ABSTRACT #177
INVESTIGATION OF THE INNERVATION OF THE CANINE MITRAL VALVE BY IMMUNOHISTOCHEMICAL STAINING. GJ Culshaw, AT French, GT Pearson, BM Corcoran. Royal (Dick) School for Veterinary Studies, University of Edinburgh, Edinburgh, UK.

Sympathetic, parasympathetic and sensory nerve fibres have been identified in mitral valves from many species but not from dogs. This innervation suggests valvular movement and tone are not entirely passive.

The aims of this study were to use immunohistochemistry to confirm, map and characterise innervation within mitral valves from dogs of different ages.

Anterior mitral valve leaflets were collected from 11 healthy dogs. Four dogs were less than 1 year of age, 4 dogs were 5 years of age and 3 dogs were estimated as 9–10 years of age. Innervation was assessed qualitatively and semi-quantitatively.

Innervation was confirmed in leaflets in all 3 groups but it was markedly reduced in the aged group. Myxomatous valve disease (MVD) was present in the valves of dogs 5 years of age and older. Innervation was densest proximally and mainly associated with the epimysial, perimysial and endomysial layers of the muscle within the valve and with blood vessels. Innervation was reduced within the middle zone of the valve and absent distally. Chordal innervation was not identified. Nerve fibres were mostly sympathetic, some were parasympathetic and some sensory.

This study confirms that the canine mitral valve is innervated. Innervation is mainly sympathetic and within muscle and around blood vessels proximally suggesting a role in valvular function and health. Zonal distribution of innervation is similar to that in other species. Density of innervation reduces with age or MVD. Further investigation of the role of innervation in the pathophysiology of MVD is required.

ABSTRACT #178
RETROSPECTIVE DESCRIPTION OF CANINE VENTRICULAR SEPTAL DEFECT. JA Abbott[1], K Hawkes[1], MT Small[2], CE Atkins[2], TC DeFrancesco[2], RL Pyle[1], BW Keene[2]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

In order to describe the clinical characteristics of canine ventricular septal defect (VSD), we reviewed case records of patients that

had been presented to one of two veterinary teaching hospitals in the southeastern United States. Patients with a diagnosis of VSD were retrospectively identified through a search of medical records and the echocardiographic case log. For one institution[1], the review period was May 1, 2000–December 31, 2004, and for the other[2], January 1, 1991–December 31, 2004. Cases were included if a Doppler echocardiographic diagnosis of VSD was recorded. Complex malformations that include a VSD and constitute defined diagnostic entities such as tetralogy of Fallot and atrioventricular canal were excluded. Clinical findings were abstracted from the medical record. Breed predispositions were evaluated through calculation of odds ratios (OR) and associated 95% confidence limits (CL) using the unaffected caseload of one institution as the control sample. Case outcome as of December 2006 was determined from review of medical records and telephone calls placed to veterinary clients and referring veterinarians.

Fifty-three cases were identified of which 31 (59%) were male. Forty-seven (89%) patients were identified as one of 32 different pure breeds and 6 were mixed-breed dogs. The Shiba Inu (OR: 278 [95% CL: 94.47, 820.81]), Bloodhound (OR: 37 [95% CL: 13.12, 106.16]), Old English Sheepdog (OR: 20 [95% CL: 4.83, 85.05] and Chihuahua (OR: 6 [95% CL: 1.87, 19.38]) were amongst purebred dogs that were over-represented. The median (range) age at which the VSD was identified was 5 (2–132) months. Most (85%) patients were presented for evaluation of a subclinical heart murmur or for pre-breeding evaluation while 8 (15%) were presented because of family history or for evaluation of signs of tachypnea or exercise intolerance. Systolic murmurs were heard during examination of 45 (85%) of the patients while 7 (13%) had to-and-fro murmurs; 1 (2%) patient did not have a murmur. The arterial pulse was described as strong or bounding in 5 (9%) patients. The echocardiographic end-diastolic left ventricular dimension exceeded the upper limit of a published reference interval in 7 (13%) patients. Seven (13%) patients had moderate or severe aortic valve insufficiency. Associated lesions included pulmonary hypertension in 4 (8%), pulmonic stenosis in 5 (10%), subvalvular aortic stenosis in 5 (10%) and tricuspid valve dysplasia in 3 (6%). Four patients died or were euthanized as a result of heart disease; the median (range) age at the time patients were lost to follow-up, died of non-cardiac causes, or the study ended was 31 (2-151) months. Two (4%) VSD closed spontaneously.

Canine VSD often is benign. Breed predispositions are evident which suggests a genetic basis for the malformation in some cases.

ABSTRACT #179
SURVIVAL STUDY AND ASSESSMENT OF PROGNOSTIC FACTORS IN DOGS WITH IDIOPATHIC DILATED CARDIOMYOPATHY. FL Yamaki[1], EC Soares[1], GG Pereira[1], VCM Oliveira[1], DAR Moreira[2], MHMA Larsson[1]. [1]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, Brazil. [2]Instituto Dante Pazzanese de Cardiologia, São Paulo, SP, Brazil.

Dilated cardiomyopathy (DCM) is one of the most common acquired cardiovascular diseases of dogs. Few studies have been described in dogs with the purpose of determining prognostic indicators for DCM, and predicting prognosis in any given single patient continues to be a challenge. Therefore, the aim of this study was to evaluate survival time, and to find factors influencing prognosis in dogs with DCM.

Fifty dogs with DCM were prospectively evaluated by physical examination, ten-lead electrocardiography, thoracic radiography, 24-hour Holter monitoring, and echocardiography. The animals were followed-up for at least 150 days or until death. Thirteen clinical variables were studied, including left ventricle shortening fraction, and presence of non-sustained ventricular tachycardia on Holter monitoring. Kaplan-Meier method was used for estimating survival curves. Comparisons of Kaplan-Meier curves were made using Log-Rank test. A multivariate analysis based on exponential distribution was performed, and Cox regression method was used in order to determine independent prognostic factors.

The mean and median survival times were, respectively, 347 days and 223 days (range 5 to 1021 days). Probability of survival at six months was 51%, at 1 year was 37%, and at 2 year was 13%. The survival time was significantly longer ($p = 0.046$) in English Cocker Spaniel. Atrial fibrillation ($p = 0.037$), ventricular ectopy on ten-lead electrocardiography ($p = 0.022$), and non-sustained ventricular tachycardia on Holter monitoring ($p < 0.001$) were associated with increased mortality.

Survival time was variable, but the prognosis was usually poor. The presence of non-sustained ventricular tachycardia in Holter monitoring was the most significant prognostic indicator.

ABSTRACT #180
DEVELOPMENT OF A MULTIVARIATE STATISTICAL MODEL TO PREDICT CONGESTIVE HEART FAILURE IN CANINE MITRAL VALVE DISEASE. CA Reynolds, DC Brown, MA Oyama. Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

The progression of canine mitral valve disease (MVD) is variable, and predicting the risk and time to onset of congestive heart failure (CHF) in asymptomatic dogs is challenging. Accurate prediction would permit intervention and ostensibly improve outcome. The purpose of this study was to retrospectively develop a logistic regression model that identifies which variables are likely to best distinguish dogs with and without CHF due to MVD, and to then evaluate the ability of these variables to predict risk of CHF in a cohort of asymptomatic dogs followed over time. To help identify critical variables, medical records of 82 dogs with MVD were retrospectively evaluated and physical examinations, thoracic radiographs, and echocardiograms were reviewed. Forty five dogs (55%) were diagnosed with CHF based on thoracic radiographs and 37 dogs (45%) were asymptomatic. The association between presence or absence of CHF and age, tricuspid regurgitation velocity, left ventricular dimension at end-diastole, left ventricular dimension at end-systole, left atrial size (LA:Ao), vertebral heart size, heart rate, and serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) were examined by multiple logistic regression. NT-proBNP had the strongest association with the presence of CHF ($p < 0.001$), and the other variables were individually added to determine their significance and effect on the model. The combination of NT-proBNP and LA:Ao yielded the best goodness-of-fit value (Hosmer-Lemeshaw GOF = 0.39). Analysis of the receiver operating characteristic curve indicated that the combination of LA:Ao and NT-proBNP was highly accurate in differentiating dogs with CHF from those without CHF. The area under the curve for the ROC curve was 0.98. There was a 10% probability of CHF when LA:Ao is 1.7 and NT-proBNP is 541 pmol/L; a 45% probability when LA:Ao is 1.8 and NT-proBNP is 1086 pmol/L; and a 100% probability when LA:Ao is 2.8 and NT-proBNP is 2192 pmol/L. The developed statistical model identifies critical variables, namely NT-proBNP and LA:Ao, that should be prospectively and longitudinally examined in a cohort of dogs with asymptomatic MVD with the goal of predicting when any individual dog is going to experience the first episode of CHF.

ABSTRACT #181
ECCENTRIC MYOCARDIAL WALL THICKENING AND INCREASED INTERSTITIAL FIBROSIS UNDERLIE CARDIAC DYSFUNCTION IN LONG-TERM TYPE 1 DIABETIC CANINES. Linnea Lentz, Sheree Beam, Heidi Millner, Kathryn Hilpisch, Jennifer Heisel, Ruth Klepfer, Renee Gerhart, Teri Sorenson, Mike Miller, Rodolphe P Katra. Medtronic, Minneapolis, MN.

Diabetic cardiomyopathy is an increasingly recognized disease associated with cardiac dysfunction, even in the absence of hypertension or coronary disease. The mechanism and myocardial substrate for this dysfunction, however, remain poorly understood. We hypothesize that hypertrophy and increased fibrosis in the diabetic (DB) heart promote myocardial stiffening and underlie cardiac dysfunction.

To test this hypothesis, a total of 33 partial pancreatectomy Type 1 DB canines (mean age 8.0 ± 1.1years) and 10 control canines (CTRL, mean age 8.0 ± 1.8years) were studied. DB canines were diabetic for 6.5 ± 1.4years. Of the total canines in this study, 10 DB

and 6 CTRL canines were studied by echo to assess cardiac dysfunction. The remaining 23 DB and 4 CTRL canines had their hearts excised, weighed, sectioned and measured, for morphological and histological assessment according to standard techniques.

In general, DB had significantly larger mean left ventricular (LV) wall thickness and smaller chamber diameter (13.2 ± 0.8 and 29.3 ± 4.3 mm, respectively) compared to CTRL (12.1 ± 1.3 and 34.8 ± 3.9 mm, $p < 0.03$), despite being age- and heart- weight matched. Interestingly, regional analysis revealed heterogeneous LV thickening in DB, localized to apical posterior regions (12.0 ± 1.9 vs. 10 ± 1.2 mm, $p < 0.04$) and basal anterior regions (14.4 ± 2.1 vs. 11 ± 1.4 mm, $p < 0.01$) compared to CTRL. No significant differences in LV thickness were observed at septal or lateral regions. Histological analysis showed no notable myocyte atrophy, myocardial disarray or replacement fibrosis. However, regional analysis revealed a significant increase in interstitial fibrosis in DB compared to CTRL (Collagen score: 2.6 ± 0.6 vs. 1.2 ± 0.3 AU, $p < 0.01$), with larger levels ($p < 0.01$) localized at the epicardium relative to other regions, in CTRL and DB. Notably, echo assessment of cardiac function demonstrated slower early diastolic transmitral velocity (E) (0.7 ± 0.1 vs. 0.9 ± 0.2 m/s, $p < 0.05$) and smaller E/A ratio (1.1 ± 0.2 vs. 1.4 ± 0.3, $p < 0.05$) in DB compared to CTRL, confirming the diastolic dysfunction.

These data suggest that in Type 1 DB canines, heterogeneous cardiac remodeling, eccentric myocardial thickening, and passive stiffness due to interstitial fibrosis may play a role in altering diastolic function, thereby, contributing to manifestation of the diabetic cardiomyopathy phenotype.

ABSTRACT #182
EFFECT OF SHIPPING TEMPERATURE ON CANINE N-TERMINAL PROHORMONE ATRIAL NATRIURETIC PEPTIDE & N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE. G Farace[1], A Beardow[1], C Carpenter[1], K Yeung[1], M Zieba[1], SJ Ettinger[2], SD Forney[2]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA.

The current tests for canine N-terminal prohormone atrial natriuretic peptide (NT-proANP) and N-terminal prohormone brain natriuretic peptide (NT-proBNP) are ELISA assays that are run in a reference lab setting rather than at the petside. This means that samples have to be drawn from the patient and shipped to a facility for testing. Unfortunately both peptides are degraded by a range of peptidases and the half-life of both peptides is a matter of hours. This means that in order for the sample to reach the reference lab in the same state as when it was drawn some thought has to be given as to the conditions the sample is shipped under. One approach would be to add protease inhibitors to the sample to prevent, or at least slow, degradation. In the human field a number of protease inhibitors have been shown to be useful including EDTA, thiorphan, and aprotinin. EDTA can be easily added to a sample by collecting blood into an EDTA collection tube but this limits the sample type to plasma only. Requiring the routine addition of inhibitors adds complexity and cost to the process and is unlikely to enjoy complete compliance from the community.

Another option is to control the temperature of the sample during transit since reducing the temperature decreases peptidase activity and thus slows sample degradation. This approach has been taken by Veterinary Diagnostics Institute (VDxI) (Irvine, CA) who provide a shipping kit containing cold packs to ensure that the plasma sample is kept between −20 and 4 °C in transit.

The aim of this study was to evaluate the effect of temperatures between −60 and 37 °C on both peptides. Samples were drawn from healthy animals and from those with cardiac disease. Samples were then kept at −60, 4, 25 and 37 °C for 3–5 hours and 23–25 hours before being assayed according to the kit instructions.

NT-proANP shows little or no change after 3–5 hours at any temperature analyzed. After 23–25 hours all the samples at 37 °C show a minimum decrease of 25%, however no significant decrease is seen in the aliquots at −60, 4 and 25 °C.

NT-proBNP shows degradation in the first 3–5 hours at 25 and 37 °C with some samples, but this was not the case with all samples. At the later timepoint all the 37 °C sample aliquots were at least 50% lower than the aliquots at −60 °C. At 25 °C 2/3 of the samples also had a 50% drop compared to the −60 °C aliquot. There was no significant difference between the −60 °C and the 4 °C aliquot.

This data suggests that in order to be confident that the values obtained from a sample are accurate, plasma samples should be shipped using cold packs to ensure that the sample is at or below 4 °C. However, it is not necessary to use dry ice as there doesn't appear to be a difference between samples shipped at 4 °C and those at −60 °C over a 23–25 hour time period.

ABSTRACT #183
ANALYTICAL VALIDATION OF A COMMERCIALLY AVAILABLE CANINE N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE ELISA. M Zieba[1], A Beardow[1], C Carpenter[1], G Farace[1], K Yeung[1], SJ Ettinger[2], SD Forney[2]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA.

In the past few years NT-proBNP has risen to prominence as a marker for cardiac disease in both dogs and humans. A number of studies have been published looking at the utility of the marker to diagnose cardiac disease. Currently the only assay available for the determination of N-Terminal Prohormone Brain Natriuretic Peptide (NT-proBNP) in dogs is the CardioScreen™ NT-proBNP assay (Guildhay Ltd., UK).

The assay has a quoted limit of detection of 42 pmol/L and the inter-assay and intra-assay variations are both around 7–8%. There is, however, no mention of linearity and so the accuracy of the assay with samples significantly higher than the top standard is unknown. The objective of this study was to independently evaluate the performance of the assay and also to determine the validity of the standard curve with samples well above the highest point of the standard curve.

We ran 10 replicates of 14 different samples from both healthy and sick dogs with NT-proBNP concentrations ranging from 28 to 4039 pmol/L to evaluate intra-assay precision and found that with samples greater than 800 pmol/L the coefficient of variation (CV) was 4–8% which is slightly better than the manufacturer's claim; however, with samples below 800 pmol/L the precision was poorer, ranging from 12 to 20%. The 28 pmol/L was exceptionally poor with a CV of 141%, however this sample is below the assay limit of detection which could explain the poor precision.

Inter-assay variation was examined by running 10 replicates of 4 different samples on 3 different kit lots of plates. The samples used ranged in concentration from 365 to 2215 pmol/L. The three samples which were at or higher than 800 pmol/L had an inter-assay CV of around 7.5% while the low sample was again higher with a CV of about 16%.

The linearity of the standard curve was assessed using 6 samples ranging from 2801 to 6246 pmol/L. Each sample was run neat and diluted 1:5 and 1:10. In the case of the highest sample the difference between the neat and diluted values is close to 40% which may indicate that at the very high end the values obtained are not truly accurate and samples should be diluted and re-run. However, the NT-proBNP concentration is far away from the suggested diagnostic cut-off so this may not be a major concern. The lower samples are generally in good agreement between the neat and diluted samples with the diluted sample values being within 20% of the neat value.

We also investigated the effect of changing the incubation time from the recommended 24 hours down to as low as 3 hours and found no significant effect on precision; though the flatness of the standard curve with incubation times below 7 hours means that while the assay can be shortened, using the current kit components, the incubation step should not be shortened below 7 hours.

Overall, the kit is robust and performs according to the manufacturer's specifications.

ABSTRACT #184
EFFECT OF GENDER STATUS ON NT-PROHORMONE BRAIN NATRIURETIC PEPTIDE LEVELS IN DOGS. S Leach, DM Fine, HE Durham, VK Ganjam. University of Missouri, Columbia, MO.

Natriuretic peptides, hormones secreted in response to myocardial stretching and hypoxia, are becoming an increasingly important tools in identifying and characterizing cardiac disease. Amino-terminal prohormone brain natriuretic peptide (NT-proBNP) is increased in dogs with a variety of cardiac diseases including chronic myxomatous valve degeneration, dilated cardiomyopathy, and congestive heart failure. Both age and renal dysfunction have been associated with increased NT-proBNP levels in humans. Recently, reports in the human literature indicate that gender related differences in sex hormones also affects NT-proBNP levels. We hypothesized that these gender-mediated differences occur in dogs as well, with testosterone being inversely related and estradiol having little if any correlation with NT-proBNP.

Nine healthy intact adult dogs (6 males, 3 females) presenting for a routine spay or neuter were recruited into this study. All dogs received a complete physical examination, echocardiogram, and BUN/creatinine prior to surgery. Samples for NT-proBNP and sex hormone assays were obtained prior to neutering and at recheck examination 8 to 25 days post-operatively. NT-proBNP assay was performed using a sandwich enzyme immunoassay by a commercial laboratory (Veterinary Diagnostics Institute). Sex hormones were measured in-house using a radioimmunoassay kit. The minimum sensitivity of the testosterone assay was 0.04 ng/ml, and estradiol was 8 pg/ml.

The NT-proBNP concentrations were as follows (mean $\pm$ SD pmol/L): intact males (IM) 302.0 $\pm$ 73.1, castrated males (CM) 412.0 $\pm$ 102.9, intact females (IF) 380.7 $\pm$ 153.1, and spayed females (SF) 475.7 $\pm$ 106.0. The data was analyzed using a one-way ANOVA and post-hoc Tukey test; $p < 0.05$ was considered significant. There was no significant difference between males and females at baseline ($p = 0.13$), or post-surgery ($p = 0.41$). All subjects showed a significant increase in NT-proBNP levels post-surgery ($P = 0.012$). There was a significant increase in NT-proBNP in CM vs. IM ($p = 0.04$), but not in SF vs. IF ($p = 0.08$).

Testosterone (T, ng/ml) and estradiol (E_2, pg/ml) results were as follows (mean $\pm$ SD): IM-T 2.92 $\pm$ 1.8, CM-T $\leq$ 0.04. IM-E_2 8.29 $\pm$ 4.0, CM-E_2 1.7 $\pm$ 2.6. IF-T and SF-T $\leq$ 0.04. IF-E_2 14.49 $\pm$ 5.7, SF-E_2 4.5 $\pm$ 4.2. There were significant reductions in T ($p = 0.02$) and E_2 ($p = 0.001$) levels following surgery. Linear regression analysis of pre- and post-surgical sex hormone levels and NT-proBNP failed to show any correlations.

Preliminary results of this study do not support our initial hypothesis; however, this may be due to the small sample size, particularly from females. Alternatively, the increase in NT-proBNP may be related to a surgical or anesthetic effect. Sample collection is on-going and future sampling will include post-surgical echocardiograms, and renal bloodwork.

ABSTRACT #185

EVALUATION OF BRONCHIAL COLLAPSE IN DOGS WITH CHRONIC VALVULAR HEART DISEASE.

G Kramer[1], B McKiernan[2], R Burk[3]. [1]Atlantic Coast Veterinary Specialists, Bohemia, NY. [2]Southern Oregon Veterinary Specialty Center, Medford, OR. [3]Animal Medical Center at Cooper City, Cooper City, FL.

The purpose of this study was to prospectively evaluate the degree of bronchial collapse in dogs with moderate to severe chronic valvular disease and to serve as a preliminary feasibility study for the development of bronchial stenting as a therapeutic option in this patient population.

Dogs selected for inclusion in the study had evidence of moderate to severe (3 to 4+) mitral regurgitation (MR) via echocardiography and history of coughing without radiographic evidence of pulmonary edema, pneumonia or tracheal collapse. The echocardiograms, radiographs and bronchoscopies were performed on all the dogs at Atlantic Coast Veterinary Specialists. The chest radiographs and the bronchoscopies were independently evaluated by two of the authors (Burk and McKiernan, respectively) without having specific knowledge of the clinical cases. Four dogs were evaluated in this study at the time of the abstract submission deadline.

The results are included in the table below. All dogs had collapse of the left principal bronchus (LPB), left cranial lobar bronchus (LB1) and left caudal lobar bronchus (LB2). Two dogs had partial collapse of a right-sided bronchus. Dynamic ventral compression of LPB, LB1 and LB2 was evident and appeared to be secondary to cardiac motion.

	MR	TR	PH^{+}	LAD^{++}	LAE^{+++}	MBC^{++++}	LPB*	LB1*	LB2*
Case 1	4+	2+	none	severe	severe	moderate	4+	3+	4+
Case 2	4+	0	NA	severe	severe	none	1+	2+	3+
Case 3	3+	1+	none	mild	mild	none	2+	3+	4+
Case 4	4+	4+	moderate	severe	severe	none	2+	1+	4+

$^{+}$left atrial dilation (echo), $^{+}$pulmonary hypertension, $^{++}$left atrial enlargement (radiographic), mainstem bronchial collapse (radiographic), *estimates of collapse (bronchoscopic).

In conclusion, there appears to be an association, in this small population of dogs studied, between bronchial collapse and chronic valvular heart disease and that cardiac motion dynamically worsens the degree of collapse. Bronchoscopy was more sensitive at detecting the presence of collapse compared to radiography. Bronchial stenting may be a possible therapeutic option in this population of dogs, but careful patient selection will be needed due to collapse of multiple airways seen in each of the dogs. The underlying pathophysiologic process that results in the bronchial collapse is unclear and requires more study.

ABSTRACT #186

NEUROHORMONAL MEASUREMENTS IN HEALTHY DOGS AND DOGS WITH ACQUIRED VALVULAR OR MYOCARDIAL DISEASE.

DD Sisson[1], BJ Bulmer[1], PF Solter[2], RR Panico[1], R Prošek[3], MA Oyama[4], DF Hogan[5]. [1]Oregon State University, Corvallis OR. [2]University of Illinois, Urbana IL. [3]Homstead FL. [4]University of Pennsylvania, Philadelphia PA. [5]Purdue University, West Lafayette IN.

Alterations in circulating concentrations of neurohormones have been well characterized in humans with heart disease and heart failure but have been only partially characterized in dogs. To further characterize neuroendocrine changes, blood was collected by jugular venipuncture from dogs with congestive heart failure due to dilated cardiomyopathy (CHF-DCM, N = 30) or degenerative valvular disease (CHF-DVD, N = 44); dogs with DCM but without CHF (E-DCM, N = 30); dogs with DVD but without CHF (E-DVD, N = 45); and from 81 healthy dogs (Controls).

Dogs were evaluated via physical exam, ECG, thoracic radiographs, and echocardiography. Plasma renin activity (PRA), and serum aldosterone (ALDO), NT-proANP (ANP), and BNP were measured by RIA. Epinephrine (EPI) and norepinephrine (NOREPI) were measured by HPLC. Log-transformed data was analyzed via one-way ANOVA and using Bonferroni's multiple comparison tests with significance at $P < 0.05$*. Results in the table are reported as geometric means, medians and with [95%CI].

	Controls	E-DVD	E-DCM	CHF-DVD	CHF-DCM
ANP nmol/ml	0.47 (0.47) [0.42–0.52]	0.95 (0.92)* [0.76–1.18]	0.68 (0.72) [0.53–0.87]	1.81 (1.75)* [1.47–2.22]	1.26 (1.40)* [1.00–1.58]
BNP pg/ml	6.1 (9.0) [4.6–8.0]	11.0 (13.3) [6.8–17.8]	16.9 (16.3)* [9.1–31.6]	15.4 (20.2)* [9.5–24.8]	24.6 (25.1)* [17.4–34.8]
PRA ng/ml/hr	0.65 (0.80) [0.50–0.83]	0.91 (0.95) [0.67–1.24]	1.13 (0.90) [0.81–1.59]	2.98 (3.41)* [1.72–5.15]	6.01 (7.90)* [3.80–9.50]
ALDO pg/ml	34 (39) [25.8–44.0]	49 (63) [30.3–78.2]	80 (68) [46.0–138.4]	66 (81)* [42.9–100.9]	263 (214)* [156–445]
EPI pg/ml	133 (133) [114–157]	290 (318)* [232–364]	129 (126) [93–178]	314 (302)* [245–402]	211 (204)* [166–267]
NOREPI pg/ml	254 (263) [220–291]	445 (416)* [378–522]	276 (300) [202–378]	574 (621)* [461–715]	631 (625)* [533–745]

NT-proANP, BNP, EPI, NOREPI, PRA and ALDO were increased in dogs with CHF due to DCM or DVD. NT-proANP, EPI and NOREPI were increased in dogs with DVD without CHF and BNP was increased in dogs with DCM without CHF.

ABSTRACT #187
GENE EXPRESSION OF ADRENOMEDULLIN IN CANINE NORMAL TISSUES AND DISEASED HEARTS. N Kanno, K Asano, K Teshima, M Seki, K Edamura, S Tanaka. Nihon University, Kanagawa, Japan.

Adrenomedullin (AM), originally isolated from human phenochromocytoma, is a potent endogenous vasodilating and natriuretic peptide. The AM produced by the proteolytic cleavage of its precursor (Prepro-AM) consists of 52 amino acids and a COOH-terminal amide structure, which is the common structure of the calcitonin gene-related peptide superfamily. The prepro-AM has a unique 20 amino acid residues in the NH_2 terminus, pro-adrenomedullin N-terminal 20 peptide (PAMP). The PAMP, a novel hypotensive peptide, inhibits catecholamine secretion from sympathetic nerve terminus. The purposes of this study were to determine the cDNA sequences of canine AM and PAMP, to investigate their tissue distribution, and to evaluate whether mRNA expression of canine AM increases in association with spontaneous cardiovascular diseases.

Total RNA was extracted from canine normal heart, and was used in the RT-PCR procedure to isolate prepro-AM cDNA. Sequence analysis of the isolated cDNA was conducted. The AM mRNA expressions in various tissues from 2 healthy Beagles were investigated by the RT-PCR. In addition, the mRNA levels of AM in the atria and ventricles obtained from 2 dogs with patent ductus arteriosus (PDA) and 6 dogs with chronic mitral valvular diseases (CMVD) were compared with those from normal dogs.

