). Overall, the calves fed high milk levels gained the most weight and the incidence of respiratory disease was low (0.93%: 2/215 of all enrolled calves). Milk yield, health and reproduction data will be presented.