The canine prepro-AM cDNA sequence and deduced amino acids were 1,541 base pairs and 188 residues, respectively. The cDNA sequences of canine AM and PAMP showed high homologies with those of the other mammalian species. The expressions of AM mRNA were detectable in the various normal tissues. In the cardiac tissues from the dogs with PDA and CMVD, mRNA levels of AM were elevated similar to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP).

ABSTRACT #188
MUTATIONAL ANALYSIS OF FOUR SARCOMERIC GENES IN THREE BREEDS OF CATS WITH HYPERTROPHIC CARDIOMYOPATHY. KM Meurs[1], M Norgard[1], J Haggstrom[2], M Kittleson[3]. [1]Washington State University College of Veterinary Medicine, Pullman, WA. [2]The Swedish University of Agricultural Sciences, Uppsala, Sweden. [3]University of California, Davis School of Veterinary Medicine, Davis, CA.

Hypertrophic cardiomyopathy (HCM) is an inherited disease in the Maine Coon (MC) and Ragdoll (RD) breeds. It is thought to be inherited in the Norwegian Forest Cat (NWF) and British Shorthair (BS) as well. We have previously identified two unique causative mutations in different regions of the myosin binding protein C (MYBPC3) gene in the MC and RD breeds. However, not all affected MC and RD cats with HCM have their known mutation and neither of these mutations has been identified in affected NWF or BS. Genetic heterogeneity has been observed in human HCM and $>$ 400 mutations have been identified, most commonly in one of 8 sarcomeric genes.

We hypothesized that a mutation in one of a select set of sarcomeric genes (MYBPC3, ß-myosin heavy chain (MYH7), troponin I (TNI), essential myosin light chain (MYL3)) would be associated with the development of HCM in each of NWF and BS cats and MC cats with HCM but without the known MYBPC3 mutation (A31P). PCR based sequencing of all exonic and splice site regions for these 4 genes was performed with DNA samples from three affected cats from each breed. The resulting sequencing data were compared to the corresponding sequence for all genes in 2 control cats and the published normal feline sequence. Base pair changes were considered to be causative for HCM if they met the following criteria: were present in at least some of the affected cats but not in the controls, changed a conserved amino acid and changed the amino acid to one of a different polarity, acid/base status or structure.

No significant differences were observed within the exonic and splice site regions of these genes between the affected cats and the control cats or the published feline sequence. Several polymorphisms were observed within each gene but did not segregate with disease or change the amino acid.

Since causal sarcomeric mutations have been previously identified in two breeds it is anticipated that other mutations will be identified in other breeds. This study ruled out causative mutations in the 4 genes from representative cats from these three breeds. Additional studies with other sarcomeric genes and other breeds are warranted.

ABSTRACT #189
WEEKLY VARIABILITY OF PLASMA AND SERUM NT-PRO-BNP MEASUREMENTS IN NORMAL DOGS. HB Kellihan[1], MA Oyama[2], CA Reynolds[2], RL Stepien[1]. [1]University of Wisconsin School of Veterinary Medicine, Madison, WI. [2]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration has been used to identify cardiovascular causes of dyspnea in dogs. However, the day-to-day variability of circulating NT-proBNP in dogs is not known. The purpose of this study was to examine the weekly variability of NT-proBNP concentration in normal dogs.

Thirty-seven clinically normal dogs from 2 study sites were examined prospectively. All dogs had normal cardiac auscultation and no significant abnormalities on 2-D, M-mode and Doppler echocardiographic examinations. Serum samples from site A (n=19) and plasma samples from site B (n=18) for NT-proBNP assay (Canine CardioCare, Veterinary Diagnostics Institute, Irvine, CA) were obtained from dogs at one week intervals for a total of 3 samples.

The median age of the patient population was 5 years (range: 0.8-10 years), whereas the median weight was 22 kilograms (range: 5-50 kilograms). There was no significant difference in median NT-proBNP for all dogs between week 1 (378 pmol/L [25-75% interquartile range: 219-485 pmol/L], week 2 (366 pmol/L [231-496]), and week 3 (367 pmol/L [223-469]) (p=0.99). However, the coefficients of variation (CV=standard deviation/average) for individual dogs ranged from 0-51% (median CV=12%). Eighteen of 37 dogs (49%) had CV $<$12%, 15 of 37 dogs (41%) had CV of 12-30%, and 4 of 37 dogs (11%) had CV $>$30%. Six of 37 dogs (16%) possessed one NT-proBNP reading that exceeded the manufacturer's current upper normal value of 566 pmol/L. Two of 37 dogs (5%) possessed two readings $>$566 pmol/L, and in 2 of 37 dogs (5%) all three weekly results were $>$566 pmol/L. The median serum NT-proBNP concentration of dogs from study site A was significantly higher than plasma concentrations of dogs from study site B, (Site A, 420 pmol/L [25-75% interquartile range: 296-542 pmol/L], vs. Site B, 257 pmol/L, [162-432]; $p \leq 0.001$), suggesting either a difference between serum and plasma concentrations vs. a regional difference in the study population.

Weekly variability of NT-proBNP measurement is generally low; however, in a small percentage of dogs, variability has the potential to confound a single result and serial measurements are recommended.

ABSTRACT #190
NT-PRO BNP IN DOGS WITH ATRIAL FIBRILLATION. EA Shipley, AB Saunders, SG Gordon, RM Roland, LT Drourr, SE Achen, MW Miller. Department of Small Animal Clinical Sciences and Michael E. DeBakey Institute, Texas A&M University, College Station, TX.

Atrial fibrillation (AF) complicates heart failure secondary to acquired heart disease in dogs. NT-proBNP release occurs in response to increased filling pressures and wall stress. Humans with AF have higher NT-proBNP concentrations, even when clinically stable, compared to those in sinus rhythm (SR). We hypothesized dogs with AF would have elevated NT-proBNP compared to dogs in SR. If NT-proBNP concentrations are higher in AF, then this may affect cutoff values or case management. The purpose of this study was to compare NT-proBNP in dogs with AF secondary to severe chronic degenerative valve disease (CVD) and dilated cardiomyopathy (DCM) to a similar group of dogs in SR. NT-proBNP was measured using a commercial laboratory (Veterinary Diagnostics Institute, Irvine, CA). Thoracic radiographs and echocardiography confirmed severe CVD and DCM. 12 dogs with AF (7 CVD, 5 DCM) were evaluated 17 times; 10 dogs in SR (7 CVD, 3 DCM)

were evaluated 15 times. There was no significant difference in age, ISACHC score or furosemide dose between CVD AF and SR groups or DCM AF and SR groups. Heart rate had no effect on NT-proBNP. When all NT-proBNP values were analyzed, AF was 2405.94 ± 737.82 (mean ± SD) compared to SR (1900.40 ± 880.83; P = 0.08). Dogs were classified as stable or unstable based on presence or absence of clinical signs, blood work and thoracic radiograph analysis, and medication adjustments; there were too few numbers to determine clinical significance. Dogs with multiple samples only had their first evaluation included in further analysis.

	N	Age (yrs) mean ± SD	Sex	ISACHC (II, IIIA, IIIB)	Furosemide (mg/kg/day) mean ± SD	NT-proBNP (pmol/L) mean ± SD	NT-proBNP (pmol/L) range
CVD AF	7	12.1 ± 1.21	4M3F	5, 1, 1	3.71 ± 2.67	2102.00 ± 934.86	429–3000
CVD SR	7	10.2 ± 1.60	3M4F	4, 2, 1	3.84 ± 1.63	2067.85 ± 932.27	181–3000
DCM AF	5	5.4 ± 3.51	4M1F	1, 4, 0	4.28 ± 3.81	2816.80 ± 409.65	2084–3000
DCM SR	3	6.7 ± 3.06	3M	0, 2, 1	6.48 ± 2.30	2462.67 ± 930.69	1388–3000

Median NT-proBNP concentration in dogs with AF was comparable to dogs in SR. Although not statistically significant, there was a trend towards higher NT-proBNP concentrations in dogs with AF versus SR and with DCM when compared to CVD. Further studies are warranted to evaluate NT-proBNP concentrations in dogs with AF. Findings may facilitate therapeutic decision making.

ABSTRACT #191

ASSESSMENT OF NTproBNP CONCENTRATION IN ASYMPTOMATIC CATS WITH CARDIOMYOPATHY. PR Fox[1], MA Oyama[2], K MacDonald[3], CA Reynolds[2]. [1]Animal Medical Center, New York, NY. [2]University of Pennsylvania, Philadelphia, PA. [3]Animal Care Center, Rohnert Park, CA.

Myocytes express and release natriuretic petptides in response to pressure or volume overload. Diagnosis of cardiomyopathy in asymptomatic cats is challenging and generally requires echocardiographic examination. We aimed to determine whether NTproBNP blood concentrations were elevated in cats with asymptomatic myocardial disease (HCM, DCM, UCM).

Asymptomatic cats with a heart murmur or gallop rhythm were each evaluated by a board certified cardiologist using medical history, physical examination, thoracic radiography, and echocardiography. Healthy cats similarly evaluated and free of cardiovascular disease comprised a control group. A central laboratory blinded to diagnosis performed serum NTproBNP assays. Control vs cardiomyopathy NTproBNP concentrations were compared by Mann Whitney test. Spearman correlation assessed NTproBNP concentrations vs echocardiographic measurements in cardiomyopathy cats. ROC analysis was used to evaluate NTproBNP outcomes.

Median NTproBNP differed between control (n = 14) vs cardiomyopathy (n = 23) groups (P < 0.0001). Median NTproBNP [interquartile range] was 24 pmol/L [24–45.5 pmol/L] for control cats and 283 pmol/L [154–603 pmol/L] for cardiomyopathy cats. Correlations (BNP vs echo) were LVWd (P = 0.18), LVd (P = 0.497), LVs (P = 0.424), %FS (P = 0.978), IVSd (P = 0.434), LA/Ao (P = 0.199) and vertebral heart score (P = 0.9).

NTproBNP > 70 pmol/L possessed 87.5% sensitivity, 100% specificity, 100% positive predictive value, and 87.5% negative predictive value for asymptomatic cardiomyopathy vs controls (fitted ROC area under curve, 0.982 [SE = 0.0193]). Therefore, NTproBNP determination may aid clinical evaluation of asymptomatic cats with myocardial disease.

ABSTRACT #192

NT-PRO-BNP CONCENTRATION IN PRECLINICAL (ISACHC 1A & 1B) CHRONIC DEGENERATVIE ATRIOVENTRICULAR VALVE DISEASE. LT Drourr, SG Gordon, RM Roland, AB Saunders, SE Achen and MW Miller. The Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Congestive heart failure (CHF, ISACHC Class 2 & 3) can be difficult to diagnose in dogs with longstanding preclinical (ISACHC Class 1A or 1B) chronic degenerative atrioventricular valve disease (CVD). NT-proBNP serum concentrations (pmol/L) are elevated in CHF due to CVD and may help confirm that diagnosis if accurate discriminatory cutoffs are established. Many small veterinary reports have described the utility of NT-proBNP for discriminating between dyspnea due to a primary respiratory disease versus CHF. It is more difficult, yet clinically relevant, to determine cutoffs that accurately differentiate dogs with CHF due to CVD from those with preclinical CVD and concurrent respiratory disease. Tarnow et. al. 2007 reported that mean NT-proBNP in Cavalier King Charles Spaniels (CKCS) with ISACHC 1A & 1B 257 ± 92 (mean ± 1SD) was similar to healthy control dogs (259 ± 123) and significantly less than CKCS with CHF due to CVD (1474 ± 646). They concluded that NT-proBNP is not significantly elevated in CKCS with preclinical CVD. These findings coupled with other reported cutoffs in the range of 300–500 pmol/L could lead to the over diagnosis of CHF in dogs with preclinical CVD.

This study reports the NT-proBNP median and range in dogs with various degrees of cardiac remodeling due to preclinical CVD and the correlation between NT-proBNP and various echocardiographic and radiographic indices of cardiac remodeling and Doppler derived E:Ea as an index of cardiac filling pressures. Eighteen dogs with preclinical CVD were evaluated a total of 24 times; 15 of the 18 dogs were CKCS, mean age was 8.2 ± 2.8 years, and 60% were male. NT-proBNP concentrations were not normally distributed. The median NT-proBNP was 508 with an interquartile range of 323–793, a minimum of 200, and maximum of 2255. Dogs with an increased LVIDd and/or LVIDs (7/24) had significantly elevated NT-proBNP when compared to those whose LVIDd and LVIDs were normal, with a median NTproBNP of 1247 (interquartile range 503–1861) and 371 (interquartile range 279–626) respectively. There was a significant correlation between NT-proBNP and 2D derived La:Ao ratio. There was no significant correlation between NT-pro BNP and VHS, M-mode derived La:Ao ratio, LVIDd and LVIDs (indexed to body surface area), or Doppler derived E:Ea ratio.

NT-proBNP is elevated to various degrees in preclincal CVD and is not correlated to many common indices of cardiac remodeling. Its true utility may lie in its correlation to important clinical endpoints such as onset of congestive heart failure. Larger prospective studies are warranted to further evaluate the clinical utility of this novel test.

ABSTRACT #193

ECHOCARDIOGRAPHIC CHARACTERIZATION OF LEFT VENTRICULAR RADIAL AND CIRCUMFERENTIAL WALL MOTION IN HORSES USING STRAIN, STRAIN RATE, AND DISPLACEMENT BY 2D SPECKLE TRACKING: METHODOLOGY AND RELIABILITY. CC Schwarzwald[1], KE Schober[2], JD Bonagura[2]. [1]Vetsuisse Faculty, University of Zurich, Switzerland. [2]College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Echocardiographic assessment of left ventricular (LV) function in horses is currently limited to subjective evaluation and calculation of two-dimensional ejection phase indices. The goal of this study was to show the feasibility, describe the techniques, and determine the reliability of 2D speckle tracking (2DST) for characterization of LV radial and circumferential wall motion that could be useful for assessment of regional and global LV systolic and diastolic function in horses.

Six healthy, adult horses were included. Repeated echocardiographic examinations were performed by two independent observers in unsedated horses, using a GE Vivid 7 echocardiograph. A right-parasternal short-axis view of the LV at the level of the chordae tendineae was imaged in 2D mode at a frame rate between 59 and 73 FPS. 2DST analyses were performed blinded and in random order using the GE EchoPac Software (v6.1.2). Measurements included circumferential and radial peak-systolic strain (SC, SR); circumferential and radial strain rate during systole (SrC-S; SrR-S), early diastole (SrC-E; SrR-E), and late diastole (SrC-A; SrR-A); and maximum systolic radial displacement (DR). All mea-

surements were reported by the software as average over each of six LV wall segments. Global strain, global strain rate, and global displacement were then calculated as averages over all segments. Reliability of 2DST was assessed by calculating measurement variability, within-day interobserver variability, between-day intraobserver variability, and between-day interobserver variability of all variables. Variability was reported as coefficient of variation (CV) in per cent.

2DST analyses were possible for all recordings in which at least one complete cardiac cycle was available. The automated tracking seemed accurate during systole but inaccurate during early diastole based on visual assessment by the operator. Generally, reliability was higher for SR and SrR compared to SC and SrC. For segmental SR, SrR-S, and DR, measurement error, within-day interobserver variability, between-day intraobserver variability, and between-day interobserver variabilitiy, respectively, were 2.4–12.4% [range of CV], 7.6–33.1%, 11.1–22.4%, and 8.8–26.5%. For global SR, SrR-S, and DR they were 4.1–6.3%, 12.6–15.3%, 12.0–16.1%, and 12.0–15.7%.

We conclude that global SR, SrR-S, and DR can be reliably characterized in horses by use of 2DST. Circumferential strain and strain rate measurements are less reliable. Diastolic strain rate measurements may not be valid due to inaccurate tracking, possibly related to undersampling. The use of longitudinal strain and strain rate by 2DST, determination of the clinical value of 2DST for assessment of LV systolic function and LV synchrony at rest and during stress-testing, and its applicability to horses with cardiac disease will require further investigation.

ABSTRACT #194
COMPARISON OF LV TORSION, 2-D STRAIN AND STRAIN RATE IN SMALL, MEDIUM AND LARGE BREED DOGS. Y Fujii, H Takano, Y Wakao. Azabu University School of Veterinary Medicine, Kanagawa, Japan.

Strain (St), strain rate (SR) and LV torsion have been recognized as newly-developed non-invasive parameters to assess cardiac contractility. Large breed dogs tend to have lower FS compared with small breed dogs. We hypothesized that LV radial motion was less important and that twisting motion may play a more important role in contractility in large breed dogs. If so, quantification of LV twist could be more accurate method to evaluate contractility. The purpose of this study is to assess and compare LV torsion, St and SR in dogs with different body weight (BW) and breeds. Sixty-one clinically healthy dogs of 7 different breeds were used. Dogs were divided into 3 groups; small, medium and large breed. Speckle tracking imaging (Vivid 7 dimension, GE) was used to obtain LV circumferential and radial St and SR, along with systolic rotation, rotation rate and torsion in addition to standard M-mode measurements.

All parameters of 2-D St and SR except for peak systolic radial St negatively correlated with BW and body surface area (BSA). Parameters for torsion and rotation also negatively correlated with BW and BSA, which revealed that rotating motion was not predominant in systolic function in large breed dogs compared with smaller breed dogs. Stroke index derived from stroke volume divided by BSA did not demonstrate any correlation with BW or BSA. Therefore, a relatively lower LV motion in large breed dogs effectively ejected sufficient cardiac output. Peak systolic radial St may be a good parameter for contractility without influence of BW.

ABSTRACT #195
PLASMA AND URINARY LEVELS OF 6-KETO-PROSTAGLANDIN$F_{1\alpha}$ IN DOGS WITH MYXOMATOUS MITRAL VALVE DISEASE. CE Rasmussen,[1] AV Sundqvist,[1] CT Kjempff,[1] I Tarnow,[1] M Kjelgaard-Hansen,[2] TS Kamstrup,[1] A Sterup,[1] TM Soerensen[1] and LH Olsen[1]. [1]Department of Basic Animal and Veterinary Sciences and [2]Department of Small Animal Clinical Sciences, University of Copenhagen, The Faculty of Life Sciences, Frederiksberg, Denmark.

Endothelial dysfunction might be involved in the pathogenesis of myxomatous mitral valve disease (MMVD) in dogs. A decreased plasma concentration of the nitric oxide metabolites nitrate and nitrite have been found in Cavalier King Charles Spaniels (CKCS) with mitral regurgitation, suggesting an endothelial dysfunction in dogs with MMVD. It is speculated that the vasodilator prostacyclin also plays a role in the pathogenesis of MMVD.

The aims of this study were to validate an enzyme immunoassay for a metabolite of canine prostacyclin (6-keto-prostaglandin(PG)$F_{1\alpha}$) and to compare plasma and urinary 6-keto-$PGF_{1\alpha}$ in dogs with different degrees of MMVD.

The study included 76 privately owned dogs: 34 CKCS, 32 Dachshunds and 10 control dogs of different breeds not predisposed to MMVD. All dogs went through clinical and echocardiographic examination. The degree of MMVD was estimated by echocardiographic evaluation of the degree of mitral valve prolapse, mitral regurgitation and heart dimensions. Spontaneous urine samples were collected and blood was drawn from the jugular vein. 6-keto-$PGF_{1\alpha}$ was measured in plasma and urine by enzyme immunoassay (Amersham 6-Keto-Prostaglandin $F_{1\alpha}$ Enzyme-immunoassay Biotrack (EIA) System, GE Healthcare). Two-way analyzes of variance (ANOVA) was used to test the influence of different MMVD parameters, body weight, age and gender on the urinary and plasma concentration of 6-keto-$PGF_{1\alpha}$ in CKCS and Dachshunds. Wilcoxon signed ranks-tests were used to assess if the concentrations of 6-keto-$PGF_{1\alpha}$ in the control dogs were different from concentrations in CKCS and Dachshunds.

The intra- and inter-assay coefficient of variation of the enzyme immunoassay was 5% and 8% at high concentrations of 6-keto-$PGF_{1\alpha}$, and 20% and 24% at low concentrations of 6-keto-$PGF_{1\alpha}$, respectively. The range of plasma and creatinine standardized urinary 6-keto-$PGF_{1\alpha}$ concentration was 24.1–185.2 pg/ml and 77.2–732.2 pg/μmol urinary creatinine, respectively. The severity of MMVD, body weight, age and gender were not associated with plasma or urinary 6-keto-$PGF_{1\alpha}$; however, the control dogs had a significantly lower creatinine standardized urinary 6-keto-$PGF_{1\alpha}$ than the CKCS and Dachshunds.

In conclusion, the enzyme immunoassay seemed valid for measuring canine 6-keto-$PGF_{1\alpha}$ in plasma and urine. Plasma or urinary 6-keto-$PGF_{1\alpha}$ does not appear to be influenced by the degree of MMVD in CKCS or Dachshunds. However, the cause of lower creatinine standardized urinary 6-keto-$PGF_{1\alpha}$ in control dogs compared to CKCS and Dachshunds remains to be established.

ABSTRACT #196
Abstract withdrawn.

ABSTRACT #197
MEASUREMENT OF REGIONAL MYOCARDIAL FUNCTION IN HEALTHY SMALL BREED DOGS USING TWO-DIMENSIONAL STRAIN IMAGING. NM Ponzio, KE Schober, JD Bonagura. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Conventional echocardiographic indices of left ventricular (LV) systolic function are load-dependent and can be unreliable in mitral regurgitation. Strain and strain rate (SR) are echocardiographic indices of regional myocardial systolic function considered relatively load independent. This study tested the hypothesis that 2D strain could be used to quantify LV systolic function in healthy dogs. Older ($\geq$6 years) small-breed dogs (n = 24), weighing 16 kg or less, were examined by 2D strain analysis (GE Vivid 7) and conventional echocardiography. Regional longitudinal and radial myocardial strain were quantified in multiple longitudinal (n = 6) and radial (n = 6) myocardial segments. Global LV longitudinal strain also was evaluated. Intraobserver and interobserver reproducibility were calculated.

Overall, 98.3% (283/288) of myocardial segments could be analyzed. Mean global longitudinal strain was −19.09%. Longitudinal mean strain and SR ranged across the myocardial segments ranged from −9.40% to −28.87% and −1.05 to −3.65 1/s while radial mean strain and SR ranged from 41.50 to 46.79% and 2.82 to 3.01 1/s, respectively. A gradient was identified for longitudinal strain and SR, with values decreasing from apex to base. Radial strain values for the anterior septal and septal segments were significantly lower than for other radial segments. Variability within observers was low (coefficient of variation $<$10%) but was higher between observers (difference/mean for 9/13 segments $<$25%).

2D strain analysis is feasible in healthy, awake dogs. Additional studies are needed in dogs with spontaneous mitral valve disease. These investigations should consider reproducibility of this technique and sensitivity of strain for detection of LV systolic dysfunction.

ABSTRACT #198
ASSESSMENT OF CARDIAC FUNCTION IN DOGS WITH DOXORUBICIN-INDUCED CARDIOMYOPATHY AFTER A SINGLE INTRACORONARY STEM CELL TRANSPLANTATION. MG Sousa[1], D Paulino-Júnior[2], JPE Pascon[2], GB Pereira-Neto[2], T Champion[2], R Carareto[1], AA Camacho[2]. [1]College of Veterinary Medicine, The Federal University of Tocantins State, Araguaina, Brazil. [2]College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil.

Stem cell transplantation has been shown to improve cardiac function in several experimental models and even in clinical practice in human beings. These benefits are likely to be the result of several factors, including myocardial neovascularization. In dogs, however, data is still lacking about the benefits of stem cell therapy to improve cardiac contractility or relaxation. Therefore, this study was aimed at evaluating how cardiac function performs after a single intracoronary injection of autologous bone marrow-derived stem cells in a group of dogs with doxorubicin-induced cardiomyopathy with moderate systolic and diastolic dysfunction.

Seven mature dogs (1 male; 6 females), with mean weight of 17.4 kg, were enrolled in the study. Because of chronic doxorubicin therapy, these animal were shown to have moderate systolic and diastolic compromise at baseline, with mean fractional shortening of 19%, mean left-ventricular systolic diameter of 2.92 cm, and mean isovolumic relaxation time of 54.5 msec. Dogs were randomly divided into two groups: G1 with 4 dogs that were given stem cells, and G2 with 3 dogs that were evaluated as controls. Stem cells were obtained from the bone marrow. Briefly, the marrow was aspirated, centrifuged in ficoll, washed several times and diluted to permit intraconoray delivery, which was accomplished with a catheter inserted through the femoral artery under fluoroscopy guidance and general anesthesia. Mean stem cell recovery was 22.6%, whereas cellular viability reached 96.2%. At least 60 million cells were delivered into the left coronary artery of each dog. After the procedure, every animal was evaluated for 6 months at monthly intervals. We recorded the left ventricular internal diameter at systole (LVIDs), fractional shortening (FS), ejection fraction (EF), pre-ejection period-to-left ventricular ejection time ratio (PEP/LVET), isovolumic relaxation time (IVRT), and the Tei index of myocardial performance (TEI). Analysis of variance was used to check for significant changes along time. Also, an unpaired T test was used to compare groups at every single moment.

Results are listed in Table 1. One of the dogs that were given stem cells presented unexplained sudden death 12 days after the procedure. In G1, a significant decrease was seen in LVIDs ($P = 0.0005$), PEP/LVET ($P = 0.0044$), IVRT ($P = 0.0180$), and TEI ($P = 0.0012$), as well as a significant increase in FS ($P = 0.0004$), and EF ($P = 0.0005$). On the contrary, G2 not only did not show improvement in the echocardiographic parameters as some of them also worsened during the experimental time.

Results allowed concluding that stem cell transplantation is a feasible technique with promising results in the treatment of canine doxorubicin-induced cardiomyopathy. Further studies are needed to determine what are the benefits of stem cell therapy in a large population of dogs, and well as in animals with idiopathic dilated cardiomyopathy.

ABSTRACT #199
THE PHARMACODYNAMICS OF ENOXAPARIN IN NORMAL CATS. Van De Wiele CM, Hogan DF, Green HW III, Dreher K. Purdue University, School of Veterinary Medicine, West Lafayette, IN.

Systemic arterial thromboembolic disease is common in the cat most often associated with underlying cardiac disease. Many antithrombotic drugs have been considered in the prevention of thromboembolic disease in cats with the low-molecular weight heparins (LMWH) gaining particular interest over the past decade. The LMWH are fragments of standard heparin which allow continued inhibition of factor Xa with greatly reduced inhibition of factor IIa. For this reason, standard coagulation assays such as aPTT are not altered during LMWH therapy. Pharmacokinetic monitoring of LMWH therapy utilizing anti-Xa assays has been evaluated in veterinary studies with the conclusion that very frequent dosing is required to maintain a therapeutic drug effect. However, it has been established in humans and other animal species that there is no correlation between anti-Xa levels and antithrombotic effect. Additionally, there have been no clinical trials in cats to evaluate a therapeutic drug effect. The purpose of this study was to determine if enoxaparin (Lovenox®) demonstrated an antithrombotic effect in the cat when dosed at the current standard dosing protocol of 1 mg/kg SQ q 12 hrs for 5 consecutive days.

Ten normal, purpose bred cats were used for this venous stasis model and divided into 3 groups: Group A; ($n = 4$) control (untreated), group B; ($n = 3$) 4 hr post final dose (peak), and group C; ($n = 3$) 12 hr post final dose (trough). The model was created by injecting 5 μCi of ^{125}I-fibrinogen (^{125}I-fib) IV followed by 500 μg/kg tissue thromboplastin IV and subsequent isolation of a segment of the abdominal vena cava. The isolated venous segment was maintained for 20 minutes then removed and the amount of thrombus formed was determined. The extent of thrombus formation was objectively measured by the wet weight of the thrombus normalized to segment length and the percent of ^{125}I-fib accreted within the thrombus compared to the ^{125}I-fib within the entire segment. The degree of thrombus inhibition and anti-Xa levels were determined for groups B and C.

The median normalized thrombus wet weight and %^{125}I-fib were 0.565 mg/mm and 42.0%, 0.000 mg/mm and 0.0%, and 0.390 mg/mm and 12.2% for group A, group B and group C, respectively. The median percent thrombus inhibition values for groups B and C were 100.0% and 72.5%, respectively. Anti-Xa levels were not available at the time of writing so final statistical analyses are pending.

The population numbers are very small in this study and this will most likely negatively impact statistical analysis. However, we conclude that this data suggests that enoxaparin does result in a measurable antithrombotic effect in the normal cat at a dosing interval commonly employed, and at a dosing interval less frequent than suggested from previous studies within the literature.

ABSTRACT #200
HEMODYNAMIC EFFECTS OF THE COMBINATION OF CARVEDILOL AND PIMOBENDAN IN DOGS. M Uechi, N Isayama, T Mizuno, T Ebisawa, S Yamano, M Mizuno, T Mizukoshi. Nihon University, Kanagawa, Japan.

The objective was to evaluate the influence on cardiac function of a combination of carvedilol, which is a β-blocker and pimobendan, which is a Ca sensitizer. Six Beagle dogs (8.7–12.8 kg) were used. The dogs were divided into 4 groups that were placebo, the carvedilol group, pimobendan group, and the combination group. Placebo and carvedilol (1 mg/kg) and/or pimobendan (0.4 mg/kg) were orally administered. Heart rate, arterial blood pressure, left ventricular fractional shortening, and left ventricle internal diameter were measured before and after 3-hour administration. Heart rate was not different in placebo (94 ± 21 bpm), the carvedilol group (88 ± 11 bpm), the pimobendan group (99 ± 22 bpm), and the combination group (100 ± 19 bpm). The pimobendan group (59 ± 3%) and combination group (55 ± 3%) revealed significant elevation of fractional shortening compared with the control (41 ± 5%) and the carvedilol group (40 ± 11%). In the combination group (125 ± 15 mmHg), ($P < 0.01$) blood pressure fell significantly compared with the placebo (141 ± 14 mmHg), carvedilol group (131 ± 18 mmHg), and pimobendan group (139 ± 22 mmHg). It is suggested that pimobendan indicated a positive inotropic effect in the presence of carvedilol without affecting the heart rate, and the vasodilator action of both medicines was enhanced by the combination of carvedilol and pimobendan.

ABSTRACT #201
EVALUATION OF PIMOBENDAN FOR THERAPY OF CANINE PULMONARY HYPERTENSION. KJ Atkinson, DM Fine, LA Thombs, HE Durham, LG Schultz. University of Missouri, Columbia, MO.

Pulmonary hypertension (PHT) is characterized by elevated pulmonary arterial pressure that results in clinical signs such as syncope, right-sided heart failure, and death. Pimobendan, a phosphodiesterase III inhibitor and calcium channel sensitizing agent causes positive inotropy and vasodilation, which may be advantageous in PHT. In humans, amino terminal-prohormone brain natriuretic peptide (NT-proBNP) levels reflect PHT disease severity.

The hypothesis of this study was pimobendan therapy would decrease the severity of PHT and improve patient's quality of life (QOL). The study was conducted as a prospective short-term, double-blinded, randomized, crossover design, with a long-term, open-label component. Ten client-owned dogs with a peak tricuspid regurgitant pressure gradient (TRPG) 50 mmHg on echocardiogram and clinical signs such as cough, syncope, and ascites were enrolled. The etiology of the pulmonary hypertension was myxomatous atrioventricular valve disease in 8 of the dogs, and chronic pulmonary disease in 2. The dogs were examined on days 0, 14, 21, 35, and 91, with completion of a QOL questionnaire, NT-proBNP levels, thoracic radiographs, and echocardiography. In the 35-day short-term phase, dogs were allocated at random to receive either a placebo or pimobendan (0.18–0.3 mg/kg, PO q12 h) for 14 days. Following a 1 week washout period, the dogs received the alternative treatment for 14 days. Following the 35 day short-term period all dogs received pimobendan for an 8 week period. Data were analyzed using a two period crossover design. Differences for each parameter were assessed by the appropriate F test and the significance level was set at $p < 0.05$.

Short-term comparison of pimobendan vs. placebo: Pimobendan resulted in a significant decrease in the peak TRPG ($p = 0.0061$). 10/10 dogs had a decreased TRPG on pimobendan therapy in comparison to when they were on placebo, with a mean decrease of 16.37 ± 12.89 mmHg. All dogs demonstrated a significant decrease in NT-proBNP levels ($p = 0.0023$) while receiving pimobendan, with a mean difference of 708.8 ± 508.6 pmol/L. A significant improvement in the summed QOL score was noted with pimobendan therapy in 9/10 dogs ($p = 0.016$).

Long-term comparison of day 0 vs. 91: The TRPG was significantly decreased at completion of the study ($p = 0.03$). 9/10 dogs had a decreased TRPG, with a mean decrease of 14.0 ± 15.6 mmHg. No significant changes in NT-proBNP levels ($p = 0.34$) or the summed QOL scores ($p = 0.41$) were seen. These data suggest that pimobendan therapy is well tolerated in this small study population. Pimobendan therapy led to a short-term amelioration in clinical signs, a reduction in NT-proBNP levels, and a decreased TRPG. Failure to show an improvement in the clinical signs and NT-proBNP levels long-term may be due to the progressive nature of the underlying PHT, or the small sample size.

ABSTRACT #202
TRANSARTERIAL DUCTAL OCCLUSION USING THE AMPLATZ® CANINE DUCT OCCLUDER IN 23 CASES. SE Achen, SG Gordon, AB Saunders, RM Roland and LT Drourr, MW Miller. The Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas.

Transarterial ductal occlusion using the Amplatz® canine duct occluder (ACDO) was first reported by Nguyenba et al in 2007. Herein we report the results of Amplatz canine duct occluder deployment in 23 dogs with PDA. The study population consisted of 17 females and 6 males, ranging in age from 2.5 to 102 months (median 5.6 months) and weighing between 2.3 and 37 kilograms (median 5.7 kg). Sixteen dogs had isolated, uncomplicated left-to-right shunting PDA. Concurrent congenital cardiac defects included mild pulmonic stenosis and subaortic stenosis in two dogs, mild pulmonic stenosis in two dogs, moderate subaortic stenosis in one dog mild subaortic stenosis in one dog and suspected mitral valve dysplasia in one dog.

PDA morphology was classified by angiography, followed by ductal occlusion with a single Amplatz canine duct occluder. All dogs had a type II PDA, with 22 dogs having type IIA morphology and 1 dog having type IIB morphology. Angiographic ductal length was 17 ± 6.9 mm (mean $\pm$ 1 SD). The mean diameter of the ductal ampulla was 8.5 ± 3.7. Mean minimal ductal diameter was 3.1 ± 1.6 mm (range 1.2–5.9 mm). Mean ACDO diameter to angiographic minimal ductal diameter ratio was 1.8 ± 0.6. Median fluoroscopy time was 6.9 minutes (range 2.6–25.7 minutes). Appropriate device deployment was achieved in 22 of 23 dogs. Device deployment was not achieved in one dog due to the need for a larger device and inability to place an appropriately sized catheter. That dog's PDA was subsequently closed by surgical ligation. Angiography was performed and recorded 5–10 minutes following device deployment in 21 dogs documenting complete occlusion in 16 dogs and trivial flow in 5 dogs (not recorded in 1 dog). Based on transthoracic color Doppler echocardiography performed the day following the procedure, complete occlusion was achieved in all dogs. The only complication was femoral artery tearing in 2 dogs, with no significant bleeding and successful ligation at the completion of the procedure. We conclude that ductal occlusion using an Amplatz canine duct occluder is a safe and efficacious therapy for PDA in dogs.

ABSTRACT #203
PERCUTANEOUS ATRIAL SEPTAL DEFECT OCCLUSION USING THE AMPLATZER® SEPTAL OCCLUDER IN 9 DOGS: SHORT AND MID-TERM OUTCOME. SG Gordon, MW Miller, RM Roland, AB Saunders, LT Drourr and SE Achen. The Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Percutaneous atrial septal defect (ASD) occlusion in the dog was first reported by Sanders et al. in 2005. Herein we report the results of Amplatzer® septal occluder deployment in 10 dogs with ASD seen between 01/11/05 and 01/10/07. Breeds included Standard Poodle (7/10), Boxer (2/10) and Dalmatian. All dogs were female with a mean age of 3.9 ± 2.4 (mean $\pm$ SD), weighing 21 ± 3.5 kg. All dogs had isolated left to right shunting septum secundum ASD. Six dogs had left base systolic heart murmurs ranging in grade from I-IV/VI. All dogs had echocardiographic evidence of moderate (4/10) to severe (6/10) right atrial and ventricular enlargement. Four of 6 dogs had evidence of pulmonary hypertension (PH) with Doppler estimated systolic pulmonary artery pressures of 60 ± 28 mmHg. Eight dogs had no clinical signs, 1 dog had exercise intolerance and 1 dog had congestive heart failure.

All devices were deployed via a right jugular approach. The femoral vein approach was attempted in the initial dog but was unsuccessful. This dog was subsequently occluded from the right jugular. Transthoracic echocardiographic estimates of ASD size were 12.7 ± 5.4 mm with a range of 7–22 mm. Device size selection was based on the stop flow balloon inflation estimate of ASD size. Devices were successfully deployed in 9 of 10 dogs. One dog had inadequate ASD rim tissue which precluded occlusion. This dog was subsequently recovered and offered open heart repair which the owners declined. Deployed Amplatzer® septal occluder size was 16.4 ± 3.4 mm with a range of 12 to 22 mm. Median fluoroscopy time was 17.5 minutes with an interquartile range of 9–33 minutes and absolute range of 7–112 minutes. Transthoracic color Doppler echocardiography performed the day following the procedure revealed complete occlusion in 4 of 9 dogs, trivial to mild residual flow in 4 of 9 dogs and moderate residual flow in one dog. Follow-up echocardiograms (6.0 ± 4.0 months after the procedure) were available for 7 of 9 dogs. Right atrial and ventricular enlargement resolved in 3 of 7 and reduced to mild in the remaining 4 of 7 dogs. PH resolved in all 4 dogs in which it was documented prior to device deployment. Severity of residual ASD flow was unchanged and judged to be hemodynamically insignificant. The only complication was inadvertent device deployment into the right ventricle in 1 dog requiring catheter based retrieval. Following device retrieval this dog was successfully occluded. All dogs were discharged on aspirin 1.3 ± 0.6 mg/kg PO q 24 hrs for 6 months. The total mean follow-up time in these 9 dogs is 14.2 ± 9.1 months. Clinical signs resolved in the two symptomatic dogs and no dog has had mid-term complications.

ABSTRACT #204
A RETROSPECTIVE STUDY OF PROGNOSIS OF MEDICAL OR SURGICAL TREATMENT IN DOGS WITH MITRAL REGURGITATION. T Ebisawa, M Uechi, T Mizuno, S Yamano, M Mizuno, T Mizukoshi. Nihon University, Kanagawa, Japan.

The objective was to compare the prognosis of medical treatment and mitral valve plasty (MVP) using cardiopulmonary bypass

(CPB) in dogs with mitral valve regurgitation (MR) with pulmonary edema. Fifty-three cases that underwent medical treatment and 16 cases that underwent MVP from April 2005 to June 2007 were entered in the study from 5 animal hospitals. The average age at the time of the initial pulmonary oedema onset was 140 ± 31 months. The average body weight was 5.7 ± 3.5 kg. Cardiac murmur was detected at grade 4.3 ± 0.6 in the mitral valve area. ISACHC classification was II (n = 9), IIIa (n = 30), and IIIb (n = 28). It was cured with diuretics, cardiotonic drugs, and vasodilators at the time of the initial pulmonary edema. Maintenance medical treatments administered were diuretics, cardiotonic drugs, vasodilators, and carvedilol. Forty-one of 53 patients died throughout the survey period, and the mortality was 77%. Mean length of life was 222 ± 214 days. The center survival period was 156 days (1–854 days). Six of 20 MVP cases died during the operation or postoperative hospitalization, and 14 dogs were discharged. The perioperative average age was 123 ± 10 months. The average weight was 5.5 ± 2.2 kg. Two dogs died of a reason other than cardiac disease after discharge. The center survival period was 450 days (233–955 days). With medical treatment alone, MR dogs died from pulmonary edema by an average of 7 month; however, MVP cases survived more than 12 months.

ABSTRACT #205
HOW CAN RIGHT VENTRICULAR FUNCTION BE OPTIMIZED DURING CARDIAC RESYNCHRONIZATION THERAPY? Y Nishijima, A Pedraza-Toscano, A Pozzi, RL Hamlin. The Ohio State University, Columbus, OH.

Sites and sequence for Cardiac Resynchronization Therapy (CRT) which yield optimal cardiac functions, and effects on right ventricular (RV) function, are unknown. This study determined the pacing site(s) and interventricular delay(s) that produce maximal RV function in normal dogs. The right atrium (RA) was paced at 120 bpm in 6 healthy dogs to measure the effects with a physiologic activation sequence. For single-site pacing (SSP), 1 of 2 RV sites (apex and base) or 1 of 5 left ventricular (LV) sites (anterior and posterior base, anterior and posterior mid free-wall, and apex) were paced. For multi-site pacing (MSP), LV pacing was initiated by stimulating either 1 of 5 pacing sites or all 5 pacing sites simultaneously. RV function was assessed by $dRVP/dt_{max}$, and the shortest QRS duration, and compared to values during RA pacing alone. Values of $dRVP/dt_{max}$ and QRS duration were compared (repeated measures ANOVA). With SSP, maximal $dRVP/dt_{max}$ occurred with pacing the RV base alone (22% greater than RA pacing, but not statistically significant), and least with pacing LV apex alone (−34%, compare to RV base alone). With MSP, RV function was optimal with the longest interventricular delay (32 ms), and when pacing the RV base with any of the LV sites. QRS durations for all pacing sites were significantly longer compared to RA pacing. Conclusions: Optimizing LV contraction hinders RV contraction. RV function is optimized with increasing interventricular asynchrony. CRT can be optimized to improve RV function.

ABSTRACT #206
OUTCOMES FOLLOWING CARDIOPULMONARY RESUSCITATION IN 68 ANIMALS (MARCH 2007–DECEMBER 2007). GJ Buckley, EA Rozanski, LM Freeman, JE Rush. Tufts Cummings School of Veterinary Medicine, North Grafton, MA.

Studies evaluating survival following cardiopulmonary arrest (CPA) in animals are limited. The purpose of this study was to evaluate the patient characteristics, return of spontaneous circulation (ROSC), extended survival and discharge from the hospital after cardiopulmonary arrest.

The log of cardiopulmonary resuscitation (CPR) efforts in the ICU and Emergency Room was searched for all canine and feline cases occurring between March-December, 2007. Data recorded from patients' medical records included signalment, whether or not the patient achieved ROSC, whether spontaneous circulation was maintained for more than 20 minutes (defined as extended survival) and whether the patient was discharged from the hospital. Animals could be enrolled more than one time if greater than 20 minutes of spontaneous circulation occurred between CPA episodes. Chi square analysis was used to compare the frequency of ROSC and extended survival between species, and independent t-tests were used to compare age between animals with and without ROSC or extended survival. $P < 0.05$ was considered significant.

Seventy-two separate CPR events were recorded, involving 68 patients including 54 dogs and 14 cats. Four dogs had two separate arrests included in the analysis. Age was known for 62 animals, with a mean = 9.0 ± 4.4 yrs. ROSC occurred in 47 CPR events (65%). There was no significant difference in frequency of ROSC between cats (71%) and dogs (64%; p = 0.59). Age was not significantly different between animals with ROSC (9.5 ± 4.2 yrs) and those without (mean = 8.0 ± 4.5 yrs; p = 0.22). Extended survival was achieved in 33 CPR events (46%). No significant differences in extended survival were detected between dogs (50%) and cats (23%; p = 0.08), nor was there a significant difference in age between animals with and without extended survival (p = 0.23). Of 47 animals achieving ROSC, 15 died from a subsequent cardiopulmonary arrest, while 27 were euthanized. Five animals (7%; 5 dogs and 0 cats) were discharged from the hospital. This study demonstrates that ROSC was common in dogs and cats undergoing CPR in an ICU setting. Extended survival was also common but animals survived to discharge at a lower rate. Future directions in CPR should focus on improved supportive care following ROSC as well as improvements in client communication regarding cardiopulmonary resuscitation outcomes.

ABSTRACT #207
TOO YOUNG TO DIE? PRESENTATION OF AND COMPLICATIONS IN ENGLISH SPRINGER SPANIELS UNDERGOING ARTIFCIAL PACING. Sonja Fonfara[1], Joao Loureiro[1], Simon Swift[1], Rachel James[2], Nuala Summerfield[3], Joanna Dukes-McEwan[1]. [1]Small Animal Teaching Hospital, University of Liverpool, Neston, Wirral, UK, [2]Nantwich Veterinary Hospital, Nantwich, Cheshire, UK, [3]North Downs Specialist Referrals Ltd., Caterham, Surrey, UK.

In the UK, English Springer Spaniels (ESS) are one of the most common breeds presented for artificial pacing.

We reviewed indication, age, troponin I levels and complications in ESS receiving pacemakers in comparison to other dogs presented for pacing between 2003 and 2007.

Forty-three dogs were paced in that time period, 8 (19%) were ESS. After Labradors, ESS were the second most common breed presenting with 3rd degree atrioventricular block (AVB) (n = 6). One ESS with atrial standstill had evidence of myocardial failure before pacing. One ESS had sinus arrest; 50% of the ESS (4/8) were < 3 years old at presentation, which was significantly younger than other dogs (mean 9 years, 2/35 < 5 years). Troponin I levels ranged between 0.27–180 ng/mL (normal < 0.15 ng/mL) in ESS, which was similar to other dogs. However, young ESS had highest troponin I levels. Major complications, such as lead dislodgement, exit block and infection occurred in all of the <3 year old ESS, in contrast to 11% of other dogs paced. However, ESS with major complications were still alive 1.5 and 2 years after procedure. Only one ESS developed heart failure following pacing, regardless of initial troponin I level.

ESS presented for pacing were significantly younger than other dogs. ESS with elevated troponin I at presentation suggesting significant myocardial damage developed major complications after procedure. However, these animals had a marked improvement of clinical signs and quality of life and are still alive 1 and 2 years after procedure.

ABSTRACT #208
RADIOGRAPHIC, ECHOCARDIOGRAPHIC, AND NEUROHORMONAL EFFECTS OF VVI PACING IN DOGS WITH THIRD DEGREE ATRIOVENTRICULAR BLOCK. BJ Bulmer[1], DD Sisson[1], MA Oyama[2], PF Solter[3]. [1]Oregon State University, Corvallis, OR. [2]University of Pennsylvania, Philadelphia, PA. [3]University of Illinois, Urbana, IL.

Artificial pacemaker (AP) implantation is the mainstay of therapy for treatment of third degree atrioventricular block (3 AVB). Despite several reports detailing long-term survival for dogs with APs there is little data detailing the radiographic, echocardiographic or neurohormonal consequences in this patient population.

Complete baseline (3 AVB) and 4 weeks post fixed rate (100) ventricular demand (VVI) pacing were available for comparison from 9

dogs previously enrolled in a study of physiologic pacing. Compared to 3 AVB, VVI pacing reduced radiographic heart size (12.25 ± 2.0 vs 10.92 ± 1.1, p = 0.01) and pulmonary parenchymal score (median 1, range 0–3 vs median 0, range 0–1, $p < 0.05$). Echocardiographically determined left atrial size (3.21 ± 0.5 vs 2.77 ± 0.3 cm, $p < 0.01$), left atrial to aorta ratio (1.50 ± 0.4 vs 1.28 ± 0.3, p = 0.02), left ventricular (LV) diastolic dimension (4.41 ± 0.6 vs 3.81 ± 0.5 cm, $p < 0.001$), fractional shortening (43.6 ± 8 vs 22.2 ± 7%, $p < 0.001$), and LV posterior wall systolic dimension (1.56 ± 0.2 vs 1.36 ± 0.2 cm, p = 0.03) were reduced during VVI pacing. LV systolic dimension (2.46 ± 0.4 vs 3.00 ± 0.5 cm, $p < 0.01$) was significantly larger during VVI. Serum aldosterone levels were significantly lower during VVI pacing (158 ± 127 vs 49 ± 31 pg/ml, p = 0.04). There were no significant differences between 3 AVB and VVI pacing in aortic dimension, interventricular septal dimensions, LV posterior wall diastolic dimension, NT-pro atrial natriuretic peptide, brain natriuretic peptide, plasma renin activity, endothelin-1, epinephrine or norepinephrine concentrations. These results suggest compared to 3 AVB, fixed rate VVI pacing at 100 beats per minute reduces radiographic and echocardiographic cardiac dimensions.

ABSTRACT #209

AMBULATORY ELECTROCARDIOGRAPHIC MONITORING AND OUTCOME IN 338 ASYMPTOMATIC DOBERMAN PINSCHERS (1999–2007). M.L. O'Sullivan, M.R. O'Grady, A.R. Pastor, C. Walker. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

The purpose of this study was to describe the frequency and character of ventricular arrhythmias on ambulatory ECG (Holter) monitors in a population of asymptomatic Doberman Pinschers and to determine whether any of the indices related to ventricular premature contractions (VPCs) were predictive of the combined endpoint of sudden death (SD) or congestive heart failure (CHF).

Recordings from 388 Dobermans were previously collected between 1999 and 2007. Exclusion of those known to be in CHF at the time of the recording and those with recording duration less than 8 hours resulted in a total of 370 asymptomatic Dobermans. Holter analysis was previously performed using the Pathfinder digital Holter analysis system (Delmar Reynolds Medical Ltd.) with manual review and editing. Data collected from the medical record included birth date, gender, coat color, body weight (BW), Holter date, length of Holter recording, total number of VPCs, couplets, triplets, salvos (> 4 VPCs in a row), ventricular tachycardia (VT) (> 5 VPCs in a row with rate > 100 bpm), bigeminy (pattern of alternating sinus beat and VPC), and number of VPCs displaying R on T (earliest VPCs). The numbers of the above events per hour (hr) were calculated by dividing the total number by the length of the recording. Date and manner of outcome was collected from the medical record or from phone calls to the owners or primary care veterinarians. Outcomes were obtained on 338 out of the 370 dogs. Outcomes were classified as SD, CHF, non-cardiac death, lost to follow-up, or still alive and asymptomatic as of May 1, 2007. Univariate and multivariate Cox proportional hazards analysis was used to determine if any Holter indices were predictive of the combined endpoint of SD or CHF. Dogs in the other outcome categories were censored. Significance level was $P < 0.05$.

Of the 338 dogs, 58% were female and 42% were male. Median age and BW were 5.9 years (range 0.5–13.6) and 34.1 kg (range 22.7–57.5), respectively. Median Holter length was 22.8 hours. Percentage of asymptomatic dogs that had VPCs, couplets, triplets, salvos, VT, or bigeminy present were 68%, 24%, 12%, 10%, 7%, and 12%, respectively. 68 dogs (20%) experienced SD or CHF, while 270 (80%) were censored with a total of 168 dogs being still alive. On univariate analysis, many variables were directly associated with SD or CHF, including age, BW, VPCs/hr, VPCs with R on T/hr, couplets/hr, triplets/hr, presence of salvos, VT, and bigeminy. On multivariate analysis while accounting for age and BW, the variables that remained most significant in the face of combining variables were presence of salvos or VT, and bigeminy.

The presence of some degree of ventricular arrhythmia was found to be very common in a population of asymptomatic Dobermans; however, this group may be very biased as it constitutes a voluntary group. The presence of salvos, VT, or bigeminy appears to be particularly significant in terms of prediction of potential for SD or CHF.

ABSTRACT #210

EVALUATION OF A 5-MINUTE ECG IN COMPARISON TO THE 24-HOUR-ECG TO DIAGNOSE DCM IN DOBERMAN PINSCHERS. G. Wess, A. Schulze, J. Simak, M. Killich, V. Butz, K. Hartmann. Clinic for Small Animal Internal Medicine, LMU University of Munich, Germany.

Dilated cardiomyopathy (DCM) in Doberman Pinschers is characterized by a protracted, slowly progressive occult phase during which ventricular and occasionally atrial premature contractions first appear, followed by development of progressive left ventricular dysfunction and usually progressively more severe ventricular tachyarrhythmias. The incidence of sudden death, caused by ventricular tachycardia-fibrillation, prior to the onset of congestive heart failure (CHF) is at least 30%. Subsequent to CHF, death is usually the result of CHF but can occur suddenly. Diagnosis of the occult phase is based upon detection of more than 50 VPCs in a 24-hour ECG (Holter). Holter analysis requires special equipment, sufficient experience and is expensive, time consuming and is not always available.

The purpose of this study was to compare a 5-minute ECG to the Holter monitor results as a gold standard to diagnose occult cardiomyopathy in Doberman Pinschers.

A total of 498 Holter and 5-minute examinations of 324 Doberman Pinschers (53.1% female, 46.9% male) were compared for this study. Each dog was assessed by a 5-minute ECG, Holter examination and echocardiography at each examination. A cut-off value of > 50 VPCs/24-hours on Holter examination was considered diagnostic for occult cardiomyopathy.

Holter examinations revealed > 50 VPCs/24 hours in 123 examinations (24.7%). 61.8% of these examinations with > 50 VES/24-hours had one or more VPCs in the 5-minute ECG. No VPCs in 5 minutes were found in 47 examinations, in which the Holter exam had diagnosed occult cardiomyopathy (38.2%). A 5-minute ECG with at least one VPC had a sensitivity of 61.8%, a specificity of 96.5%, a positive predictive value of 85.4% and a negative predictive value of 88.5% for the presence of > 50 VES/24 hours.

Sensitivity of a 5-minute ECG is 61.8% too low to be a good screening test for occult Doberman Pinscher cardiomyopathy and can not replace a 24-hour ECG. Many dogs with > 50 VPCs/24 hours had not even one VPC during 5 minutes. However, if at least one VPC is detected during a 5-minute ECG, the suspicion that the dog could have a cardiomyopathy should be high and the dog should be further assessed by a 24-hour Holter examination and echocardiography.

ABSTRACT #211

EVALUATION OF CARDIAC LOADING CONDITION BY PLASMA ANP AND NT-PRO-BNP AFTER MITRAL VALVE PLASTY IN DOGS. H. Yamamoto, M. Uechi, T. Ebisawa, Y. Sakamoto, K. Harada, K. Asano, T. Mizukoshi, T. Mizuno, M. Mizuno, S Yamano. Nihon University, Kanagawa, Japan.

The objective was to evaluate whether plasma ANP and NT-pro-BNP detect a reduction in the cardiac volume load in dogs with moderate and severe mitral regurgitation before and after mitral valve plasty (MVP) using cardiopulmonary bypass (CPB). The cases were eight dogs (BW 3.4–10.6 kg) in which MVP was performed from April 2006 to September 2007. All cases indicated cough, cardiac enlargement and pulmonary oedema by X-ray, and the preoperative ISACHC classification was II to IIIa. These were difficult to maintain with medication. MVP using a CPB was performed by annuloplasty of the mitral valve and rebuilding of the chordae tendineae by artificial sutures. Plasma ANP and NT-pro-BNP were measured preoperatively, and postoperatively at 1 and 3 months. Preoperative ANP was 263 ± 74 pg/ml (n = 8), and NT-pro-BNP was 3846 ± 1664 pg/ml (n = 4). The clinical symptom was improved remarkably 1 month postoperatively, and the ISACHC classification decreased in Ib. ANP (127 ± 59 pg/ml and 121 ± 52 pg/ml, n = 8, $P < 0.001$) and NT-pro-BNP (2415 ± 2146 pg/ml and 1202 ± 568 pg/ml, n = 4) 1 and 3 months postoperatively, respectively, significantly decreased compared with preoperatively. A significant reduction in the LA/Ao ratio and heart size was shown by echocardiography, and X-ray, respectively. Medication was withdrawn with the improvement of clinical signs in 4 of 8 cases. ANP and/or NT-pro-BNP levels after cardiac surgery were useful as an indicator of cardiac load in dogs.

ABSTRACT #212
PLASMA CYTOKINE LEVEL IN DOGS UNDERGOING CARDIOPULMONARY BYPASS. T Mizuno, M Uechi, T. Ebisawa, H. Kamiyama, Y. Sakamoto, K. Harada, H. Yamamoto, K. Asano, M Mizuno, S Yamano, T Mizukoshi. Nihon University, Kanagawa, Japan.

Various inflammatory mediators are activated by triggers such as surgical injury at the time of heart surgery, endotoxemia, ischemia, and the contact of the blood in the CPB circuit surface. The inflammatory response of the whole body by the inflammation mediator causes postoperative renal failure, respiratory failure, hemorrhage, nerve damage, cardiac arrhythmia, myocardial disorder, and it progresses to serious multi-organ failure. The objective was to clarify whether inflammatory mediators such as IL-6, IL-10, and TNF-α after open heart surgery using cardiopulmonary bypass (CPB) are activated in dogs. Open heart surgery was performed in 9 dogs (BW3.0–7.3 kg) using CPB from April 2007 to December 2007. The plasma cytokine level was measured preoperatively at 5 minutes after heparin administration, 5 minutes after CPB, after rebeating of the heart, 15 minutes after protamine administration, and after weaning of CPB at 3, 6, 12, 24, and 48 hours. IL-6 was elevated from preoperatively (1.3 ± 2.4 pg/ml) after 3 hours weaning of CPB (281 ± 189 pg/ml). IL-10 was elevated from preoperatively (2.6 ± 4.2 pg/ml) to 15 minutes after protamine (54.2 ± 47.5 pg/ml). There was no difference in TNF-α in all operative periods. Inflammatory cytokine was elevated in dogs in which cardiac surgery had been performed using CPB. This suggests the dogs were in an acute inflammation condition after surgery. It is postulated that the elevation of the inflammatory cytokine develops into a postoperative systemic inflammatory response syndrome.

ABSTRACT #213
PHARMACOKINETICS OF SINGLE-DOSE ORAL PREGABALIN ADMINISTRATION IN NORMAL DOGS. V Salazar, CW Dewey, WS Schwark, BL Badgley, RD Gleed, WA Horne, JW Ludders. Cornell University College of Veterinary Medicine, Ithaca, NY.

Pregabalin, an $\alpha_2\delta$ ligand of neuronal voltage-gated calcium channels, is currently used to treat epilepsy and neuropathic pain in humans. Based on experimental rodent models and human clinical trials, pregabalin may be a useful drug for both anticonvulsant and pain management in dogs. Nevertheless, pharmacokinetics studies have not been performed for pregabalin in this species. The objective of this study was to describe the pharmacokinetics of pregabalin in normal dogs after a single oral dose (4 mg/Kg).

Six adult research dogs (Labrador Retriever and Greyhound mix) were used. All studies were approved by Cornell University's Institutional Animal Care and Use Committee (IACUC). All dogs, after jugular vein catheterization, were given a single oral dose of pregabalin (4 mg/kg). Blood samples were collected at 0 (before drug administration), 15, 30, 60, 90, 120, 180, 240, 360, 480, 720, 1440 and 2160 minutes after drug administration. Plasma pregabalin concentration was measured by HPLC. Various pharmacokinetic variables were measured by a commercially available software applied to the plasma concentration-time data.

No obvious adverse effects associated with the oral administration of pregabalin were observed. In all dogs, except for one, plasma pregabalin remained within the therapeutic range (for seizures) in humans (2.8–8.2 μg/ml) between hour 1 and 8 after administration. The pharmacokinetic parameters studied were (mean ± SD and range) area under the curve (AUC) = 78.2 ± 14.5 μg-h/ml (56.5–92.1 μg-h/ml); absorption half-life ($T_{1/2}$abs) = 0.48 ± 0.33 h (0.25–1.11 h); elimination half-life ($T_{1/2}$el) = 6.81 ± 0.55 h (6.21–7.40 h); time over the presumed minimum effective concentration (T > 2.8 μg/ml) = 10.83 ± 2.48 h (6.97–14.47 h); maximum plasma concentration (C_{max}) = 6.8 ± 1.2 μg/ml (4.6–7.9 μg/ml); time for C_{max} to occur (T_{max}) = 1.9 ± 1.1 h (1.0–4.0 h); volume of distribution (V_d) = 0.49 ± 0.07 L/Kg (0.43–0.59 L/Kg); clearance (Cl) = 50.8 ± 9.6 ml/h/Kg (42.0–65.3 ml/h/Kg); mean residence time (MRT) = 10.4 ± 0.8 h (9.1–11.3 h). Values were predicted for steady state plasma levels using 8 h or 12 h dosing intervals respectively: (Css_{max}) = 12.2 ± 2.5 μg/ml; 9.5 ± 1.2 μg/ml; (Css_{min}) = 6.5 ± 1.2 μg/ml, 3.6 ± 0.8 μg/ml; (Css_{ave}) = 8.9 ± 1.7 μg/ml, 6.5 ± 1.2 μg/ml.

In conclusion, in normal dogs, a 4 mg/Kg oral dose of pregabalin is well tolerated and achieves plasma drug concentrations within the therapeutic range reported to control seizures in people between hour 1 and 8 after administration. Based on the favorable pharmacokinetics and tolerability demonstrated for oral pregabalin in this study, further study of efficacy for the treatment of neuropathic pain and seizures is warranted.

ABSTRACT #214
PREGABALIN THERAPY FOR REFRACTORY IDIOPATHIC EPILEPSY IN DOGS. CW Dewey[1], S Cerda-Gonzalez[1], JM Levine[2], BL Badgley[1], JM Ducote[3], GM Silver[4], JJ Cooper[2]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY. [2]Texas A&M University College of Veterinary Medicine, College Station, TX. [3]Animal Neurology and Neurosurgery of Texas, Carrollton, TX. [4]Massachusetts Veterinary Referral Hospital, Woburn, MA.

Idiopathic epilepsy (IE) refractory to phenobarbital (PB), potassium bromide (KBr), or a combination of these drugs remains a significant clinical problem in dogs. Pregabalin (PG) is a new antiseizure drug shown to be effective in rodent seizure models and human clinical trials. Considered the "next generation" of gabapentin, PG's pharmacologic effects have been linked to its interaction with the α2δ subunit of neuronal voltage-gated Ca^{++} channels. By decreasing Ca^{++} influx, synaptic release of excitatory neurotransmitters (e.g., glutamate) is reduced.

Based on pharmacokinetic data from normal dogs, a PG dose of 2–4 mg/kg q 8 hrs was administered to 6 dogs with suspected IE refractory to PB, KBr, or the combination of these drugs. Dogs starting at the lower end of dosing were increased by 1 mg/kg weekly to a maximum of 4 mg/kg if tolerated. To qualify for study inclusion, dogs had to satisfy the following criteria, in addition to having characteristic clinical features of IE: accurate seizure logs for at least 3 mos prior to the study; a minimum average of 2 seizures/mo; and plasma [PB] and/or [Br] within therapeutic range. Four of the 6 dogs were receiving both PB and KBr, and one dog each was receiving sole PB or KBr therapy. The mean [PB] was 24.9 ug/mL (range, 19.9–29.4 ug/mL) and the mean [Br] was 2.01 mg/mL (range, 0.6–2.81 mg/mL). Five of the 6 dogs exhibited cluster seizures as their typical pattern. Seizure frequency for 3 mos prior to and 3 mos following the addition of PG treatment was compared for each dog.

Five dogs achieved a final dosing level of 4 mg/kg, and 1 dog achieved 3 mg/kg. One dog left the study at the owner's request at 38 days following a cluster episode and was considered a drug failure. Seizures were reduced in the post-PG period in the remaining 5 dogs, with seizure reduction ranging from 23% to 79% (mean = 52%). Four of these 5 dogs were responders (i.e., a minimum of 50% seizure reduction) with a mean seizure reduction of 59.3%. Side effects attributed to PG were reported in 5 dogs, most notably sedation and ataxia. One dog (on PB and KBr) developed severe pancreatitis after garbage ingestion at the end of the study and was euthanized-this was not considered a PG-related side effect. Plasma PG concentrations were measured via HPLC for all dogs at various times during the study (between 30 and 90 days); with the exception of 1 dog (2.6 ug/mL), all dogs had concentrations within the range considered therapeutic for humans (2.8–8.2 ug/mL). Although preliminary, results of this investigation suggest that PG may be an effective add-on therapy for dogs with refractory IE and that side effects are similar to those reported for its predecessor, gabapentin.

ABSTRACT #215
DISPOSITION OF LEVETIRACETAM IN CATS. MB Carnes[1], DM Boothe[1], TW Axlund[2]. [1]Auburn University College of Veterinary Medicine; Auburn, AL. [2]Metropolitan Veterinary Referral Group; Akron, OH.

Levetiracetam is a unique anticonvulsant drug that has been used effectively in humans and shows promise in canine patients. The pharmacokinetics have been determined and safety evaluated in humans, dogs, rodents and rabbits with no apparent side effects at recommended dose regimens. The purpose of this study was to determine if therapeutic concentrations of levetiracetam can be achieved and maintained throughout a reasonable dosing interval (either orally or intravenously) at doses not associated with adverse effects in the cat. The study design was a prospective, randomized,

double cross-over. Ten healthy purpose-bred cats were administered levetiracetam (20 mg/kg) either orally or intravenously. Blood was collected at times 0, 10, 20, 40 minutes, 1, 1.5, 2, 3, 4, 6, 9, 12 and 24 hours. Plasma levetiracetam concentrations were determined by high performance liquid chromatography and expressed as mean ± standard deviation. No adverse events were noted during the course of the study. Mean (± sd) values were peak concentration (mcg/ml) 25.54 ± 7.97 (oral C_{max}) and 37.52 ± 6.79 (IV C_0), elimination half-life (hr) 2.95 ± 0.95 (oral) and 2.86 ± 0.65 (IV), mean residence time (hr) 5.65 ± 1.25 (oral) and 4.57 ± 0.94 (IV), apparent volume of distribution (L/kg) 0.52 ± 0.09, clearance (ml/kg/min) 2.0 ± 0.60. The oral bioavailability (%) was determined to be 100 ± 0.39. Drug concentrations were in the therapeutic range determined for humans (5–45 mcg/ml) for at least 9 hours. Based on these results, a 20 mg/kg oral or IV dose of levetiracetam every 8 hours in cats is expected to result in plasma concentrations within the therapeutic range established for humans. This study supports the use of levetiracetam as an alternative anti-epileptic drug in cats; however, further studies are indicated to evaluate the efficacy of levetiracetam in seizure management in this species.

ABSTRACT #216

***TOXOPLASMA GONDII* ANTIGEN RECOGNITION PATTERNS IN SEROPOSITIVE DOGS WITH AND WITHOUT ACUTE POLYRADICULONEURITIS.** N Holt[1], L Pearce[1], P Cuddon[2], JR Hawley[1], MR Lappin[1]. [1]Colorado State University, Fort Collins, CO and [2]Alameda East Veterinary Hospital, Denver, CO.

Acute Canine Polyradiculoneuritis (ACP) is the most commonly recognized peripheral neuropathy in dogs and is thought to have multiple causes. The disease in dogs is similar to Guillain-Barre Syndrome in humans, which has been associated with molecular mimicry to antigens of infectious agents such as *Campylobacter jejuni* and *Toxoplasma gondii*. In a previous study, prevalence rates of antibodies to selected infectious agents were calculated for dogs with and without ACP and an association between ACP and positive *T. gondii* IgG antibody titers determined by ELISA was shown. The objective of this follow-up study was to use western blot immunoassay (WB) to compare *T. gondii* antigen recognition patterns between *T. gondii* IgG positive dogs with and without ACP to determine whether a unique pattern exists in dogs with ACP.

Archived sera from *T. gondii* IgG ELISA positive dogs were selected based exclusively on sample availability. Sera from 24 dogs with clinical findings consistent with ACP evaluated by one of the investigators (PC) and 12 age- and state-matched dogs without clinical evidence of ACP were assayed in an optimized IgG heavy chain-specific, WB assay using *T. gondii* RH1 strain as the antigen source. A single investigator determined the apparent molecular masses of the recognized antigens using digital images of the blots and a commercially available software program.

Serum antibodies from 8 affected dogs and 11 control dogs bound to *Toxoplasma gondii* antigens. Overall, antigens with apparent molecular masses of 67, 61, 58, 45, 36, 33, 24, 9 and 6 kD were recognized by the 19 positive dogs. Antigen recognition patterns varied between the groups.

	Molecular mass (kD)								
Group	67	61	58	45	36	33	24	9	6
Dogs with ACP	1	1	1	1	2	3	1	1	5
Dogs without ACP	3	3	4	7	0	7	3	1	8

An antigen with an apparent molecular mass of 36 kD was recognized by two dogs with ACP but none of the control dogs. Further work is indicated to determine whether this finding is reproducible in a larger sample set and to determine whether this antigen is immunologically similar to those associated with Guillain-Barre Syndrome in humans.

ABSTRACT #217

BETA-AMYLOID PATHOLOGY IN THE CEREBRAL CORTEX OF THE DOG. G Santamarina[1], D Insua[1], ML Suarez[1], M Sarasa[2], P Pesini[2]. [1]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, University of Santiago de Compostela, Lugo, Spain. [2]Araclon Biotech, Zaragoza, Spain.

We studied the presence of β-amyloid (βA) cortical deposits in a group of young (n = 7), aged not-cognitively-impaired (NCI, n = 14), and aged cognitively-impaired (CI, n = 5) dogs. βA was visualized by immunohistochemistry. The extension of the deposits was estimated by area fraction fractionator in one frontal lobe section per dog.

No βA labeling could be detected in the brain of young dogs. Extensive βA deposits were found in all the aged-CI dogs. Additionally, seven aged-NCI dogs presented moderate to high βA deposits in spite of no apparent cognitive symptoms. The βA deposits appeared as diffuse plaques that spread across the deep cortical layers from the limit with the subjacent white matter. In the 5 aged-CI dogs these plaques were also seen in more superficial cortical layers where they tended to acquire a denser and better delimited appearance. Many small-diameter cortical blood vessels were intensely stained in 8 of the 12 dogs with βA deposits, including 4 without cognitive impairment. In contrast, 7 aged dogs were free of cortical βA deposits. These results suggest that βA deposits is not a mere consequence of normal aging but exogenous and/or genetic factors could determine the susceptibility of the individuals to develop amyloid pathology at a given age. The finding of βA angiopathy and a moderate to heavy βA burden in some aged animals lacking a positive diagnostic of cognitive impairment reminds similar reports on the presence of extensive βA deposits in non demented human brains. It is possible that early symptoms of the canine counterpart of AD could pass unnoticed to the owners and underline the importance to find reliable biomarkers for the disease.

ABSTRACT #218

POLYMICROGYRIA IN FOUR STANDARD POODLES: A CASE SERIES WITH HISTOPATHOLOGIC, ELECTROENCEPHALOGRAPHIC AND MAGNETIC RESONANCE IMAGING FINDINGS. C Jurney[1], J Haddad[1], N Crawford[2], AD Miller[3], AM Komaromy[1], TJ Van Winkle[1], CH Vite[1], P Sponenberg[2], KD Inzana[2], GC Johnson[4], DP O'Brien[4]. [1]University of Pennsylvania College of Veterinary Medicine, Philadelphia, PA. [2]Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA. [3]Cornell University College of Veterinary Medicine, Ithaca, NY. [4]University of Missouri, College of Veterinary Medicine, Columbia, MO.

This case series describes four standard poodles with polymicrogyria, including a 5 year old spayed female, a 3 month old intact male, a 3.5 month old intact male and a 4 month old intact male. Previously this disorder has been reported in only four dogs (all Standard Poodles) and in cattle. Polymicrogyria is a disorder of cortical migration resulting in abnormal, small, disorganized gyri. Further disruption is present histologically at the cortex, with loss of normal layering. Clinical signs in all dogs included cortical blindness and behavioral abnormalities. Subjects also had other neurologic abnormalities including gait changes, decreased pupillary light responses and partial seizures. Magnetic resonance imaging (MRI) of the 5 year old patient allowed for visualization of multiple disorganized gyri in the occipital lobes, which were especially apparent on T2 weighted coronal plane images. Electroencephalogram (EEG) of this patient revealed epileptiform discharges, including both spike and spike and wave discharges with voltage maximum potentials over the parietal/occipital region. The EEG supported that a repetitive behavior displayed by the dog was a complex partial motor seizure. MRI on the 4 month old male showed asymmetric hydrocephalus. At necropsy, only one of the four animals had hydrocephalus. All dogs had occipital lobe involvement; two dogs had involvement of other lobes as well. The cases presented here demonstrate a significantly longer survival time than previous reports; as well providing an antemortem diagnosis via magnetic resonance imaging and electroencephalogram.

ABSTRACT #219

DETECTION OF CANINE DISTEMPER VIRUS BY HEMI-NESTED PCR IN VARIOUS CLINICAL SAMPLES FROM DOGS WITH NEUROLOGICAL SIGNS. HA Amaral[1],

A Cortez[1], LJ Ricktzenhain[1], MR Funada[1], RM Soares[1], EL Durigon[2], MHMA Larsson[1]. [1]Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, Brazil. [2]Universidade de São Paulo, Instituto de Ciências Biomédicas II, São Paulo, SP, Brazil.

Canine distemper is a worldwide, highly contagious disease, which often induces severe neurological signs. The aim of this study was to evaluate different biological samples (conjunctival and genital swabs, urine and peripheral blood mononuclear cells/PBMCs) by Reverse Transcription-hemi-nested PCR.

All collections were done from dogs with neurological manifestation, examined at the Veterinary Hospital of Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (HOVET-FMVZ-USP), from October 2004 to May 2007. Only dogs without vaccine or vaccinated more than 50 days before the samples were collected, to avoid positive results from vacinal virus.

Fragments of the nucleoprotein (NP) gene of canine distemper virus were detected in 43 from 50 dogs evaluated by hemi-nested-PCR. A greater number of positive results were obtained from genital swabs (40), followed by conjuctival swabs and urine (37) and PBMCs (33). Sensitivity in detecting positive results was increased by using two clinical samples together (genital swab and urine), especially in dogs that had not shown extra neural signs or during the convalescent and late stage of canine distemper.

ABSTRACT #220

POSTOPERATIVE COMPLICATIONS AND OUTCOME IN 14 DOGS TREATED FOR HYDROCEPHALUS BY VENTRICULOPERITONEAL SHUNTING. Alberta de Stefani, Luisa De Risio, Lara Matiasek, Alejandro Lujan Feliu-Pascual. The Animal Health Trust, Newmarket, UK.

Ventriculoperitoneal (VP) shunting is indicated for the treatment of hydrocephalus independently of the underlying cause. Outcome and postoperative complications following this procedure have been reported in a limited number of cases in the veterinary literature to date. The purpose of this study was to describe the outcome, type and rate of complications in 14 dogs presented with severe neurological signs secondary to obstructive hydrocephalus and treated by VP shunting (Medronic PS Medical Snap Shunt Assemblies).

The clinical database (2001–2006) was searched for dogs that underwent VP shunting. Inclusion criteria were complete medical record, progressive CNS signs unresponsive to medical treatment, normal metabolic profile, negative antibody titers and/or CSF PCR for *Toxoplasma gondii*, *Neospora caninum* and *Canine Distemper virus*, MRI scan of the brain, VP shunting and detailed follow-up. Fourteen dogs met the inclusion criteria. There was no breed predisposition. Mean age was 5 years (6 months to 11 years). Most common clinical signs at presentation included lethargy, obtundation, seizures and compulsive walking. Obstructive hydrocephalus was diagnosed as congenital in 5 dogs and acquired (intraventricular tumors, intraventricular hemorrhage, and inflammatory disease) in 9. Four dogs developed complications 1 week to 17 months postoperatively. Complications included ventricular catheter migration, infection, shunt underdrainage, kinking of the peritoneal catheter, valve fracture and skin necrosis of the abdomen. Three dogs underwent 1 or more successful revision surgeries and 1 dog was treated with antibiotics. Of the 5 dogs diagnosed with congenital hydrocephalus, 3 were euthanized due to relapse of clinical signs (severe seizures) 2 to 9 months following surgery, 1 dog was euthanized due to renal failure 12 months after surgery, and 1 dog was still alive at the time of writing 16 months postoperatively. Of the 9 dogs with acquired hydrocephalus, 7 were euthanized due to lack of improvement/worsening of the neurological signs 2 days to 19 months postoperatively (mean 8.6 months), 1 was euthanized due to cardiac failure 10 months postoperatively and 1 was still alive at the time of writing 6.5 years postoperatively. All but 1 dog were discharged within 1 week of surgery, showed a significant neurological improvement and regained a good quality of life. Thirteen dogs were alive at 1 month postoperatively, nine dogs were alive 3 months postoperatively, and 6 dogs were alive 12 months postoperatively.

Ventriculoperitoneal shunting was successful in relieving the neurological signs and improving quality of life of the majority of the dogs. Several postoperative complications occurred in 29% of dogs which resolved following revision surgery and/or medically.

ABSTRACT #221

SHORT- AND LONG-TERM OUTCOME IN 63 DOGS TREATED CONSERVATIVELY OR SURGICALLY FOR DISC ASSOCIATED WOBBLER SYNDROME. S De Decker, SFM Bhatti, L Duchateau, MC Tshamala, VA Martlé, I Van Soens, SA Van Meervenne, JH Saunders, LML Van Ham. Ghent University, Ghent, Belgium.

Disc associated wobbler syndrome (DAWS) is the most typical and predominant wobbler syndrome. There is little known about the results and risk factors associated with non-surgical treatment of this specific wobbler syndrome.

Medical records of dogs with DAWS treated conservatively or surgically (ventral slot) were retrospectively analyzed. Diagnosis was confirmed by myelography in all dogs. Sixty-three dogs were included. Fifty-one dogs were treated conservatively and 12 surgically. Follow-up information was obtained by recheck examinations or telephone questionnaire. A success score of 1 to 10 was given and a successful outcome was defined as a success score of 8 or higher. This definition included only dogs that did not show worsening of clinical signs after DAWS was diagnosed. In the conservatively treated group the following potential risk factors were evaluated: age, duration and severity of clinical signs, number of protruded intervertebral discs, and additional radiographic abnormalities. Because of the limited number of dogs, these factors were not evaluated in the surgically treated group. Statistical analysis was performed using the Fischer exact test or the Wilcoxon rank sum test.

A successful outcome was achieved in 45% of conservatively treated dogs. Seventy-five percent of surgically treated dogs achieved an initial successful outcome. Due to recurrence of clinical signs in 66% of these dogs, only 42% of the surgically treated dogs obtained a definitive successful outcome. Although not significant, surgically treated dogs had a longer life expectancy (mean, 19 months) than conservatively treated dogs (mean, 9 months). Eighty-five percent of conservatively treated dogs, who had to be euthanized because of DAWS, were so euthanized in the first year after diagnosis. Outcome of conservatively treated dogs was negatively influenced by severity of clinical signs and additional radiographic abnormalities and not by age, disease duration and number of protruded intervertebral discs.

The results of this study suggest that conservative treatment of DAWS can be considered in mildly affected cases without additional radiographic abnormalities and that the first year after diagnosis is a critical period to obtain a successful outcome.

ABSTRACT #222

CERVICAL VERTEBRAL MALFORMATION-MALARTICULATION SYNDROME IN THE BERNESE MOUNTAIN DOG: CLINICAL AND MAGNETIC RESONANCE IMAGING FEATURES. JS Eagleson[1], J Diaz[2], SR Platt[1], M Kent[1], F Gruenenfelder[1], NJ Sharp[2], SJ Schatzberg[1]. [1]University of Georgia College of Veterinary Medicine, Athens, GA. [2]Canada West Veterinary Specialists, Vancouver, BC, Canada.

The Bernese Mountain Dog (BMD) has been described sporadically in the literature as a breed affected by cervical vertebral malformation-malarticulation (CVMM) syndrome. However, the clinical and imaging features of CVVM in BMDs have not been characterized. The purpose of this retrospective report was to describe the clinical presentations and the MR imaging characteristics in a small, unrelated group of BMDs with CVMM.

Seven BMDs (four males, three females) diagnosed with cervical myelopathy were evaluated using MR imaging at two referral hospitals (2003–2007). Age ranged from 18 months to 7 years (median = 5.2 years); weight ranged from 40.0 kg to 57.3 kg (mean = 50.1 kg). Four dogs had a neuroanatomic localization of C1-C5. Of the four dogs, three presented with upper motor neuron paresis and general proprioceptive ataxia in all four limbs, and one dog presented with paraparesis and pelvic limb proprioceptive ataxia. All four dogs with C1-C5 localizations were ambulatory. Three dogs had a C6-T2 neuroanatomic localization, two of which were ambulatory but tetraparetic with general proprioceptive ataxia in the pelvic limbs; the other dog was severely tetraparetic and non-ambulatory. Three of seven dogs were neck guarding at the time of evaluation. All dogs had progressive clinical signs ranging in duration from 3 days to 3 months.

Spin echo T1-weighted (T1W) and T2-weighted (T2W) images were obtained using a 1.5 Tesla magnet in 6 dogs and using a 1.0 Tesla magnet in 1 dog. All dogs had evidence of intervertebral disc degeneration, ventral extradural spinal cord compression associated with intervertebral disc protrusion ± dorsal longitudinal ligament hypertrophy, dorsolateral extradural spinal cord compression associated with articular process hypertrophy (bilateral in 5 dogs and unilateral in 2 dogs), and some degree of intramedullary hyperintensity on T2W images at the level of spinal cord compression. All dogs had more than one compressive site (ventral or dorsolateral or both) within the region of C3-4 to C6-7. Dorsolateral compression was more significant than ventral compression in 6 of the 7 dogs.

In conclusion, CVMM in the BMD has features similar to CVMM in both the Doberman Pinscher and the Great Dane.

ABSTRACT #223

CEREBROSPINAL FLUID NEUROTRANSMITTER CONCENTRATIONS AFTER SUBARACHNOID HEMORRHAGE IN DOGS: EFFECT OF TREATMENT. JR Coates[1], SR Platt[2], DM Eifler[1], KR Bulsara[3], University of Missouri, [1]College of Veterinary Medicine and [3]School of Medicine, Columbia, MO. [2]University of Georgia, College of Veterinary Medicine, Athens, GA.

Subarachnoid hemorrhage (SAH) is characterized by decreased cerebral blood flow, subsequent cerebral vasospasm and ischemia, and high mortality in people. Impaired endothelial and neuronal nitric oxide (NO) release further lead to inflammation and excitoxicity triggered by excitatory amino acids, glutamate and aspartate. Immunosuppression using cyclosporine A ameliorates cerebral vasospasm, as well as upregulation of NO synthase using statin treatment. We hypothesized that dogs with SAH have alterations in CSF concentrations of glutamate, aspartate, GABA and glycine which are indirectly but positively affected by use of cyclosporine and simvastatin. CSF concentrations of neurotransmitters were investigated as biomarkers of neuronal injury and indicators of beneficial treatments for CNS ischemia.

A double SAH model was induced in dogs by 2 injections (3 ml) of autologous blood into the cerebellomedullary cistern (CMC) 24 hours apart. Dogs were assigned to one of three groups: Control-untreated ($n = 4$); simvastatin (Zocor, Merck Inc., 20 mg/kg SID PO) only ($n = 4$); simvastatin (20 mg/kg SID PO) and cyclosporine A (Sandimmune, Sandoz Inc., 6 mg/kg SID PO) ($n = 4$). Drugs were administered 24 hours after the second injection for 10 days. CSF was collected from the CMC before each injection and on days 3, 7, and 10 and immediately stored at −80 °C. The CSF samples were reacted to produce 1-cyanobenz[f]isoindole derivatives. Derivatives were analyzed with electrochemical HPLC. Data analysis used repeated measures model and included factors for treatment, time and a treatment time interaction term (PROC MIXED in SAS; $p < 0.05$). Multiple comparisons were adjusted for using Tukey's test.

In the control group, glutamate significantly increased to highest levels by day 3 and then returned to baseline, whereas glycine, GABA and aspartate were not significantly altered from baseline at any time point. There was significant decremental effect of simvastatin alone ($p = 0.0004$) and in combination with cyclosporine ($p = 0.0007$) on day 3 glutamate concentrations when compared to the control group. A significant incremental effect of combination treatment on day 3 glycine levels was noted compared to control group ($p = 0.006$). No significant differences in GABA and aspartate levels were noted between treatment groups on any of the sample days.

Although precise roles of these neurotransmitters have not been elucidated in pathophysiology of canine CNS ischemia, their alterations from baseline suggest further investigation. A combination of immunosuppression and NO synthase upregulation may be useful in ameliorating elevated glutamate levels in CNS ischemia. Results may be confounded by small sample size and concurrent independent effects of hemorrhage and inflammation notably present in SAH models.

ABSTRACT #224

TUMOR NECROSIS FACTOR ALPHA IN THE CEREBROSPINAL FLUID OF DOGS WITH CENTRAL NERVOUS SYSTEM DISEASE. JD Parkes, KL Kline, A Fales-Williams, CA Petersen. Iowa State University College of Veterinary Medicine, Ames, IA.

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine essential in the inflammatory phase of the innate immune response. Cells that express TNF-α include, but are not limited to, B cells, macrophages, neutrophils, plasma cells and astrocytes. Most of the veterinary research on cytokine expression in canine central nervous system (CNS) disease has been performed using reverse transcription-polymerase chain reaction (RT-PCR) on biopsies of CNS tissue or cells from cerebrospinal fluid (CSF). Production of pro-inflammatory cytokines in situ in CSF of canine patients with CNS disease has not been investigated to date. The purpose of this prospective study was to evaluate whether TNF-α could be detected in measurable quantities in CSF samples in situ in dogs with CNS disease and to compare levels to those of normal, healthy dogs. Our hypothesis was that TNF-α would be measured in appreciable quantities in CSF and that the levels would be statistically different between various CNS disease processes in dogs.

Thirty-seven client owned dogs that presented to the Iowa State University Veterinary Teaching Hospital with signs of CNS disease underwent collection of CSF as part of their diagnostic work up. These samples were routinely analyzed; cell count, cytology and protein level. A small aliquot of CSF from each patient (approximately 100 μL) was reserved for TNF-α production analysis. The samples were centrifuged at high speed for clarification and frozen at −80 degrees centigrade until time of assay. CSF samples from 8 normal, healthy dogs that presented to the hospital from the local humane society for routine spay or castration were used as the negative control group. Samples were analyzed via quantitative sandwich enzyme immunoassay technique using the Quantikine® Canine TNF-α Immunoassay (R&D Systems, Minneapolis, MN, USA). Study dogs were allotted into one of 6 groups based on disease process. These included intervertebral disk disease (9), vascular disease (2), inflammatory disease (5), idiopathic epilepsy (9), neoplasia (6) and other CNS disease such as congenital disease and trauma (4). Ages of study dogs ranged from 1 to 12 years. Statistical analysis was performed with SAS version 9.1 using Wilcoxon rank sum and Kruskal-Wallis tests.

TNF-α could be consistently measured in the CSF samples of both normal and diseased dogs via ELISA kit with a median of 20 pg/ml and a 95% confidence interval of 15–27 pg/ml. A dog with acute intervertebral disk disease had the highest single TNF-α level at 47 pg/ml. There was no statistically significant difference in TNF-α levels among the groups of diseased dogs or between normal and diseased groups.

In conclusion, levels of TNF-α could be consistently measured in the CSF in both diseased and normal dogs using a commercially available ELISA kit. Levels of TNF-α were not significantly different between diseased and normal groups or among dogs in diseased groups.

ABSTRACT #225

CORRELATION OF MR IMAGING MENINGEAL ENHANCEMENT OR CSF FLAIR-SUPPRESSION WITH CSF ANALYSIS IN DOGS. FA Wininger[1], SP Holmes[2], RS Bagley[1], AV Chen[1], DG Hicks[1] and JN Bryan[1]. [1]Veterinary Clinical Sciences-Washington State University, Pullman, WA. [2]Veterinary Clinical Sciences University of Florida, Gainesville, FL.

To determine if a correlation exists between meningeal enhancement or CSF FLAIR-suppression present on MR imaging and results of CSF analysis, a retrospective assessment of dogs with intracranial signs who had both intracranial MR imaging and CSF analysis were reviewed. Inclusion criteria for cases were the following: clinical signs consistent with an intracranial neurolocalization, a complete MR brain study and CSF analysis. Recorded historical parameters included signalment, duration of clinical signs, medications administered, neuro-localization and diagnosis at time of discharge. MR studies were evaluated by a single radiologist independent of the knowledge of CSF analysis results. The presence of meningeal enhancement on T1 weighted imaging after administration of a paramagnetic contrast agent (gadolinium) and incomplete suppression of the CSF signal in the lateral ventricles on FLAIR sequences was subjectively evaluated. Meningeal enhancement was subjectively graded as absent, mild, moderate or severe. Quantitatively, intensity comparisons were made between 5 points in the lateral ventricles, the temporal muscles and the surrounding air. Both atlanto-occipital and lumbar puncture sites were accepted in

the study and analysis included nucleated cell count, protein, glucose, creatinine kinase concentrations, and cytologic description. Patients with spinal causes of CSF alterations on lumbar puncture or marked blood contamination in their CSF tap (> 2000 RBC/ul) were excluded from the study.

25/78 (32%) dogs had detectable contrast enhancement of the meninges. 3/3 dogs with severe enhancement and 11/12 dogs with moderate enhancement had abnormal CSF protein and/or cell counts, while 6/10 patients with mild enhancement had similar CSF changes. Of the remaining 53 dogs without detectable contrast enhancement of the meninges, 26 (49%) had abnormalities on CSF analysis.

Thirty-seven of 78 dogs (47%) had incomplete suppression of CSF signal on FLAIR studies. Of these 37 dogs, however, there was no obvious correlation between incomplete FLAIR suppression and CSF analysis.

This initial retrospective evaluation suggests that moderate to marked meningeal enhancement seen with MR imaging is commonly associated with CSF alterations; however, mild enhancement (subjectively identified) is incompletely associated with CSF alterations in naturally affected animals with intracranial signs. Also, importantly, not all dogs with CSF abnormalities have meningeal enhancement present on MR imaging as close to 50% of dogs in this study had no meningeal enhancement in the presence of CSF abnormalities.

ABSTRACT #226

COMPLETE OPTICAL NEUROPHYSIOLOGY IN VIVO AND IN VITRO. Fred A Wininger[1], Jennifer L. Schei[2], Stephen A Greene[1] and David M. Rector[1]. [1]Dept VCAPP, [2]Physics and Astronomy Dept, Washington State University, Pullman, WA.

To assess neuro-physiologic function in clinical patients, electrical stimulation and recording of neural membrane potential are commonly used in veterinary medicine. Optical means of neural stimulation and recording have many potential advantages over traditional electrophysiologic assays. Optical stimulation does not elicit an electrical artifact, which despite removal methods masks early neural events associated with the stimulation. Using focused optics, it is possible to direct focal stimulation anywhere in a two or three dimensional field of view, or even provide patterned stimulation. Since a contact tissue interface is not needed, optical stimulation is possibly less invasive, with the potential to transmit through other tissues. Optical recording can measure both direct neural activation and hemodynamically coupled changes, affording the benefit of excellent temporal and spatial resolution respectively.

We extracted walking leg nerves from lobsters and placed them in a nerve chamber. Electrodes within the chamber were used to record the electrical responses to stimulation. We illuminated the nerve with an LED through an optical window in the chamber which allowed for detection of changes in the polarization of light through crossed polarizers (birefringence). The nerve was stimulated using both electrical and optical methods. Electrical stimulus was applied with varying pulse widths via electrodes within the chamber. For optical stimulation, an infrared diode laser was situated close to the nerve surface, and perpendicular to its longitudinal axis.

For our *in vivo* studies, we optically stimulated the vagus nerve of Sprague-Dawley rats. Previous studies have shown success in stimulating the sciatic nerve inducing a motor unit action potential by similar means. A lateral approach to the vago-sympathetic trunk allowed for placement of the diode laser adjacent to the nerve. Electrocardiography was concurrently performed along with neural electrical recording distal to the stimulation site.

We tested several different optical power levels, pulse widths, and distances from the nerve to determine the optimal conditions for optical stimulation. As many as 500 triggered action potentials were electrically recorded following optical stimulation. We also observed birefringent signal change to electrical and optical stimulation concomitant with the electrical response. In the rat model, precipitous, synchronous decreases in heart rate were induced with both optical and electrical stimulation.

Data from these experiments suggest that the infrared laser is capable of depolarizing the axons and generating action potentials within a lobster nerve and the rat vagus nerve. Since we could simultaneously stimulate and record neural activation with optical signals, this study paves the way for neurophysiology without the need for wires or electrical conduction in veterinary patients.

ABSTRACT #227

DETECTION OF CEREBRAL METABOLITES IN A CANINE MODEL OF ISCHEMIC STROKE USING 1H MAGNETIC RESONANCE SPECTROSCOPY. BT Kang[1], DP Jang[2], JH Lee[1], DI Jung[1], SH Gu[1], CY Lim[1], JH Yoo[1], C Park[1], YB Kim[2], EJ Woo[3], ZH Cho[2], HM Park[1]. [1]Konkuk University College of Veterinary Medicine, Seoul, South Korea. [2]Gachon University of Medicine and Science, Neuroscience Research Institute, Incheon, South Korea. [3]Kyung Hee University, Department of Biomedical Engineering, Yongin, South Korea.

Proton magnetic resonance spectroscopy (^{1}H MRS) provides *in vivo* biochemical information on tissue metabolites. However, there have been no prior studies using ^{1}H MRS in dogs with ischemic stroke. The purpose of this study was to investigate the serial metabolic changes of ^{1}H MRS in the cerebrum of ischemic dogs, and to correlate the metabolic changes with the immunohistochemical features of the ischemic lesion. An ischemic stroke was induced in five health laboratory Beagle dogs by permanent middle cerebral artery occlusion using a silicone plug. ^{1}H MRS was serially performed three times with a 1.5-tesla MR system: before, 3 days after and 10 days after the stroke. Immunohistochemical staining was performed to determine the expression of neuronal nuclei (NeuN) and glial fibrillary acidic protein (GFAP) at both the ipsilateral and contralateral cerebral cortex. Reduced levels of *N*-acetyl-asparate ($P < 0.05$), choline (Cho), creatine (Cr) and myo-inositol (mI), and a marked increase in the lactate (Lac) level ($P < 0.01$) were found at three days after the stroke. At 10 days after the stroke, the increased levels of Lac ($P < 0.01$) were maintained over time; however, the other metabolites partially recovered. The changes of Cr, Cho and mI were not statistically significant ($P > 0.05$). There was a significant loss of NeuN and GFAP immunoreactivity at the ischemic core. ^{1}H MRS may be to a useful diagnostic tool for the evaluation of ischemic stroke in dogs; our results demonstrated that it showed the prominent metabolic features of an ischemic brain.

ABSTRACT #228

BRAIN VOLUME ANALYSIS AND IDENTIFICATION OF CEREBELLAR ATROPHY IN DIFFERENT BREEDS OF DOGS USING MAGNETIC RESONANCE IMAGING. R. Thames[1], N.J. Olby[1], I. Robertson[1], T. Flegel[2], D. Henke[3]. [1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, [2]University of Leipzig, Leipzig, Germany, [3]University of Bern, Bern, Switzerland.

Neurodegenerative diseases affecting the cerebellum are well documented in several different breeds of dogs. When extreme, the cerebellar atrophy associated with such diseases can be detected on magnetic resonance imaging (MRI). However, there is little published information regarding the changes in cerebellar size that occur with age or relating its volume and cross sectional area (CSA) to other regions of the brain in different breeds. As a result, identification of cerebellar atrophy on MRI is subjective. We hypothesize that the CSA of the cerebellum on mid-sagittal images maintains a consistent ratio with CSA of other regions of the brain in all breeds of dogs of all ages and that this ratio can be used to identify cerebellar atrophy on MRI.

The proportions of the volumes and mid-sagittal CSA of the forebrain, brainstem, and cerebellum were quantified and compared in different breeds and ages of normal dogs and in dogs diagnosed with cerebellar degenerative disorders. Brain MRIs of normal dogs were obtained from the IAMs Pet Imaging Centers' database. Images of dogs diagnosed with cerebellar degenerative disorders were recruited from neurologists. All images were uploaded into the OsiriX DICOM viewer program. The CSA of the forebrain, brainstem, and cerebellum was manually traced from every T1 weighted axial image and the T2 weighted mid-sagittal image. Volumes of each brain region were calculated from the axial images by multiplying the CSA of each image by the slice thickness. Comparisons between groups were made using the student's t test.

Four groups of normal dogs aged from 5–10 years (Labrador Retrievers (n = 10), Beagles (n = 8), toy breeds (n = 10) and brachycephalics (n = 7)) were compared. The volumes of the different brain regions as well as total brain volumes were different between breeds, but the proportion of each region to the total brain volume was not significantly different. The same trend was observed when

comparing the midsagittal CSAs. Labrador Retrievers from three different age groups were compared to evaluate the effect of age on cerebellar size. There was no observed effect of age on the relative size and proportion of the cerebellum in these dogs. The ratio of the midsagittal CSA of the cerebellum to the CSA of the brainstem was consistent between different breeds and ages of dogs. This ratio was calculated in 15 normal and 22 American Staffordshire Terriers diagnosed with cerebellar degeneration and was significantly different between these two groups ($p < 0.0001$). The ratio was also calculated in an affected Scottish Terrier and an Old English Sheepdog and clearly identified cerebellar atrophy in both dogs. We conclude that the ratio between the cerebellar and brainstem midsagittal CSA is a useful indicator of cerebellar atrophy in dogs. CSA can readily be measured from MRI making this a rapid and accessible diagnostic test.

ABSTRACT #229

VALIDATION OF A GRADING SYSTEM FOR MENINGEAL ENHANCEMENT; 200 DOGS. V Penning[1], K Suckling[1], K Evans[1], K Chandler[1], R Cappello[1]. [1]Queen Mother Hospital for Animals, Royal Veterinary College, North Mymms, Hatfield, Herts, UK.

Pathological enhancement of the meninges on MRI following administration of an intravenous paramagnetic contrast agent most commonly reflects inflammatory, infectious or neoplastic processes. However apparent enhancement may also be seen due to oblique sectioning through dural vessels. Therefore in humans pachymeningeal enhancement of less than 3 cm in linear segments is considered normal, also the meninges covering the falx cerebri and tentorium cerebelli normally enhance in approximately 50% of humans.

The aim of the study was to use a modified from Quint et al. (1996) grading system for the patterns of gadolinium enhancement in the meninges in the normal canine brain, inflammatory central nervous system disease, structural malformation and neoplasia.

The grading system for pachymeningeal enhancement ranged from Grade 1 to 6 and for leptomeningeal enhancement ranged from Grade 0–5 The MRI was performed with a 1.5 tesla Philips Gyroscan NT Intera was used with a slice thickness of 3.5–4.5 mm with a 10% interslice gap. The meningeal covering of the cerebral convexities, the falx cerebri, the tentorium cerebelli, the cerebellum and the medulla oblongata were graded. Cytology and protein concentration were recorded on cerebrospinal fluid taken from the cisterna magna and a grade was assigned for cytology from 0–4 and for protein from 0–3).

Two hundred dogs were reviewed retrospectively. The signalment of the dogs, history, neurological localization, MRI and cerebrospinal fluid analysis were recorded. There were 105 males and 85 females. The age range was 3–180 months, mean 60 months. Eighty-two dogs were considered to be normal, 37 were diagnosed with inflammatory CNS disease and 20 with intracranial neoplasia. Sixty-one cases had structural malformations, infarctions, metabolic disease and spinal lesions. Statistical analysis using Kruskal Wallis with follow-up pair wise Mann Whitney U tests with the significance set at $P < 0.005$ was performed. The total pachymeningeal enhancement grade was significantly higher in the inflammatory group compared to the control group ($P = 0.00$). Leptomeningeal enhancement was significantly higher in the inflammatory compared to the neoplastic and control groups ($P = 0.00$). Increases in CSF white blood cell count (WBC) and protein correspond to increased meningeal enhancement grades when over the threshold of 20WBC/mm3 (cytology grade 3) and 50 mg/dl (protein grade 2) or when activated macrophages, plasma cells or leucocytes are present (cytology grade 2). Pachymeningeal enhancement occurred over the cerebral convexities and falx cerebri in over half the dogs with normal brains. Different patterns of meningeal enhancement are seen with pathological conditions. Further studies are required to confirm the differences, to confirm why some normal areas enhance and to describe the pathology that enables the meninges to enhance.

ABSTRACT #230

SUBACUTE NECROTIZING ENCEPHALOPATHY IN YORKSHIRE TERRIERS IS ASSOCIATED WITH A COMBINED RESPIRATORY CHAIN COMPLEX I AND IV DEFECT. A Fischer[1], K Baiker[2], S Hofmann[3], S Medl[4], W Schmahl[3], MF Bauer[3], K Matiasek[3]. [1]Section of Neurology, Department of Small Animal Medicine, Ludwig Maximilians University, Munich, Germany. [2]Chair of General Pathology & Neuropathology, Ludwig Maximilians University, Munich, Germany. [3]Institute of Diabetes Research, Academic Hospital Munich-Schwabing, Germany. [4]Small Animal Referral Clinic, Babenhausen, Germany. Institute of Clinical Chemistry & Molecular Diagnostics, Ludwigshafen City Hospital, Ludwigshafen, Germany. The Animal Health Trust, Newmarket, UK.

Subacute necrotizing encephalopathy (SNE) is a fatal neurodegenerative disorder in Yorkshire Terriers and Alaskan Huskies that reveals striking similarities to human Leigh- (LS) and Leigh-plus-MELAS syndromes. The resemblance, thereby, bases on morphological observations, whereas the molecular proof of a mitochondrial (mt) defect, known to underlie LS, still is pending in dogs. The present investigation, therefore, was aimed specifically to screen for a mitochondrial involvement in canine SNE.

Fresh muscle samples and brain tissue were available from two female Yorkshire Terriers, aged six and nine months. Muscle probes underwent spectrophotometric assessment of respiratory chain complexes (RCC) I through IV normalized for citrate synthase. Mitochondrial DNA was extracted from muscle and brain tissue and inspected for gene rearrangements via Southern blot analysis. Thereafter, mitochondrial tRNA-genes and ATP6-gene were amplified and sequenced. Levels of heteroplasmia were assessed through restriction fragment length polymorphism.

Both animals presented with a combined defect of RCC I and IV. Mitochondrial DNA analyses revealed a homoplasmic A-to-G transition at position 2691 within mitochondrial tRNA(UUR) gene. However, the same mutation was detected in 3 out of 43 healthy controls. Major gene rearrangements of mtDNA and ATP6-gene mutations were excluded.

This investigation, for the first time, lends biochemical proof to the suspected mitochondrial dysfunction in canine SNE. Evidence of combined RCC defects is very suggestive of a depressed mitochondrial protein synthesis rather than abnormal structural proteins or assembly factors. Thus, we sequenced all 22 mt-tRNAs and detected a polymorphism in mt-gene for the leucine-bearing tRNA(UUR). Even though this A2691G transition exactly resembles human MELAS-mutation, that causes LS amongst a plethora of other syndromic encephalopathies, it appears to be a neutral polymorphism in canine species. Apart from tRNAs all transcription elements in mitochondria are nuclear encoded. Further investigations of the genetic background of SNE, therefore, will have to address nuclear candidate genes that encode for transcription factors such as the elongation factor gene (EFG)-1.

ABSTRACT #231

IMMUNOPHENOTYPING OF GRANULOMATOUS MENINGOENCEPHALITIS IN 4 DOGS. KM Vernau, W Vernau, RA LeCouteur, RJ Higgins, BK Sturges, PJ Dickinson, DR Westworth, DK Naydan, PF Moore. School of Veterinary Medicine, University of California, Davis, California, CA.

Granulomatous meningoencephalomyelitis (GME) is a devastating idiopathic inflammatory disease affecting the CNS of dogs. Definitive diagnosis is based on characteristic histopathological findings. A previous study assessed formalin-fixed brain from dogs with GME using a restricted panel of reagents. Lesions consisted of a mixture of macrophages and lymphocytes, with a predominance of T and few B lymphocytes. The purpose of this study is to further elucidate the pathogenesis of GME by more fully characterizing the inflammatory cells in the brains of dogs with GME.

Four dogs with histopathologically confirmed GME were identified. A complete necropsy was done in all dogs. Based on MRI, sections of fresh brain lesions were frozen in OCT medium and adjacent sections immersion-fixed in 10% neutral buffered formalin. Immunophenotyping of inflammatory cell infiltrates was done on both formalin-fixed and adjacent fresh frozen brain sections, using panels of antibodies specific for canine leukocyte markers.

In all dogs, immunophenotyping demonstrated that perivascular cuffs in the parenchyma contained a mixed population of inflammatory cells, with lymphocytes, macrophages, dendritic cells and plasma cells predominating. B lymphocytes accounted for the ma-

jority of lymphocytes with lesser numbers of a mixture of CD4+ and CD8+ T lymphocytes. Macrophages and dendritic cells were activated as evidenced by upregulation of CD4 and CD80, and CD80 and CD86 expression respectively. In the brain parenchyma and meninges was a similar but less severe inflammatory infiltrate. These data suggest that a viral etiology is unlikely and that humoral immunity plays a major role in the pathogenesis of GME.

ABSTRACT #232
IDENTIFICATION OF NOVEL TARGETING PEPTIDES FOR CANINE GLIOMA. BK Sturges[1], PJ Dickinson[1], OH Aina[2], D York[1], RA LeCouteur[1], KS Lam[2]. [1]School of Veterinary Medicine, University of California, Davis, CA. [2]University of California, Davis, Cancer Center, Sacramento, CA.

Targeting of cancer cells using tumor specific peptides has many advantages over similar techniques using antibodies, including reduction in non-specific binding and immunogenicity and smaller size. Using screening of random, one-bead one-compound combinatorial libraries, peptides with specific binding to canine glioma cells (J3TBg) were identified *in vitro*. Similar peptides have been shown previously to bind to the α3 integrin subunit that plays an important role in tumor cell adhesion, invasion and metastasis. We hypothesized that the peptides would also bind specifically to experimental (J3TBg) gliomas *in vivo* and to spontaneous canine gliomas.

Using biotin-conjugated peptides and secondary labeling with streptavidin-Alexa-488 fluorochromes, *in vivo* binding of peptides to control (J3TBg) orthotopic tumors in nude mice and spontaneous canine gliomas was demonstrated on cryostat sections. Delivery of peptides in live animals was investigated using 6–8-week-old nude mice with established J3TBg xenograft tumors. Intravenous administration of peptides labeled with the fluorochrome Alexa-680 was done and tissue distribution was monitored at the near-infrared spectra using the Kodak IS2000MM image station. Mice were serially imaged at 15 minutes, 6 hours, and 24 hours for uptake and washout of peptide. The peptide probe displayed highly specific tumor uptake within 15 min and lasted for 24 hours. Some kidney and bladder signal also was noted at 15 min, but was not detectable at 24 hours.

Preferential binding of peptides to cell surface molecules of canine glioma cells may provide a rational system for the development of targeted therapies for both canine and human gliomas.

ABSTRACT #233
ALLOPREGNANOLONE THERAPY IN FELINE NIEMANN-PICK TYPE C. Charles H Vite[1], Wenge Ding[1], Caroline Bryan[1], Patricia O'Donnell[1], Karyn Cullen[1], David Aleman[1], Sergey Magnitsky[2], Harish Poptani[2], G. Diane Shelton[3], Mark Haskins[1], Tom van Winkle[1], Synthia Mellon[4], Steven Walkley[5]. [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. [2]School of Medicine, University of Pennsylvania, Philadelphia, PA. [3]School of Medicine, University of California, San Diego, La Jolla, California, CA. [4]University of California, San Francisco, California, CA. [5]Albert Einstein College of Medicine, Yeshiva University, Bronx, New York, NY.

The only large animal model of Niemann-Pick type C (NP-C) disease is a colony of cats housed at the School of Veterinary Medicine of the University of Pennsylvania. The disease in cats, due to a point mutation in the NPC1 gene, is a homologue of the disease in children with similar clinical, biochemical, and neuropathologic abnormalities. We have extended the natural history study of feline NP-C disease progression and successfully used allopregnanolone to partially treat neurologic and hepatic components of the disease. Statistically significant differences between age-matched NP-C and normal cats were identified in measures of weight gain, onset of neurological dysfunction, post-rotatory nystagmus, nerve conduction velocity, brain stem auditory evoked response testing, liver enzyme concentrations, chitotriosidase activity, nuclear magnetic resonance measures of the brain's gray and white matter, and brain, liver, lung, and peripheral nerve histology. All these differences serve as markers of disease and demonstrate that the feline model has been developed to a point where it is a significant resource for evaluating experimental therapies of NP-C disease.

We have treated 10 NP-C cats with allopregnanolone and have shown significant improvements in many of our measures of NP-C disease. Cats that began treatment with allopregnanolone after 3 weeks of age (AlloLate) showed significant improvements in tibial nerve conduction velocity, liver enzyme concentrations, chitotriosidase activity, and brain and liver pathology. However, there was no improvement in onset and severity of signs of neurologic dysfunction or in lifespan. A cohort of cats treated with allopregnanolone at 1, 3, 7, 14, and 21 days (AlloEarly) are currently being assessed to determine whether the earlier administration of allopregnanolone improves neurologic disease in an animal model other than mice. Data suggests that the early administration of allopregnanolone may delay the onset of neurological dysfunction in cats.

Our continuing goals are to use this well-characterized large animal model to assess the efficacy of experimental therapies of NP-C disease and to better understand NP-C disease pathophysiology.

ABSTRACT #234
EFFECT OF CYCLOSPORINE AND PREDNISONE TREATMENT IN GOLDEN RETRIEVER MUSCULAR DYSTROPHY. A. Uriarte, I. Barthelemy, J.-L. Thibaud, S. Blot. Ecole Veterinaire d'Alfort, France.

Duchenne Muscular Dystrophy (DMD) is a lethal X-linked disorder induced by a mutated dystrophin gene producing a deficient protein. A canine relevant homologous disease is termed Golden Retriever Muscular Dystrophy (GRMD). To date, no curative treatment exists although different therapeutic modalities such as drugs, gene and cell therapies have been developed to amend the dystrophic phenotypes. However several of these protocols require immunosuppressive regimens such as cyclosporine (CsA) combined with prednisone to prevent rejection of the cells or the transgene.

The aim of the study was to determine the effects of this regimen regarding the clinical evolution of dystrophic dogs, the muscle pathology as well as the muscle force.

Eight dogs were obtained from a GRMD colony at the Veterinary School of Alfort and were cared for according to the European Guide for Care and Use of Laboratory Animals: 4 GRMD dogs were treated since 2 months of age with CsA (20 mg/kg/d) and prednisone (2 mg/kg/d), and 4 GRMD dogs were untreated controls. A clinical locomotion status was evaluated once a month since 2 months of age until 9 months of age, using a specific scale based on the intensity of splaying the digits, palmigrade/plantigrade stance, stiffness of the gait, joint ankylosis, muscular contractures and ability to stand up and to get over a fence. At 6 and 9 months of age, a biopsy from both tibialis cranialis muscles was stained for HE and alizarin red and by immunohistochemistry to evaluate the distribution of slow and fast-twitch fibres. At 4 and 6 months of age, the maximal isometric force generated by tarsal flexor muscle of the tibialis cranialis compartment was evaluated by supramaximal percutaneous stimulation of the peroneal nerve. The average of 6 tetanic contractions was measured.

Our results show a similar evolution of the locomotion but a predominance of fast fibres, an increase of calcified myofibers and a lower force measurement for treated dogs.

In the literature, despite increases in calcified myofibers in GRMD dogs under prednisone treatment, functional benefit has been shown in DMD and GRMD. Additionally, administration of CsA (25 mg/kg/d) in mice induces a slow-to fast phenotype without affecting animal health or ambulation. However, fast fibres require dystrophin to produce force, whereas slow fibres seem not to. Fast fibres are also known to be less resistant to fatigue therefore unable to withstand sustained tetanic contractions. Collectively, these two conditions may contribute to the reduced force in CsA/prednisone treated GRMD dogs due to the observed predominance of fast dystrophic fibres.

Other effects of this immunosuppressive regimen were constant, such as papillomas, overweight and hepatic overload. In conclusion, our results indicate that CsA/prednisone treatment in GRMD dogs suggests possible detrimental consequences.

ABSTRACT #235
A NEW CHEMOTHERAPY PROTOCOL (DOSE INTENSIFYING SIMULTANEOUS CHEMOTHERAPY = DISC PROTOCOL)

FOR THE TREATMENT OF CANINE MALIGNANT LYMPHOMA: 21 CASES. I. Zenker[1], N. Eberle[2], M. Kessler[3], D. Simon[2], I. Nolte[2], K. Hartmann[1], J. Hirschberger[1]. [1]Clinic of Small Animal Medicine, Faculty of Veterinary Medicine, Ludwig Maximilian University of Munich, Munich, Germany. [2]Clinic of Small Animal Medicine and Surgery, University of Veterinary Medicine Hannover, Hannover, Germany. [3]Small Animal Clinic, Hofheim, Germany.

Drug dosage, administration interval, as well and the combination of drugs with different mechanisms of action are the mainstays of successful chemotherapy. The aim of this study was to record toxicity profile, remission rates, and individual dosage levels of a Dose Intensifying Simultaneous Chemotherapy (DISC) protocol for dogs with lymphoma. Twenty-one dogs with newly diagnosed and previously untreated lymphoma were included. Dogs were treated with a standardized protocol starting with L-asparaginase (day 0) followed by weekly simultaneous administration of vincristine, cyclophosphamide, and doxorubicin for a total of 12 treatments. Prednisolone was administered for 3 days and furosemide for 2 days with each simultaneous treatment. Dogs were treated at the same dosage level starting at 33% of the normal target dose of vincristine (0.7 mg/m^2), cyclophosphamide (200 mg/m^2), and doxorubicin (30 mg/m^2) each for 2 cycles. If a grade 1 or no toxicity occurred, the dosages were increased to the next dosage level. All dogs were treated with dosage escalations of 5–7%, starting at 33%. Response distribution in the 21 patients was as follows: complete remission 17/21 (81%); partial remission 1/21 (5%); stable disease 2/21 (9%); progressive disease 1/21 (5%). This equals an overall remission rate of 86% and is comparable to other combination chemotherapy protocols. Only 1 dog was withdrawn from the protocol due to toxicity, 2 due to lack of response, all 3 at dosage level 33%, and 1 dog was euthanized because of progressive disease at dosage level 40%. Two dogs died of causes unrelated to disease or treatment. Only 3 dogs experienced 1 episode of grade 4 toxicity each: 1 case of asymptomatic neutropenia (dosage level 45%), 1 of asymptomatic thrombocytopenia (dosage level 45%) and 1 septic episode in a diabetic dog (dosage level 33%). Other signs of toxicity were infrequent and mild (grade 1 and 2, rare asymptomatic grade 3 thrombocytopenia or anemia), with only 2 dogs requiring hospitalization for less than 48 hours. The highest dosage level of 60% was achieved in 1 dog. Four dogs reached 33%, 2 dogs 40%, 4 dogs 45%, 2 dogs 50% and 5 dogs 55% as the highest level. Highest dosage level is not yet reached in 2 dogs currently on the protocol. Median remission and survival times have not been reached at submission of this abstract. Toxicity events and remission rates of the DISC protocol are comparable to other polychemotherapy protocols. This protocol has shown a wide difference of individual dosage tolerance and further studies are needed to compare treatment outcome to that of established chemotherapy protocols.

ABSTRACT #236

EVALUATION OF OXIDANT/ANTIOXIDANT STATUS IN DOGS WITH MULTICENTRIC LYMPHOMA. SRR Lucas, MG Gimeno, CM Satsuki, VABF Wirthl. School of Veterinary Medicine and Animal Science – University of São Paulo, Brazil.

Oxidative stress is an imbalance between oxidant and antioxidant defense mechanisms in favor of the oxidants, leading to cellular damage. Under normal circunstances, oxidants serve a protective function by killing bacteria and tumor cells. However, they can have detrimental side effects and DNA oxidative damage can contribute to aging, mutagenesis and carcinogenesis. Lymphomas comprise one of the most common groups of tumors in dogs and can be used as a model for this disease in humans. Cancer patients are reported to have generalized oxidative stress and oxidative damage within tumor tissues. The aim of this study was to evaluate the oxidant and antioxidant status in dogs with multicentric lymphoma. Serum samples were obtained from 15 dogs with multicentric lymphoma, stage III to V, at the diagnosis, without previous treatment and 20 healthy dogs (control group). Oxidative status was measured by the analysis of thiobarbituric acid reactive substances (TBARS) expressed as malondyaldehyde (MDA). Total antioxidant status was measured by the use of a Randox TAS kit NX2332. Mean of MDA concentration was significantly higher in dogs with multicentric lymphoma at the diagnosis (2.96 µmol/L) than in healthy dogs (1.49 µmol/L). There was no difference in the total antioxidant status between the groups (0.965 and 0.957 mmol/L respectively).

In conclusion, dogs with multicentric lymphoma showed increase in the levels of oxidative substances but the antioxidant defense mechanisms seem to be deteriorated. Studies are necessary to confirm if this finding may contribute to prognosis and response to the treatment.

ABSTRACT #237

AUTOLOGOUS DENDRITIC CELL VACCINATION OF DOGS WITH ORAL MELANOMA USING HUMAN gp100 ANTIGEN. J. Paul Woods[1], Stephen Kruth[1], Maureen Barry[1], Steve Gyorffy[2], Juan Carlos Rodriguez-Lecompte[2], Jack Gauldie[2]. [1]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada. [2]Department of Pathology and Molecular Medicine, Center for Gene Therapeutics, McMaster University, Hamilton, Ontario, Canada.

Melanoma, the most common oropharyngeal cancer in dogs, is characterized by local invasion and early widespread metastases. Surgery and/or radiation therapy have been employed for local control; however, chemotherapy has not been successful at preventing systemic metastatic disease. Therefore, immunotherapy in the form of vaccines is being investigated for control of metastatic disease. Recent advances cloning tumour associated antigens (e.g. gp100) which are potential immunological targets and adoptive gene transfer techniques have led to new novel approaches to cancer therapy. The purpose of this prospective study was to assess the use of autologous dendritic cell vaccination using xenogenic human gp100 antigen for the treatment of canine oral melanoma.

Dogs presenting to the Veterinary Teaching Hospital of the Ontario Veterinary College with histologically confirmed oral melanoma and staging were entered into the study. Staging consisted of primary tumour size, mandibular lymph node aspirate or biopsy, and 3-view thoracic radiographs. Local disease was controlled by surgery or radiation. Dendritic cells were harvested (initially from bone marrow and later from peripheral blood), expanded ex vivo, transduced with a vector expressing a human xenoantigen (Ad-5-hgp100), and then vaccinated intradermally (3 vaccines q monthly).

Eleven dogs were entered consisting of 10 female (spayed), 1 male (neutered); mean and median age 10 years (range 4 to 17 years); various breeds, with WHO stage I (3 dogs), II (1 dog), and III (7 dogs). The overall median survival was 439 days (mean 613; range 130–2,204 days). By WHO stage the median survival was stage I (425 days), II (130 days), and III (439 days). No toxicity was observed in any of the dogs receiving the vaccines.

This study demonstrated the feasibility and safety of culturing canine dendritic cells and then transducing the dendritic cells with an adenovirus expressing a xenogenic tumour antigen for vaccinating dogs. Further studies will be needed to determine the efficacy of this immunotherapeutic approach for the treatment of oral melanoma in dogs.

ABSTRACT #238

IDENTIFICATION AND CORRELATION OF CYTOLOGIC CRITERIA TO HISTOLOGIC GRADE IN CANINE CUTANEOUS MAST CELL TUMORS. J Morrison[1], C Andreasen[2], H Flaherty[2], P Schmidt[3]. [1]Dept. Veterinary Clinical Sciences and [2]Dept. of Veterinary Pathology, Iowa St. Univ. College of Veterinary Medicine, Ames, IA. [3]Western University of Health Sciences, Pomona, CA.

Mast cell tumors are the most common cutaneous tumors recognized in dogs. Histologic grade has been considered one of the most valuable factors in determining prognosis. Cytologic evaluation of cutaneous tumors is relatively inexpensive, requires no specialized equipment, is minimally invasive and associated with little to no patient morbidity. The aim of this study was to identify cytologic criteria of mast cell tumors that correlate to their histologic grade using the Patnaik scale.

This retrospective study identified cases of mast cell tumors (n = 52) from computerized searches medical records and pathology reports. All cases were required to have at least one cytology slide of the tumor available for review. All samples were stained by clinical pathology laboratory personnel with Wright-Giemsa stain using a

standard protocol. Cytologic criteria evaluated (n = 14) included granule staining properties, predominant nuclear shape, presence and prominence of nucleoli, nuclear to cytoplasmic ratio, cytoplasmic border, cellular granule percentage, multinucleated cells, binucleated cells, mitotic figures, nuclear size, red blood cells, neutrophils, eosinophils, and overall cell size. Cytologic evaluation was performed without knowledge of histologic grade. Kendal-Tau non-parametric analysis was performed on the initial data set and the value for statistical significance was set at $p < 0.1$. Logistic regression was performed on those cytologic criteria identified as significant from the initial statistical analysis.

Multiple slides were available in 39/52 cases, so 132 total slides were reviewed. Direct smear (n = 80) and cytospin (n = 52) samples were included. Histopathology results were available for 26/52 cases. Twenty of the 26 cases had multiple cytology slides, giving a total of 64 histologic grades.

Four cytologic factors were determined to be significantly related to histologic grade in the initial analysis: presence of multinucleated cells [Kendal-Tau value = 0.42, p-value = 0.04], mitotic index [Kendal-Tau value = 0.39, p-value = 0.06], large cell size [Kendal-Tau value = 0.31, p-value = 0.09], and nuclear shape [Kendal-Tau value = 0.45, p-value = 0.03]. Logistic regression was performed and 3 cytologic factors were determined to be statistically significant: presence of multinucleated cells (p-value = 0.02), large cell size (p-value = 0.07), and nuclear shape (p-value = 0.06). All statistically significant results were obtained from cytospin samples.

Prognostic information obtained from cytology samples could significantly decrease patient morbidity since surgical planning and adjunctive therapy could be more appropriately tailored to the patient.

ABSTRACT #239

LYMPHATIC STAGING AND SENTINEL LYMPH NODE IDENTIFICATION IN CANINE ORAL MALIGNANT MELANOMA.

Deanna R. Worley, Alexander M. Reiter, David E. Holt, Dorothy Cimino Brown, Carrie Tupper, Tiffany Scanlon, Thomas Van Winkle, John R. Lewis. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

Assessment of tumor response to therapy is more accurate with standardized regional lymph node dissection and staging. Study purposes included investigating oral malignant melanoma drainage patterns via removal of ipsilateral mandibular, parotid, and retropharyngeal lymph nodes; identifying potential sentinel lymph node(s) via lymphatic mapping with concurrent isosulfan blue injection; and determining if sentinel lymph node mapping can be incorporated into routine management of oral malignant melanomas.

Patients presenting to the University of Pennsylvania for staging and resection of oral malignant melanomas were prospectively enrolled in this pilot study. All patients received routine pre-surgical staging. Isosulfan blue dye was injected peritumoral and regional lymph node dissection was done through a single incision. Comparisons were made between preoperative fine needle aspiration and histopathology, lymph node size and metastasis, and identifiable sentinel lymph nodes and metastatic nodes.

In the initial three patients enrolled to date, minimal post surgical swelling was the main complication noted. Nodal metastases were not identified histopathologically *ex vivo*, though a malignant mandibular node not present during regional lymph node dissection was identified two weeks postoperatively. Visual uptake of blue dye was seen in two mandibular lymph nodes which correlated with cytopathologic findings.

Identification of the sentinel lymph node is challenging. An ideal volume of dye to use and injection time prior to surgery are yet to be determined from this study. Further investigation is needed to determine if excisional biopsy of all potential regional lymph nodes can be minimized to a sentinel node(s).

Originally presented at the Veterinary Cancer Society Annual Conference, Ft. Lauderdale, FL, November 2007.

ABSTRACT #240

A RETROSPECTIVE SURVEY OF NEUTROPENIA IN A FELINE REFERRAL POPULATION IN THE UK.

A Hibbert, KV Tennant, SM Cue, MJ Day, AM Harvey. The Feline Centre, University of Bristol, Bristol, UK.

Neutrophils play an essential role in the host's innate immune response to pathogenic organisms and inflammation. Neutropenia may result from decreased production, increased consumption or destruction of neutrophils. The aim of this study was to retrospectively evaluate the aetiology of neutropenia in a population of feline patients in a UK referral hospital.

Forty-two cats presenting to the University of Bristol Feline Centre between January 2002 and May 2007 were identified. The age of the cats ranged from 5 months to 13 years (mean 5.1 years). Twenty-three cats were domestic shorthaired, 5 were domestic longhaired and 14 were pedigree cats (7 breeds). Twenty-three cats were male neutered, 4 were male entire and 15 were female neutered.

Neutropenia was defined as a neutrophil count $< 2.4 \times 10^9$/l (reference range 2.4–12.5 $\times$ 10^9/l). The initial neutrophil count ranged from 0.03–2.33 $\times$ 10^9/l (mean 1.29 $\times$ 10^9/l). Band neutrophils were identified in six cases (range 0.02–0.11 $\times$ 10^9/l) and toxic changes in two cases (both with toxic granulation and Dohlë bodies). Bicytopenia was present in 18 patients (neutropenia and anemia in 15 cats, neutropenia and thrombocytopenia in three cats), and two cases were pancytopenic. At presentation 13 cats were hyperthermic (rectal temperature $> 39.2\,^{\circ}C$) and one was hypothermic (rectal temperature 37.1 °C).

The aetiology of neutropenia was classified as bone marrow-related disease in 21.4% of the cases (neoplasia [5], myelodysplasia [1], maturation arrest [2] and aplastic anaemia [1]), infectious in 16.7% (FeLV [3], FIV [2], FIP [1] and FHV-1 [1]), increased demand in 16.7% (inflammatory process [6] and sepsis [1]), drug-related in 14.3% (chemotherapy induced myelosuppression [6]), immune-mediated disease in 19% (associated with IMHA [3], idiopathic neutropenia [2]) and was due to an undetermined aetiology in 19% of the cases (8 cats).

The most common class was bone marrow-related disease. Immune-mediated disease was diagnosed least frequently, within this group two cases of idiopathic neutropenia were identified; both cases were successfully treated with prednisolone (initial dose 1 mg/kg per os BID) and were alive 21 and 24 months later. Neutropenia may be a manifestation of many disease processes; evaluation of drug history and examination for infectious, inflammatory, septic and immune-mediated disease should be made prior to bone marrow analysis to enable a diagnosis.

ABSTRACT #241

PLATELETWORKS®: A BEDSIDE TEST TO MONITOR CLOPIDOGREL THERAPY IN HEALTHY CATS.

A Hamel-Jolette, M Dunn, C Bédard. Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada.

Plateletworks® is a bedside test used in human medicine to monitor platelet inhibiting drugs. As arterial thromboembolism is a common complication in cats suffering from cardiomyopathy, they are often treated with anti-platelet medication. Monitoring is may be important in such situations.

The purpose of this prospective study was to determine if the Plateletworks method can detect a decrease in platelet aggregation in cats receiving clopidogrel, an anti aggregating drug.

Nine sterilised healthy adult cats were used for this study. On day 1, a 2 ml venous blood sample was collected from the jugular vein. Immediately after blood collection, 1 ml was placed in the Plateletworks baseline tube (EDTA), 1 ml was placed in the Plateletworks ADP tube and a blood smear was prepared. Tubes were analyzed using a hematology impedance cell counter to determine platelet counts. Immediately following blood sampling, 18.75 mg of clopidogrel was administered orally to each cat once a day for three days. Venipuncture and blood analysis were repeated on day 4, in the same manner as on day 1. The percent platelet aggregation (PPA) was then calculated as follows:

$$\frac{\text{Baseline platelet count} - \text{ADP platelet count}}{\text{Baseline platelet count}} \times 100 = \text{PPA}$$

PPA on day 1 (mean $\pm$ SD) was calculated as 70.55 $\pm$ 15.59% and on day 4 was 1.52 $\pm$ 9.5%. Clopidogrel therapy caused a marked decrease in PPA ($P < 0.01$).

The Plateletworks method appears to be a promising test to monitor clopidogrel therapy in cats.

ABSTRACT #242
THROMBOELASTOGRAPHY IN HEALTHY CATS. A Montgomery, CG Couto, K Schober, P Vilar Saavedra, N Westendorf, MC Iazbik. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Thromboembolic diseases are common in cats with hypertrophic cardiomyopathy (HCM), but they are difficult to diagnose using conventional laboratory methods. Moreover, cats with HCM are usually placed on anticoagulants or antiplatelet agents to prevent thrombosis, but again we lack an adequate means of monitoring treatment efficacy using traditional methodology.

The thrombelastograph (TEG®) allows for a global evaluation of the hemostatic system; while conventional coagulation tests typically evaluate only one part of the coagulation system, the TEG simultaneously examines the interaction between platelets, clotting factors, the fibrinolytic system, and clot retraction mechanisms. Since its inception, the TEG has been widely employed in human clinical medicine and research, only recently gaining popularity in veterinary medicine.

To our knowledge, reference values for citrated native TEG in cats have not been reported in the literature. The purpose of this study was to establish TEG reference ranges in healthy cats using the whole blood citrated native technique. We sampled 28 clinically healthy adult cats based on normal PE, CBC, and hemostasis profile results and with no previous history of bleeding disorders. The sample population ranged in age from 1 to 7 years old; 14 cats were castrated males and 14 cats were spayed females. Samples were obtained in non-sedated animals via medial saphenous venipuncture using a 21 gauge butterfly catheter. A 3 ml sterile syringe prepared with 0.3 ml of 3.2% buffered sodium citrate anticoagulant was used for blood collection and samples were stabilized for 30–40 minutes at room temperature prior to being re-calcified and analyzed in the TEG according to the manufacturer's instructions. We obtained the following values (mean ± SD): R = 6.104 ± 3.646 min; K = 4.21 ± 3.21 min; angle = 47.92 ± 14.51 deg; MA = 48.06 ± 10.02 mm; G = 4974 ± 1919 d/sc; CL60 = 83.95 ± 14.43%, and LY60 = 7.86 ± 10.21%. Interestingly, there were two distinct patterns of clot maintenance, one with minimal LY60 (0.2 to 3%), which is comparable to that seen in dogs and horses, and another with marked increases in LY60 (>7%), which we suspect is due to platelet retraction mechanisms.

ABSTRACT #243
EFFECTS OF HEMATOCRIT ON THROMBOELASTOGRAPHY TRACINGS IN DOGS. P Vilar, J Hansell, N Westendorf, MC Iazbik, L Marín, and C. G Couto. The Ohio State University, Veterinary Clinical Sciences, Columbus, OH.

The main advantage of the TEG compared to other conventional tests of hemostasis (e.g., bleeding time [BT], prothrombin time [PT], activated partial thromboplastin time [aPTT], D-dimer) is the ability to assess *ex vivo* using a whole blood sample, most of the *in vivo* components that play a role in the formation of the hemostatic plug (i.e., red blood cells [RBCs], white blood cells, platelets, clotting factors, fibrinolytic proteins). Since RBCs are the major component of blood, the hematocrit (HCT) is the main factor that influences blood viscosity. Flow and/or rheological conditions of the blood affect coagulation (Kretschmer et al. 2004); and, by definition, TEG evaluates the viscoelastic properties of the clot. Therefore, our objective was to evaluate the effects of HCT on TEG parameters in order to correctly interpret the TEG tracings in patients with high or low HCT values (e.g., blood loss anemia, DIC, Greyhounds). Nine healthy dogs based on results of physical examination, complete blood count (CBC), clotting times (aPTT, PT), fibrinogen concentration, and TEG, with no previous history of bleeding disorders were included in the study. Blood samples were collected by jugular venipuncture using a butterfly collection set, and aliquoted into three 2.7 ml Vacutainer 1:9 collection tubes containing 3.2% buffered sodium citrate. Two citrate tubes were centrifuged immediately to obtain platelet rich plasma (PRP) used to serially dilute the pure red blood cell samples (PRBC's) to obtain final HCT values of 60%, 40%, and 20% in each dog; the platelet counts were maintained within the reference range ($130–450 \times 10^9$ u/L) by using PRP. A single recalcified (20 μL$CaCl_2$) citrated native TEG test per sample was performed. Five specific TEG parameters were compared: The "*R*" is the time from addition of the agonist ($CaCl_2$) until the clot starts forming; "*K*" or clot kinetics; angle or "α", is related to the fibrinogen concentration and fibrin build-up and cross-linking; "*MA*" or ultimate strength of the clot formation and finally "*G*" represents the elastic shear of the clot. The Pearson's correlation coefficients (r) for HCT and TEG parameters were "R"= 0.85; "K"= 0.24; angle = −0.99; MA = −0.99; and G = −0.81.

In conclusion, HCT showed a strong correlation with some of the TEG parameters when PLT count was within the normal limits. Therefore, HCT values should be addressed in order to obtain a correct interpretation of the TEG tracings in dogs.

ABSTRACT #244
RAPID DIAGNOSIS OF ANTIVITAMIN K INTOXICATION IN DOGS AND CATS. C. Hugnet[1], X. Pineau[2], F. Buronfosse[2], S. Queffélec[2]. [1]Clinique vétérinaire des Lavandes, La Bégude de Mazenc, France, [2]French Animal Poison Control Center (C.N.I.T.V.), Ecole Nationale Vétérinaire de Lyon, Marcy L'Etoile, France.

Antivitamin K (AVK) toxicity is a common problem in companion animal medicine. The vitamin K-dependent coagulation factors II, VII, IX and X are altered by AVK, inducing clotting disorders. Main clinical symptoms are cough, lameness, hematoma, hematuria, melena. Increased One Stage Prothrombin Time (OSPT) and Activated Partial Thromboplastin Time (APTT) are main biological alterations, but these modifications are not pathognomonic.

In France, emergency treatment consists in intravenous injection of vitamin K1 (Vitamine K1 injectable TVM®). Contrary to other formulations, this product doesn't contain cremophor as excipient, and injection-associated shock is exceptional.

We studied effects of IV administration of vitamine K1 (5 mg/kg) on OSPT and APTT kinetics during AVK intoxication and others clotting disorders (i.e. DIC, ophidian envenomation, angiostrongyliasis, Von Willebrand disease, hepatopathy and so on). We used an in-house analyzer (SCA 2000, Idexx Laboratories), with fresh blood samples collected from jugular vein.

We observed a rapid return to normal value, within 20 minutes after injection of vitamin K1, of OSPT and APTT in all suspected AVK intoxications in dogs (n = 78) and cats (n = 31). On the other hand, we didn't observe any significant influence of vitamin K injection on OSPT and APTT in other coagulation disorders (45 dogs and 18 cats).

With this simple test, veterinary practitioners can now confirm a diagnosis of AVK intoxication in 20 minutes.

ABSTRACT #245
SEQUENCING AND PARTIAL CHARACTERIZATION OF FELINE MYELOPEROXIDASE. Pressler BM, Anderson KA. Purdue University, West Lafayette, IN.

Myeloperoxidase (MPO) is a myelomonocytic granule protein responsible for generation of oxygen radicals, hydrochlorous acid, and other toxic metabolites during the respiratory burst. In addition to this microbicidal function, plasma MPO concentration is a predictor of myocardial infarction in people with chest pain, and is an autoantigen in anti-neutrophil cytoplasmic autoantibody-associated diseases. MPO has not been investigated in cats with disease as of yet, but healthy cats given propylthiouracil develop anti-MPO antibodies and a multisystemic auto-immune syndrome highly similar to a subset of hyperthyroid people given the same drug. The purpose of this study was to sequence the feline MPO gene, partially characterize the mature granulocytic protein, and compare it to the human homologue in preparation for future studies.

Total mRNA was isolated from bone marrow collected from a random-source euthanized cat using a commercial kit. cDNA was produced using both oligo(dT) and feline MPO gene-specific primers designed using published human MPO primers, but modified using the NCBI-reported feline genome. PCR resulted in six overlapping sequences which spanned the total feline MPO cDNA sequence. Sequencing of these fragments revealed a 2165 base pair coding DNA sequence with 85% homology to the human MPO sequence. Comparison of the gene to the partially sequenced feline genome shows the RNA length to be divided into a minimum of 12

exons, with 158 bp lying within a thus far non-sequenced section of the genome. The final MPO coding DNA sequence differed from the reported genome at 8 bps (1 bp deletion and 7 bp substitutions). Comparison of the predicted feline MPO amino acid sequence with the published human MPO protein supports that these bp changes are due to errors in the NCBI-reported genome rather than being single nucleotide polymorphisms or silent mutations.

Feline granulocytes were isolated from peripheral blood obtained from a healthy donor using differential centrifugation and mounted on glass slides using standard cytospin techniques. Immunofluorescent antibody staining with a FITC-conjugated anti-human MPO antibody (Dako) revealed cytoplasmic granular fluorescence similar to that seen with staining of human MPO-expressing granulocytes. MPO was presumptively isolated from the same feline donor following granulocyte isolation, cell lysis, and concentration using a concanavalin A-Sepharose column. Elution fractions were tested for peroxidase activity using the Bradley method, and the highest-activity fractions were analyzed by SDS-PAGE and Western immunoblotting. After staining with anti-human MPO antibody, 55 and 12 kD bands were detected, consistent with the molecular weights of the MPO heavy and light chains predicted by translation of the coding DNA sequence.

We were able to sequence and partially characterize the feline MPO gene and protein, and demonstrated that the feline and human DNA and amino acid sequences are significantly homologous. Knowledge of the MPO sequence will facilitate future investigations on the role of MPO in feline disease, including the suitability of human MPO in any assays developed.

ABSTRACT #246

INHERITED FACTOR VII DEFICIENCY IN BEAGLES AND MINIATURE SCHNAUZERS IN VICTORIA, AUSTRALIA AND THE USE OF REML ANALYSIS TO DETERMINE HERITABILITY OF THE DEFECT. SM Lillis, JA Charles, LC Hygate, GA Anderson and BW Parry. Faculty of Veterinary Science, The University of Melbourne, Werribee, VIC, Australia.

Inherited factor VII (FVII) deficiency is a disorder of haemostasis characterised by infrequent bleeding episodes in affected dogs. It has been documented in North America, the United Kingdom and Japan in Beagles, Miniature Schnauzers, Alaskan Malamutes and cross-bred dogs. There has been one reported case in an Alaskan Malamute in Australia.

The occurrence of inherited FVII deficiency was investigated in clinically healthy dogs in Victoria, Australia. Coagulation results of 76 Miniature Schnauzers, 53 Beagles, 44 Labrador Retrievers and 27 Greyhounds were analysed, with the Labradors and Greyhounds being used as control animals for comparison with the Beagle and Miniature Schnauzer groups.

A prothrombin time (PT) and FVII assay was performed in each animal. A dog was considered to be affected by FVII deficiency if its FVII activity was below 50% of that of a control plasma pool derived from healthy dogs of mixed breed, age and gender. A PT greater than 1.25 times that of the control plasma pool was considered to be abnormal.

There were statistically significant differences in the proportions of Beagles (15/53) and Miniature Schnauzers (13/76) with low FVII activity compared with Labrador Retrievers (2/44) or Greyhounds (0/27). There was no effect of age on FVII activity in any breed tested. Desexed Beagles had significantly higher FVII activity than entire Beagles. Twelve of the 13 Miniature Schnauzers and 8 of the 15 Beagles with low FVII activity had abnormal prolongation of the PT.

Analyses of three-generation pedigrees of 69 of the 76 Miniature Schnauzers were conducted. Heritability was determined by restricted maximum likelihood (REML) analysis using an animal model. Estimated breeding values (EBV) for the defect were calculated using a Best Linear Unbiased Prediction (BLUP) method for each of the 422 miniature schnauzers represented in the pedigrees.

A high heritability of 0.66 (SE $\pm$ 0.256) was calculated for the defect in the Miniature Schnauzers. There were insufficient pedigree data to perform comparable heritability studies in the beagle group.

These findings confirm the presence of inherited FVII deficiency in Beagles and Miniature Schnauzers in Australia. The EBV calculated will facilitate future implementation of a breeding program to eliminate or reduce the prevalence of the defect in the Miniature Schnauzer breed.

ABSTRACT #247

COMPARISON OF GEL COLUMN, CARD AND CARTRIDGE TECHNIQUES FOR DEA 1.1 BLOOD TYPING OF DOGS. Mayank Seth, Sarah Winzelberg, Karen V Jackson, Urs Giger. Section of Medical Genetics, University of Pennsylvania, Philadelphia, PA.

While many blood group systems have been described in dogs, the Dog Erythrocyte Antigen (DEA) 1 blood group with the DEA 1.1 type is generally considered clinically most important. Although naturally-occurring DEA 1.1 alloantibodies are not found, sensitization of a DEA 1.1 negative dog is rapidly elicited with transfusion of DEA 1.1 positive blood and is cause for serious hemolytic reactions with subsequent DEA 1.1 mismatched transfusions. Accordingly, DEA 1.1 blood typing of donor and patient prior to transfusion is recommended. Several DEA 1.1 typing techniques have recently been introduced using monoclonal antibodies as well as polyclonal serum from sensitized dogs. Here we report on a comparative blood typing study in dogs regarding their accuracy and ease of use.

We compared 3 commercially available DEA 1.1 typing assays: a gel column diffusion assay (GEL; DiaMed, Switzerland), a card-based agglutination test (CARD; DMS Laboratories, NJ), and an immunochromatographic cartridge (CHROM; Alvedia, France); the GEL and CHROM methods use the same monoclonal antibody. A polyclonal tube typing method (LAB; Midwest Animal Blood Services, MI) was used on 7 samples, when discrepancies were noted, and GEL and LAB typing results were identical. All assays were performed according to manufacturer's instructions. GEL and CARD reactions were graded from 0 to 4+ with $\geq$2+ being considered a positive reaction. Definitive DEA 1.1 status was based on agreement between at least 2 typing methodologies.

Blood typing was performed on EDTA samples from 52 healthy potential blood donors and 39 sick dogs. Due to persistent autoagglutination 3 dogs could not be definitively typed by at least 2 methods; interestingly the CHROM test gave a DEA 1.1 negative result in all 3 samples. Of the remaining 88 dogs, 45% were typed as DEA 1.1 negative and 55% as DEA 1.1 positive by the GEL test technique and at least one other method. Identical typing results were obtained in 84% of cases by all methods. With the CHROM test 6 samples from DEA 1.1 positive dogs gave no appreciable DEA 1.1 banding. With the CARD test $\geq$2+ agglutination was noted in samples from 5 DEA 1.1 negative dogs and $\leq$ 1+ agglutination in 3 DEA 1.1 positive dogs (plus 1 inconclusive result due to autoagglutination). In total 8 CARD reactions were graded as fine or 1+ agglutination, of which 5 were DEA 1.1 negative and 3 were DEA 1.1 positive.

We conclude that the DEA 1.1 GEL test is a simple, accurate typing method to screen dogs in the laboratory. Moreover, there is generally good agreement between laboratory and in-clinic DEA 1.1 typing methodologies, when performed by an experienced individual. Rare false negative results are observed with the CHROM assay, whereas the CARD test produces some false positive and false negative results. The results of GEL and CHROM are most easy to interpret and archive.

ABSTRACT #248

USE OF UNFRACTIONATED HEPARIN (UH) VERSUS LOW MOLECULAR WEIGHT HEPARIN (LMWH) IN CRITICALLY ILL DOGS. C Thorneloe, C Bedard, S Boysen, M Dunn. Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada.

LMWHs exert their anticoagulant activity by binding to antithrombin III and preferentially inhibiting factor X. This results in inhibition of hemostasis with minimal prolongation of PT and aPTT. These characteristics make LMWHs an interesting therapeutic alternative to UH.

The goals of this prospective randomized blinded clinical study were to 1) compare the efficacy of LMWH (dalteparin) and UH in critically ill dogs using antiXa activity and 2) demonstrate that at a fixed dosage, LMWH has a predictable effect on coagulation and does not prolong clotting times.

Dogs were assigned randomly to one of two groups. Group 1 received LMWH 150 U/kg SC TID and group 2 received UH 200 U/

kg SC TID for 48 hours. For each dog, the following parameters were measured anti-Xa activity, PT, aPTT and ATIII on day one at baseline (T0) and at 3, 8 and 24 hours and on day two at 3 and 8 hours.

Fifteen dogs were enrolled in the study; 7 received LMWH and 8 received UH. All dogs receiving LMWH had anti-Xa activity within or above the target range (0.4–1.0 U/ml) on day 1 T3 and 8. None of the dogs receiving UH had anti-Xa activity within the target range on day 1 T3 and 8. ATIII levels were similar in both groups. No prolongation in PT or aPTT was noted except for one dog in the LMWH group.

Unlike UH, LMWH yielded predictable antiXa activities in critically ill dogs, was well tolerated and in general did not result in prolonged clotting times.

ABSTRACT #249
THE EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON CANINE HEMOSTASIS AND SYSTEMIC PROSTAGLANDIN LEVELS. SL Blois, DG Allen, RD Wood, PD Conlon. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in veterinary medicine to provide analgesic and anti-inflammatory benefits to patients. The adverse effects associated with NSAID use are believed to be largely due to inhibition of the enzyme cyclooxygenase (COX)-1. As such, COX-2-selective NSAIDs were developed in attempt to limit the development of NSAID-associated adverse effects. Recent reports in the human medical literature have suggested an increased incidence of thromboembolic events associated with the use of COX-2 selective NSAIDs. There is speculation that COX-2 selective NSAIDs may lead to an imbalance in cardiovascular prostaglandin levels, with a relative increase in thromboxane versus prostacyclin. Thromboxane promotes platelet aggregation and vasoconstriction, while prostacyclin counteracts these effects.

This study examined the effects of NSAIDs on hemostasis and prostaglandin levels in healthy dogs. Ten dogs were given four NSAIDs and one placebo in a cross-over design at dosages consistent with current therapeutic recommendations. The NSAIDs administered included aspirin, carprofen, deracoxib, and meloxicam. Parameters measured before and after 7 days of NSAID administration included platelet optical aggregometry, platelet function analysis (using the platelet function analyser, PFA-100, machine), and plasma thromboxane and prostacyclin levels.

Administration of NSAIDs did not cause a significant effect on platelet function measured by the PFA-100. Maximal platelet aggregation declined mildly after deracoxib administration. Rate of platelet aggregation was not affected by NSAID administration. Plasma thromboxane levels decreased after aspirin administration compared to levels after deracoxib administration, while NSAID administration did not affect plasma prostacyclin levels. The ratio of thromboxane to prostacyclin was not altered by NSAID administration. One-stage prothrombin time (OSPT), activated partial thromboplastin time (aPTT), and fibrinogen concentration were not affected by NSAID administration.

This study showed that treatment with COX-2 selective NSAIDs in healthy dogs did not result in increased platelet function or an imbalance in plasma thromboxane and prostacyclin levels at the tested dosages.

ABSTRACT #250
CLINICAL AND CLINICOPATHOLOGIC EFFECTS OF PLATELETPHERESIS ON HEALTHY DONOR DOGS. MB Callan, EH Appleman, FS Shofer, NJ Mason, BM Brainard, RP Groman. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Plateletpheresis allows highly efficient collection of large numbers of platelets from donor dogs that can be used for the treatment of life-threatening bleeding due to severe thrombocytopenia or thrombopathia. The advantages of a platelet concentrate prepared by apheresis in comparison to the standard platelet-rich plasma/platelet concentrate prepared from a unit of fresh whole blood are greater platelet yield (3 to 4.5×10^{11} vs 1×10^{11}) and negligible red blood cell contamination. The purpose of this study was to determine if plateletpheresis using the COBE Spectra is a safe, feasible, and efficacious method for collecting platelets from dogs weighing ~20 kg and to document the clinical and clinicopathologic effects of plateletpheresis.

Plateletpheresis was performed on 14 adult healthy mixed breed dogs weighing 18 to 27.7 kg (mean 22.8 kg). Approximate target values for the collections were total platelet yield 3×10^{11}, collect volume 220 mL, and run time of 90–110 minutes. CBCs were obtained from donors at baseline, 2 hours post-apheresis, and daily for 1 week. Blood was collected at baseline and every 15 minutes during plateletpheresis for assessment of electrolytes and acid-base status (NOVA CCX) and serum citrate concentration. Dogs were monitored by continuous ECG and indirect blood pressure measurement every 5 minutes. All dogs received 10% calcium gluconate as a constant rate infusion (CRI), with rate adjusted as needed based on serial measurements of $[Ca^{2+}]$.

A high quality platelet product was safely collected from all 14 dogs, with total yields ranging from 2.7 to 4.6×10^{11} platelets in a plasma volume of 220 to 270 mL. Mean donor platelet count was decreased by > 50% 2 hours post-apheresis but returned to baseline level by day 6. There was no difference between mean HCT pre- and post-apheresis. The procedure was generally well tolerated, with no evidence of hypotension. Serum citrate concentrations progressively increased, causing blood $[Ca^{2+}]$ to decrease to < 1 mmol/L in 10 dogs despite calcium supplementation. Lip licking was noted in 3 dogs, and generalized tremors and ventricular ectopy were noted in 1 dog. Two hours following apheresis and discontinuation of calcium CRI, blood $[Ca^{2+}]$ had returned to baseline levels, although $[Mg^{2+}]$ and serum citrate concentrations remained below and above baseline levels, respectively.

The COBE Spectra may be safely utilized for plateletpheresis in dogs weighing approximately 20 kg. Due to the large amount of citrate infused to the donor, calcium supplementation with serial monitoring of blood $[Ca^{2+}]$ is recommended to limit clinical signs of hypocalcemia during the procedure.

ABSTRACT #251
EFFECTS OF ANTICOAGULANT ON pH, IONIZED CALCIUM CONCENTRATION, AND PLATELET AGGREGATION IN CANINE PLATELET-RICH PLASMA. MB Callan[1], FS Shofer[1], JL Catalfamo[2]. [1]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA. [2]Cornell University, College of Veterinary Medicine, Ithaca, NY.

In vitro platelet aggregation studies may be part of the diagnostic evaluation of a patient with suspected thrombopathia or the quality control of canine platelet transfusion products, such as apheresis platelet concentrates and cryopreserved platelets. While 3.8% sodium citrate (CIT) is the most commonly used anticoagulant for diagnostic studies, anticoagulant acid-citrate-dextrose (ACD-A) is the choice for collection of platelets by apheresis and for storage. The purpose of this study was to compare the effects of CIT and ACD-A, on pH, extracellular ionized calcium concentration $[Ca^{2+}]$, and platelet aggregation in canine platelet-rich plasma (PRP).

Blood was collected from 12 healthy mixed breed dogs into both CIT and ACD-A. The pH and $[Ca^{2+}]$ of PRP were measured by a NOVA, and platelet aggregation was assessed by optical aggregometry. Platelet agonists (final concentration) evaluated were ADP (20 μM), γ-thrombin (100 nM), and convulxin (20 nM). Washed platelets were used to evaluate the effects of pH and $[Ca^{2+}]$. Amplitude (%) and slope of aggregation were recorded.

CIT PRP was alkaline (mean pH 7.56), whereas ACD-A PRP was acidic (mean pH 7.05). The $[Ca^{2+}]$ was significantly greater in CIT (mean, 0.224 mmol/L) than in ACD-A PRP (mean, 0.188 mmol/L). ADP-induced platelet aggregation was markedly diminished in ACD-A (mean, 3%) compared with CIT (mean, 54%). Platelets collected in ACD-A from all dogs responded to γ-thrombin with only platelet shape change in contrast to platelets in CIT, which responded with shape change and aggregation (mean, 66%). Anticoagulant did not have an effect on convulxin-induced

platelet aggregation (mean, ACD-A 65% vs. CIT 69%); however, the slope was significantly less in ACD-A than in CIT.

In washed platelet suspensions at pH 7.4, there were no differences in amplitude of convulxin- or γ-thrombin-induced aggregation at various calcium concentrations, although the slope was greater with increasing $[Ca^{2+}]$ in response to γ-thrombin. Varying pH had no effect on amplitude of aggregation induced by convulxin or γ-thrombin; however, using a lower concentration of γ-thrombin, the amplitude was significantly less at pH 7.0 (mean 40%) than at pH 7.4 (mean 64%). The slope of aggregation increased with rising pH with both agonists.

Canine platelet aggregation induced by ADP and γ-thrombin was negligible in ACD-A PRP, suggesting that increased extraplatelet $[H^+]$ inhibits signaling triggered by these agonists but not by convulxin. The choice of anticoagulant may influence *in vitro* platelet function studies, leading to erroneous conclusions.

ABSTRACT #252

EVALUATION OF CANINE PLATELET CRYOPRESERVATION METHODS. EH Appleman[1], BS Sachais[2], R Patel[1], KJ Drobatz[1], RP Groman[1], D Kennedy[1], P O'Donnell[1], C Bryan[1], MB Callan[1]. University of Pennsylvania, [1]School of Veterinary Medicine and [2]School of Medicine, Philadelphia, PA.

Platelet cryopreservation allows long-term storage and immediate availability of platelet products for transfusion. The aims of this study were 1) to compare two methods of canine platelet cryopreservation, the standard rapid freeze (−80 °C) in 6% DMSO (DMSO) vs. 2% DMSO plus Thrombosol (Thrombosol - a mixture of second-messenger effectors that may lessen platelet freezing damage); and 2) to determine if either cryopreservation method produces a platelet product with acceptable function and survival as compared to fresh platelets.

Platelet concentrates (PC), containing $\sim 3 \times 10^{11}$ platelets in ~ 220 ml plasma, were collected via apheresis from 10 healthy research dogs. Each PC was split into 3 units: fresh and cryopreserved in DMSO and Thrombosol. *In vitro* platelet evaluation included optical aggregometry (agonists and final concentration: γ-thrombin 100 nM, convulxin 10 nM), baseline and post-thrombin stimulated P-selectin expression, and platelet morphology via phase microscopy. *In vivo* platelet survival was determined by administration of biotinylated platelets to 30 healthy research dogs which had not been previously transfused or pregnant; no dog received more than one platelet transfusion. Cryopreserved units were evaluated 1–10 weeks post-freezing.

Both γ-thrombin- and convulxin-induced platelet aggregation (median amplitude, % increase light transmittance) were markedly diminished in DMSO (10% and 9%, respectively) and Thrombosol (0% and 12%, respectively) cryopreserved platelets in comparison to fresh platelets (64% and 80%, respectively) ($P = 0.001$ for all cryopreserved vs. fresh). Baseline P-selectin expression was < 1% for fresh and frozen platelets. To determine if platelets could be activated, P-selectin expression was determined post-thrombin stimulation. There was no difference in thrombin-induced P-selectin expression between the cryopreserved platelets (mean, DMSO 20.9%, Thrombosol 16.5%), but both exhibited significantly less activation than fresh platelets (mean, 43.8%) ($P < 0.0001$). The percentage of discoid and spherical platelets was not different between the 3 platelet groups. Fresh biotinylated platelets survived 7–9 days in all 10 recipients, with a mean half-life of 3.8 days. In the DMSO and Thrombosol groups, 4 of 10 dogs in each group had > 1% circulating biotinylated platelets on day 7, but the mean half-life was 1.7 days and 2.5 days, respectively, both significantly less than fresh platelets but not different from each other.

Thrombosol did not provide any appreciable benefit for maintaining *in vitro* function or prolonging *in vivo* survival of cryopreserved platelets in comparison to DMSO. Cryopreserved platelets can be activated, as demonstrated by thrombin-induced P-selectin expression, and survive in the circulation long enough to potentially be of benefit in the management of life-threatening hemorrhage in severely thrombocytopenic or thrombopathic patients. Further studies are needed to assess *in vivo* function of cryopreserved platelets.

ABSTRACT #253

EFFECTS OF STORAGE TIME AND HEMODILUTION ON CANINE PLATELET FUNCTION. M.H. Kim[1,2] B. Jefferson[1], S.A. Kruth[2] and R.D. Wood[1]. [1]Department of Pathobiology, [2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

The ability to evaluate primary hemostasis can provide insight into the pathophysiology and management of a number of diseases that impair platelet function in dogs. Aggregometry is considered the gold standard assay for assessing platelet function; however, its clinical utility is limited. The Platelet Function Analyzer-100® (PFA-100) is a bedside point-of-care test, but its clinical utility remains to be tested thoroughly. According to manufacturer's guidelines, the PFA-100 requires hematocrits (Hct) greater than 0.35 L/L and processing within 4 hours of collection, which can present logistical problems in the clinical setting.

Objectives of this study were: i) Establish effects of different storage times on platelet function as determined by PFA-100 and aggregometry; ii) Establish effects of hemodilution on PFA-100 closure time (CT); and iii) Establish if addition of autologous packed red blood cells to anemic blood corrects PFA-100 CT.

Ten healthy mixed breed dogs were studied. To evaluate the effect of sample storage time whole blood was collected in 3.2% buffered sodium citrate and kept at room temperature until analysis. Collagen-ADP cartridges were used to assess CTs. Platelet aggregation was measured using a Chrono-log® Model 440 dual channel optical aggregometer with two agonists: ADP (5×10^{-5} mM) and platelet activating factor (1×10^{-7} mM). To assess the effects of hemodilution on CT, each animal's own platelet-rich plasma was added to their whole blood *in vitro* to establish Hct values of 0.35, 0.25 and 0.15 L/L. Autologous packed RBCs were transfused into the 0.15 L/L dilutions with a goal of establishing a hematocrit of 0.45 L/L. CTs were measured for both hemodiluted and Hct restored samples using collagen-ADP cartridges.

PFA-100 closure times were not significantly different at times 0, 90, 120, 240 and 360 min ($P = 0.20$). Similarly, aggregometry did not show any significant changes among comparisons at 90, 120, 240 and 360 min (all P values > 0.05). Hemodilution of blood with autologous platelet-rich plasma resulted in significantly different PFA-100 CTs among whole blood, 0.35 L/L, 0.25 L/L and 0.15 L/L hematocrit values ($P < 0.05$). The addition of autologous packed red blood cells significantly lowered the CT ($P < 0.01$) and restored it into the reference range.

We conclude that: i) Sample storage time of up to six hours does not significantly alter platelet function as assessed by PFA-100 and aggregometry testing; ii) Serial hemodilutions result in prolongation of PFA-100 closure time; and iii) Addition of autologous packed red blood cells into hemodiluted samples resulted in restoration of PFA-100 closure times to within reference intervals.

ABSTRACT #254

PERFORMANCE OF AN IN-CLINIC ELISA TEST, SNAP® FELINE TRIPLE™, FOR THE DETECTION OF ANTIBODIES TO FELINE IMMUNODEFICIENCY VIRUS, FELINE LEUKEMIA VIRUS ANTIGENS AND FELINE HEARTWORM ANTIGENS IN CATS. C Mainville, B Foster, K Gross, J Maley, M Monn, R Chandrashekar. IDEXX Laboratories, Westbrook, ME.

The SNAP® Feline Triple™ test is an enzyme-linked immunosorbent assay (ELISA) for the simultaneous detection of antibodies to Feline Immunodeficiency Virus (FIV), Feline Leukemia Virus (FeLV) antigen and heartworm (HTWM) antigen in feline serum, plasma or whole blood. The FIV antibody assay utilizes a peptide derived from the envelope protein and a recombinant p24 protein. The FeLV and HTWM assays utilize antibodies to detect p27 FeLV antigen and HTWM antigen, respectively.

The purpose of this study was to evaluate the performance of the SNAP Feline Triple test. A total of 462 samples were tested in this evaluation. Various confirmatory tests were performed to assess the true infection status of the samples for each analyte. These include PetChek® FIV for FIV antibody, PetChek® FeLV for FeLV antigen and PetChek® HTWM PF and/or necropsy for feline heartworm. Results showing the performance of SNAP Feline

Triple with these samples are summarized in the table below table below.

Comparison Test	Sample Size SNAP Triple/Reference Test +/+	−/+	+/−	−/−	Total	Sample Type	Relative Sensitivity and Specificity 95% Confidence Limit	Kappa Statistic
Heartworm Necropsy/ PetChek Heartworm PF Ag[1]	26	4*	0	208	238	Serum/ Plasma	Sen., 86.7% (95% CL 69.5%–95.2%) Spec., 100% (95% CL 97.8%–100%)	0.92
PetChek FeLV Ag[2]	97	0	3	208	308	Serum/ Plasma	Sen., 100% (95% CL 95.3%–100%) Spec., 98.6% (95% CL 95.7%–99.7%)	0.98
PetChek FIV Ab[3]	123	1	0	208	332	Serum/ Plasma	Sen., 99.2% (95% CL 95%–100%) Spec., 100% (95% CL 97.8%–100%)	0.99

[1]Necropsy/PetChek Heartworm PF Antigen Test Kit (5018.02).
[2]PetChek Feline Leukemia Virus Antigen Test Kit (5028.01).
[3]PetChek Feline Immunodeficiency Virus Antibody Test Kit (5036.00).
*3 samples with 1 male worm found at necropsy; 1 sample with 0 worms found at necropsy.
CL=Confidence Limit.

For the population of samples tested in this study, the SNAP Feline Triple test had 99.2% sensitivity and 100% specificity for the detection of antibodies to FIV compared to the microtiter plate assay (PetChek FIV). The sensitivity and specificity of the SNAP Feline Triple test for FeLV antigen was 100% and 98.6%, respectively, compared to PetChek FeLV. The sensitivity and specificity of the SNAP Feline Triple test for HTWM antigen was 86.7% and 100%, respectively, compared to PetChek HTWM PF and/or necropsy.

Thus, the SNAP Feline Triple test can be used as a rapid, in-clinic test for the simultaneous detection of antibodies to FIV and both FeLV antigen and heartworm antigen in feline samples.

ABSTRACT #255

SERO-SURVEY OF FELINE HEARTWORM, FELINE IMMUNODEFICIENCY VIRUS AND FELINE LEUKEMIA VIRUS INFECTIONS IN CATS. C Mainville[1], P Dingman[2], W Foster[1], R Chandrashekar[1], J Levy[2]. [1]IDEXX Laboratories, Westbrook, ME. [2]College of Veterinary Medicine, University of Florida, Gainesville, FL.

Cats can become infected with heartworm (*Dirofilaria immitis*) and there is emerging evidence that feline heartworm infection can cause significant pathology (Heartworm Associated Respiratory Disease or HARD) from early or transient infections. Feline Immunodeficiency Virus and Feline Leukemia Virus infections in cats can result in a broad range of clinical symptoms and can be a significant cause of death.

To better understand the prevalence of these infections in cats, a sero-survey was conducted. Serum samples were obtained from 11 clinics, shelters or IDEXX Reference Laboratories throughout the United States. All samples were collected from the population of samples submitted to each site in the course of general clinic or lab operations and were not necessarily tested for heartworm, FIV or FeLV at the site. Samples were tested for the presence of heartworm antigen, for FIV antibody, and for FeLV antigen using ELISA plate assays. A population of 19,054 samples was tested in this study. Regional results are summarized in the table below.

Region	HTWM Total Tested	HTWM # Positive	HTWM % Positive	FIV Total Tested	FIV # Positive	FIV % Positive	FeLV Total Tested	FeLV # Positive	FeLV % Positive
Northeast	2695	24	0.89	2695	86	3.19	2695	18	0.67
Midwest	3293	31	0.94	3293	70	2.13	3293	40	1.21
South	5467	76	1.39	5467	278	5.09	5467	105	1.92
West	7599	52	0.68	7599	272	3.58	7599	59	0.78
Total	19054	180	0.96	19054	706	3.71	19054	222	1.17

This study demonstrates that cats with heartworm infections are found in all regions of the United States examined. The heartworm ELISA detects antigen in cats infected with adult female heartworms and does not detect antigens in cats with only male worm infections. Therefore, the prevalence reported in this study is likely an under-representation of the number of cats infected with heartworm. The FIV ELISA assay cannot distinguish vaccinated from naturally infected cats; therefore the prevalence reported for FIV may be an over-representation of FIV infection. To the authors' knowledge, this is the largest number of cats across all regions of the United States tested in a single study for heartworm antigen. This study also demonstrates that FIV and FeLV infection continues to affect a large population of domestic cats in the United States.

ABSTRACT #256

A COMMERCIALLY AVAILABLE *GIARDIA* SPP. ANTIGEN ASSAY DETECTS THE ASSEMBLAGES ISOLATED FROM DOGS. M Clark, AV Scorza, MR Lappin. Department of Clinical Sciences, Colorado State University, Ft.Collins, CO.

Giardia spp. infection is difficult to diagnose and so fecal flotation is sometimes combined with fecal antigen testing. Dogs are most frequently infected with genetic assemblages that are distinct from those that most commonly infect humans. The objectives of this study were to determine the most common *Giardia* spp. assemblages that infect dogs and to determine whether a *Giardia* antigen assay (SNAP® *Giardia*, IDEXX Laboratories, Portland, Maine) that is labeled for use with canine feces detects the assemblages commonly isolated from dogs.

Fecal samples were collected from dogs evaluated in small animal clinics in north central Colorado. An aliquot of feces was assayed immediately using the SNAP *Giardia* assay and an aliquot was stored at 4 °C until transported to Colorado State University for performance of fecal flotation by zinc sulfate centrifugation. All samples positive for *Giardia* cysts or *Giardia* antigen were assessed in a PCR assay and genotyped using previously validated techniques.

Of the 220 samples, 25 (11.4%) were positive for *Giardia* spp. antigen or cysts. Of the 17 samples giving a positive PCR reaction, 4 were assemblage C and 13 were assemblage D. All of these samples were positive in the SNAP *Giardia* assay.

All of the dogs in this study were infected with *Giardia* assemblages most commonly isolated from dogs; assemblages usually found in people were not detected. The SNAP *Giardia* assay detected all of the assemblages C and D isolates and so is an accurate test for the detection of *Giardia* antigen in feces of dogs.

ABSTRACT #257

TREATMENT OF HEALTHY *GIARDIA* SPP. POSITIVE DOGS WITH FENBENDAZOLE OR NITAZOXANIDE. MR Lappin, M Clark, AV Scorza. Department of Clinical Sciences, Colorado State University, Ft.Collins, CO.

Giardia spp. infection is common in healthy dogs. The objective of this study was to determine whether administration of drugs with known anti-*Giardia* activity would eliminate infection in healthy dogs.

Feces from healthy dogs evaluated in clinics in north central Colorado were assayed for *Giardia* spp. antigen using a commercially available kit (SNAP® *Giardia*, IDEXX Laboratories, Portland, Maine) and by fecal flotation using zinc sulfate centrifugation. *Giardia* spp. infected dogs were administered fenbendazole at 50 mg/kg, PO, daily for five days or nitazoxanide at 25 mg/kg, PO, twice daily for five days. The *Giardia* antigen assay and fecal flotation were repeated on samples collected on days 10, 14, and 34.

Overall, 16 *Giardia* spp. infected dogs were administered fenbendazole (7 dogs) or nitazoxanide (9 dogs). Excess salivation, vomiting, or diarrhea developed in three fenbendazole-treated dogs and five nitazoxanide-treated dogs resulting in removal from the study. Of the dogs that completed the treatment protocols, six of eight (two fenbendazole; four nitazoxanide) were positive in one or both tests on Day 10, four of eight (one fenbendazole; three nitazoxanide) were positive in one or both tests on Day 14, and five of eight (two fenbendazole; three nitazoxanide) were positive in one or both tests on day 34.

As both drugs have been used safely in other studies, the cause of the potential side-effects is unclear. Of treated dogs, 62.5% were *Giardia* positive on day 34, suggesting persistent infection or re-infection.

ABSTRACT #258
INFECTIOUS AGENT PREVALENCE RATES IN DOGS WITH DIARRHEA AND RESPONSE TO ADMINISTRATION OF FENBENDAZOLE OR NITAZOXANIDE. MR Lappin, M Spindel, L Riggenbach. Department of Clinical Sciences, Colorado State University, Ft. Collins, CO.

Diarrhea associated with infectious agents is common in dogs housed in animal shelters. Empirical treatment is often prescribed because of financial limitations. The objectives of this study were to determine infectious agent prevalence rates in dogs with diarrhea and response rates to fenbendazole or nitazoxanide, drugs with a broad-spectrum against some common parasites.

Feces from dogs with diarrhea but no vomiting that were housed in two animal shelters were assayed by fecal flotation using zinc sulfate centrifugation, *Giardia* spp. and *Cryptosporidium* spp. by IFA (Meridian Laboratories, Cincinnati, Ohio), aerobic fecal culture, and electron microscopy. Dogs were randomly administered fenbendazole at 50 mg/kg, PO, daily for five days or nitazoxanide at 25 mg/kg, PO, twice daily for five days and a fecal score determined daily.

Diarrhea resolved in 7 of the 10 dogs initially administered fenbendazole; 4 of these dogs were positive for *Giardia* and the remaining 3 dogs were negative in all tests. The 3 dogs that continued to have diarrhea on day 6 were from the *Giardia* positive group; diarrhea resolved in the 2 dogs that were then administered a complete course of nitazoxanide. Diarrhea resolved in 7 of the 8 dogs initially administered nitazoxanide. *Giardia* alone (3 dogs), *Giardia*, *Isospora*, *Cryptosporidium*, and *Salmonella* spp. (1 dog), *Giardia* and *Cryptosporidium* (1 dog), or *Cryptosporidium* alone (1 dog) were detected in 6 of these 7 dogs; the seventh dog had no detectable infectious agent. The nitazoxanide treated dog with diarrhea on day 6 was normal after administration of fenbendazole; no infectious agents were detected.

Overall, there were no differences between apparent response rates to fenbendazole or nitazoxanide and both drugs were well tolerated. Results of this study suggest that the nitazoxanide protocol utilized may have activity against *Giardia* spp. and *Cryptosporidium* spp. infections in dogs.

ABSTRACT #259
'*CANDIDATUS* MYCOPLASMA TURICENSIS' DNA IN THE BLOOD OF CATS IN THE UNITED STATES. MR Lappin[1], IR Peters[2], S. Tasker[2]. [1]Department of Clinical Sciences, Colorado State University, Ft. Collins, CO. [2]School of Clinical Veterinary Science, University of Bristol, Langford, Bristol, UK.

Cats are potentially infected by three different hemoplasma species: *Mycoplasma haemofelis* (Mhf), '*Candidatus* M. haemominutum' (Mhm), and '*Candidatus* M. turicensis' (CMt), but only cats from the western USA have been assessed for DNA of CMt. Primers that amplify DNA of Mhf in a commonly used conventional PCR assay (cPCR) also amplify DNA of CMt and so it is possible additional cases have been missed. The objective of this study was to evaluate blood samples from cats in the USA for DNA of CMt.

Blood samples (n = 96) assessed in this study were selected solely on sample availability. DNA had been previously extracted, assayed in the cPCR, and stored at −80 °C. DNA extracts were thawed, pipetted into individual wells of a 96 well plate, and shipped to the University of Bristol for assessment in a real time quantitative multiplex PCR assay (qPCR) that amplifies the DNA of CMt as well as 28S rDNA as an internal control.

Adequate DNA, as detected by the presence of plentiful 28S rDNA in the qPCR, was detected in 94 of the 96 samples, and 7 of these 94 samples contained DNA of CMt by qPCR. Of these 7 cats, 6 were male and 6 were anemic. CMt positive cats were from Alabama (3), Maryland (2), Colorado (1), and New Jersey (1). Estimated ages were 1–3 years (3), 10–12 years (3), and 15 years (1). A band of the appropriate size for Mhf or CMt was amplified from 4 of the 7 CMt positive cats in the cPCR. DNA of Mhm was amplified concurrently by cPCR from 5 of the 7 CMt positive cats.

The results suggest that CMt infection may be common in cats of the USA and that the cPCR used may be falsely negative in some cats with CMt infection. Future prevalence studies for hemoplasma infections in cats should utilize sensitive assays that differentiate between the three recognized species.

ABSTRACT #260
DETECTION OF HEMOPLASMA DNA ON THE GINGIVA AND CLAW BEDS OF NATURALLY EXPOSED CATS. MR. Lappin[1], P. Dingman[2], J. Levy[2], JR Hawley[1], A. Riley[3]. [1]Department of Clinical Sciences, Colorado State University, Ft. Collins, CO. [2]College of Veterinary Medicine, University of Florida, Gainesville, FL. [3]VCA Becker Animal Hospital, Birmingham, AL.

Mycoplasma haemofelis, '*Candidatus* M. haemominutum', and '*Candidatus* M. turicensis' DNA has been amplified from fleas and '*Candidatus* M. turicensis' DNA has been amplified from saliva of some cats suggesting that transmission can be flea-associated or direct. The objectives were to determine whether hemoplasma DNA could be amplified from the gingiva or claw beds of cats.

Samples were collected from humane society cats in Colorado (n = 32), Florida (n = 27), and Alabama (n = 10) after euthanasia. Samples were collected on sterile PBS soaked swabs by gently rubbing the base of claws 3 and 4 (forelimb) and the base of a canine tooth. DNA was extracted and assayed in a previously described PCR with results confirmed by sequencing.

Hemoplasma DNA was amplified from 15 of 69 (21.7%) gingiva and 9 of 69 (13%) claw beds. Of the positive samples, 9 of 24 had mixed infections. Hemoplasma DNA prevalence rates from gingiva of cats in Colorado (6 of 32; 18.8%) did not vary from those of cats in Florida and Alabama (9 of 37; 24.3%). Claw beds of cats in Florida and Alabama (8 of 37; 21.6%) were more likely ($p = 0.03$) to be positive for hemoplasma DNA than those from cats in Colorado (1 of 32; 3.1%).

Evidence of flea infestation was not detected on the Colorado cats and so the presence of hemoplasma DNA was unlikely to be from contamination by infected flea feces. These results support the hypothesis that feline hemoplasmas may be transmitted directly.

ABSTRACT #261
USE OF DRIED BLOOD SMEARS FOR DETECTION OF FELINE HEMOPLASMAS USING REAL-TIME PCR. JE Sykes[1], SD Owens[2,3] JC Terry[1], LL Lindsay[1], N Pusterla[1]. [1]Department of Medicine & Epidemiology, University of California, Davis, CA. [2]Department of Pathology, Microbiology & Immunology, University of California, Davis. [3]IDEXX Laboratories Inc., Broderick, CA.

The objective of this study was to determine the sensitivity and specificity of real-time PCR for feline hemoplasmas when applied to DNA extracted from dried blood smears, in comparison to that for DNA extracted from larger blood samples. Blood samples were collected into EDTA tubes from 310 cats with possible or suspected hemoplasmosis, and dried blood smears from each sample were prepared. DNA was extracted from blood smears and a 160-μL aliquot of each blood sample with a robotic extractor, and subjected to real-time PCR for 18S DNA, and '*Candidatus* Mycoplasma haemominutum' (*Mhm*), *Mycoplasma haemofelis* (*Mhf*), and '*Candidatus* Mycoplasma turicensis'(*Mtc*) DNA. Using the results for whole blood as the gold standard, the sensitivity of each assay for *Mhm*, *Mhf*, and *Mtc* was 49/67 (74%), 11/13 (85%), and 11/20 (55%), respectively. The specificity of each assay was 239/249 (96%), 292/292 (100%), and 285/285 (100%), respectively. Where possible, whole blood samples should be submitted for detection of feline hemoplasmas using real-time PCR. The improved sensitivity of real-time PCR on blood smears for *Mhf* compared with that for the other hemoplasma species may reflect the higher organism burdens associated with infection with this species.

ABSTRACT #262
REAL-TIME PCR DETECTION OF HEMOTROPIC MYCOPLASMAS IN HEALTHY AND UNHEALTHY CATS FROM THE BARCELONA AREA (SPAIN). X Roura[1], IR Peters[3], L Altet[2], MD Tabar[1], M Planellas[1], O Francino[2], S Tasker[3]. [1]Veterinary Teaching Hospital and [2]Molecular Genetics Veterinary Service, Autonomous University of Barcelona, Spain. [3]School of Clinical Veterinary Science, University of Bristol, UK.

Sparse information exists regarding hemotropic mycoplasmas (hemoplasmas) in Spanish cats. Although a few surveys have previ-

ously reported their presence in wild or domestic feline species, little data exists describing the identity of these organisms. Three feline hemoplasmas have been recognized: *Mycoplasma haemofelis* (*Mhf*), '*Candidatus* Mycoplasma haemominutum' (*Mhm*) and '*Candidatus* Mycoplasma turicensis' (*Mtc*). The pathogenicity of the hemoplasmas is variable with infection varying from being asymptomatic to inducing a severe hemolytic syndrome. Severity of illness is influenced by the infecting hemoplasma species and presence of concurrent diseases/infections.

The aims of the present study were 1) to determine the prevalence of hemoplasmas in cats presenting to the Veterinary Teaching Hospital of Autonomous University of Barcelona (Spain) using species-specific real-time PCR (qPCR) assays and 2) to evaluate any associations between hemoplasma infection, clinical presentation and other co-infections. Statistical differences between groups were tested for significance by the Fisher exact test using SPSS v.14.0 software. A p-value < 0.05 was regarded as statistically significant.

Ninety-one blood samples were entered into the study and were classified, based on information in the clinical records (reason for presentation, clinical signs, physical examination findings, hematology and serum biochemistry results), as healthy (n = 44) or unhealthy (n = 47). EDTA-blood samples underwent DNA extraction, and qPCR assays were performed to detect *Mhf*, *Mhm* and *Mtc*. The cats comprised 42 males and 49 females ranging in age from 1.3 to 17.1 years (median age 5.9 years). Breeds comprised domestic shorthairs (n = 58), Persians (n = 18), Russian Blues (n = 2), British shorthairs (n = 1) and unknown (n = 12). All samples had previously been assessed for *Hepatozoon* spp., *Babesia* spp., *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., *Bartonella* spp. and *Leishmania infantum* DNA via PCR, and 54 available serum samples had been tested for FeLV antigen and FIV antibody. Organisms previously detected were *Hepatozoon felis* (n = 3), *Leishmania infantum* (n = 2), *Ehrlichia*/*Anaplasma* sp. (n = 1), FIV (n = 5) and FeLV (n = 2). Hemoplasma qPCR detected *Mhf* (n = 3; 1 healthy and 2 unhealthy) and *Mhm* (n = 9; 4 healthy and 5 unhealthy) but no *Mtc*. One unhealthy cat was co-infected with *Mhf* and *Mhm*. Health status, sex, age, breed, other vector-borne infections and FIV/FeLV status were not significantly associated with positive hemoplasma qPCR results.

The results of this study confirm the presence of two different feline hemoplasma species, *Mhf* and *Mhm,* amongst cats from the Barcelona area (Spain), albeit with a low prevalence of 3.3% and 9.9%, respectively. No evidence of *Mtc* infection was found in this sample of cats.

ABSTRACT #263

POSSIBLE EMERGENCE OF DRUG-RESISTANT VARIANTS OF *BABESIA GIBSONI* IN CASES TREATED WITH ATOVAQUONE AND AZITHROMYCIN: AN EPIDEMIOLOGICAL SURVEY BASED ON THE CYTB GENE IN JAPAN.

M Sakuma[1], K Fukuda[2], K Takayama[2], Y Kobayashi[2], A Setoguchi-Mukai[1] and Y Endo[1]. [1]Kagoshima University, Kagoshima, Japan. [2]Adtec Co., Ltd., Oita, Japan.

It was recently reported by Birkenheuer *et al*. that combination therapy with atovaquone and azithromycin showed favorable therapeutic effects on canine babesiosis caused by *Babesia gibsoni* (*B. gibsoni*). However, Matsuu *et al*. showed that treatment with atovaquone could possibly induce the emergence or *in vivo* selection of drug-resistant variants of *B. gibsoni* with nucleotide substitutions in *cytochrome b* (*cytb*) gene.

Under such conditions, we report the treatment with atovaquone and azithromycin of 4 naturally *B. gibsoni* infected dogs at Kagoshima University Veterinary Teaching Hospital. Initially, all dogs responded well to treatment and the expected improvements of clinical conditions and hematological parameters were obtained. However, 2 of the 4 cases showed a relapse of the disease at 42 and 56 days, respectively, after the initiation of therapy. The emergence of drug-resistant or *in vivo* selected variants was suspected in both cases. Accordingly, DNA sequencing analysis of the *cytb* gene was conducted using blood samples collected pre- and post-treatment. Compared to the standard sequence of *cytb* gene (DDBJ/GenBank/EMBL accession number, AB215096), *cytb* genes from the relapsed cases had different nucleotide sequences; one of them had an amino acid substitution (M121I) that has been reported to possibly be responsible for drug-resistance. Furthermore, *B. gibsoni* with M121I was a dominant type in one of the cases, even at the time of the primary onset of the disease.

From findings of clinical cases, variants of *B. gibsoni* with drug-resistance related sequences in the *cytb* gene are suspected to already exist in nature. We thus performed an epidemiological survey based on the cytb gene using blood samples collected from 58 cases in areas west of the Kanto area of Japan. Many types of single nucleotide substitution were detected; however, most induced synonymous amino acid substitutions. Nine non-synonymous amino acid substitutions were detected. Sites and frequency in the 58 cases were as follows: M43I (1.7%), M121I (3.4%), I136V (1.7%), V220I (5.2%), I226V (13.8%), A276V (1.7%), A290V (1.7%), I303V (5.2%) and P310S (1.7%). I226V, V220I and I303V were observed at relatively high incidences compared to those in other sites. As well, the three sites reported by Matsuu *et al*., M121I, V220I and I303V, were also detected in this study; M121I, which corresponds to the possible atovaquone binding site, was detected in 2 cases.

This study indicated that drug-resistant variants might be induced even in naturally infected dogs treated with atovaquone. *B. gibsoni* with the variant *cytb* gene already exists in nature, though its frequency is not high. Therefore, attention needs to be given to the appearance of drug-resistant variants in cases where atovaquone is used.

ABSTRACT #264

CLINICAL AND MOLECULAR CHARACTERIZATION OF A NOVEL CANINE *BABESIA* SPECIES.

LE Sikorski[1], AJ Birkenheuer[2], MK Holowaychuk[2], MP Littman[1]. [1]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA. [2]North Carolina State University College of Veterinary Medicine, Raleigh, NC.

A novel large un-named *Babesia* species was described recently. This *Babesia* species was first detected in a dog undergoing chemotherapy for lymphoma. It was unknown whether or not this was an isolated event or if this parasite is an under-recognized pathogen of dogs**.** The primary goals of this study were to report the historical and clinicopathological findings in five additional cases of babesiosis caused by this novel large *Babesia* sp. and characterize the *Babesia* sp. 18S ribosomal ribonucleic acid (rRNA) genes in each case.

The medical records of 5 dogs with naturally occurring disease were reviewed. Additionally, the *Babesia* sp. 18S rRNA genes were amplified, cloned, and sequenced in each case.

All five cases had a history of splenectomy prior to diagnosis. The dogs lived in North Carolina (3), New York (1), and New Jersey (1). Travel history was unremarkable in all cases. Non-specific signs including lethargy and anorexia were the most common owner complaints. Pigmenturia was noted by the owner in 3/5 cases. Mild fever was a common physical examination finding. Common laboratory findings included mild anemia (5/5 cases, HCT range 28.2–36.5%) and severe thrombocytopenia (4/5 cases, PLT range <5–28 $\times 10^3/\mu$l). Cross-reactive antibodies against *B. canis vogeli* (1/2 negative), *B. gibsoni* (Asian Genotype) (2/2 negative), and *B. conradae* (2/2 negative) were not always detectable. Four cases were treated with imidocarb dipropionate and responded well; the remaining case had a normal platelet count ($478 \times 10^3/\mu$l) and recovered without treatment with anti-protozoal medications. All of the 18S rRNA gene sequences were 100% identical to the originally described novel large *Babesia* sp.

In conclusion, dogs with pigmenturia, anemia and/or thrombocytopenia should be tested for *Babesia* by PCR assay. Serology may not be a useful method to diagnose infection with all types of pathogenic *Babesia* spp. Asplenia may represent a risk factor for clinical signs due to this novel large *Babesia* sp.

ABSTRACT #265

IDENTIFICATION OF A *BABESIA MICROTI*-LIKE PARASITE IN NORTH AMERICAN WILD CANIDS.

AJ Birkenheuer[1], B Horney[2], M Bailey[1], S McBurney[2], AE Acton[1], HS Marr[1]. [1]North Carolina State University College of Veterinary Medicine, Raleigh, NC. [2]University of Prince Edward Island, Charlottetown, PE, Canada.

Babesia microti-like organisms have recently been identified in Spanish dogs as a cause of hemolytic anemia and azotemia. Cur-

rently there are no reports of *B. microti*-like infections in domestic dogs that have not traveled to or resided in the northwestern region of Spain. However, a *B. microti*-like parasite has been described in a single fox from the northeastern United States and foxes are suspected to be the reservoir host for the parasites in Spain. Based on epidemiological studies *Ixodes hexagonus* is presumed to be the tick vector for dogs.

In order to assess the prevalence of this parasite in North American wild canids, blood samples from 149 legally trapped or injured/ill foxes from North Carolina or Canada and 3 coyotes from Canada were tested for the presence *B. microti*-like DNA by polymerase chain reaction.

Babesia microti-like DNA was detected by PCR in 37% (48/129) of the fox samples tested. *Babesia microti*-like DNA was not detected in any of the coyotes. DNA sequencing of the amplicons from 19 randomly selected cases revealed > 99.9% sequence identity with the *B. microti*-like parasite from Spain.

These results indicate that the prevalence of *B. microti*-like infections in North American foxes is similar to that described in European foxes. However, the ability of the *B. microti*-like parasite of North American foxes to infect domestic dogs and induce disease remains unknown. An underlying cause is not identified in many North American dogs with hemolytic anemia and/or renal failure despite intensive diagnostic investigations. *Babesia microti*-like parasites may represent a potential health threat to North American domestic dogs and may be an under recognized cause of hemolytic anemia and azotemia. Further studies investigating the pathogenic potential of this parasite in domestic dogs, prevalence of *B. microti*-like infections in North American domestic dogs, as well as the vector competence and capacity of North American tick species are indicated.

ABSTRACT #266

ANTI-*ANAPLASMA* SPP. ANTIBODY SEROPREVALENCE IN DISEASED DOGS AND HEALTHY BLOOD DONOR DOGS IN A VETERINARY TEACHING HOSPITAL IN A LYME ENDEMIC AREA. KA Gavin, TJ Nolan, MP Littman. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

The clinical importance of *Anaplasma* spp. infection remains unclear in sick and healthy dogs. Coinfections may play a role in expression of illness. The purpose of this retrospective study was to compare seroprevalence in hospital clinic (HC) dogs and healthy large breed blood donor (BD) dogs, and to review clinicopathologic findings in exposed sick dogs. Medical records were reviewed for variables such as signalment, lameness, fever, results of CBC, biochemical profile, urinalysis, and serologic test results for antibodies to other tick-borne diseases.

Between Nov 2006 and Dec 2007, the SNAP-4Dx (IDEXX) in-house test was used to determine presence of heartworm antigen and antibodies against *Borrelia burgdorferi* (Bb), *Ehrlichia canis*, and *Anaplasma phagocytophilum*/*platys* (Ap). From Dec 1999 through Oct 2006, anti-Ap antibodies were tested by IFA assay (ProtaTek Laboratories, AZ).

Positive Ap antibody test results were found in 17/267 (6.4%) BD dogs (mean 4.5 yrs old) compared with 54/956 (5.6%) of HC dogs (mean 6.4 yrs old) during the period of Nov 2006–Dec 2007. In addition, 4 seronegative infected HC cases had neutrophilic morulae in blood smears, joint tap, or spinal fluid cytologic examination (total HC Ap cases = 58/960 or 6.0%). During this period of time 65/345 (18.8%) BD and 180/1029 (17.5%) HC dogs were Lyme-positive. Among the Ap-seropositive dogs, anti-Bb antibodies were also found in 23/58 (39.7%) HC dogs and 8/17 (47.1%) healthy BD dogs.

Medical records of 70 Ap-seropositive or infected HC dogs from Dec 1999–Dec 2007 were studied. According to the owners, 19/70 (24.3%) dogs were not on topical ectoparasite prevention. Owners reported lameness in 22/70 (31.4%), lethargy in 18/70 (25.7%), and anorexia in 7/70 (10%) dogs. Common physical exam findings included fever ≥ 103F in 27/70 (38.6%), lameness in 23/70 (32.8%) dogs, and neurologic abnormalities in 10/70 (14.3%) dogs. Common laboratory abnormalities included thrombocytopenia (< 177 $\times 10^3$/ul) in 30/70 (42.3%) dogs, anemia (HCT < 39.9%) in 35/70 (50%) dogs, and urine protein/creatinine ratio > 0.5 in 10/20 (50%) dogs. Antibodies to Bb were found in 32/70 (45.7%) dogs and 8/13 dogs were positive for *Bartonella* spp. antibodies.

Since sampling of a variety of sick dogs was not routinely done and was biased toward cases with clinical signs suspicious for tick-borne disease, eg, fever, lameness, cytopenias, and/or proteinuria, a case-controlled study is ongoing, to compare the clinicopathologic findings in contiguous seronegative cases done in the same laboratory before and after each seropositive case.

In conclusion, clinically ill dogs were not found to be more likely seropositive for Ap antibodies than were healthy blood donor dogs. Confusion regarding cause and effect of Ap exposure and illness may be confounded by sampling bias and co-infections, especially with Lyme disease.

ABSTRACT #267

COMPARATIVE STRAIN ANALYSIS OF *ANAPLASMA PHAGOCYTOPHILUM* INFECTION AND CLINICAL OUTCOMES IN A CANINE MODEL OF GRANULOCYTIC ANAPLASMOSIS. DG Scorpio[1], JS Dumler[1], NC Barat[1], JA Anastasio[1], Daryn Daniluk[2], Kristen Caterina[2], Melissa Beall[2], Ramaswamy Chandrashekar[2]. [1]Johns Hopkins University School of Medicine, Baltimore, MD. [2]IDEXX Laboratories, Westbrook, ME.

A pilot study was conducted to determine which human or canine strain of *Anaplasma phagocytophilum* would reproduce clinical infection normally anticipated in dogs and other susceptible mammals with naturally-acquired granulocytic anaplasmosis.

Six hounds were inoculated IV with 1×10^6 of either *A. phagocytophilum* Webster (human), 98E4 Minnesota (canine), or E06 California (canine) strain bacteria. *A. phagocytophilum* was propagated in HL-60 (human leukemia) cells in vitro for 3 of the dog infections. Since infection in this manner introduces the potential confounder of HL-60 cells, we also used infected autologous neutrophils in the remaining 3 dogs. Once infected, dogs were monitored daily and clinical findings recorded. Clinical parameters were scored using a numerical grading system from 0–3, paying particular attention to signs of lethargy, anorexia, petechiation, lymphadenopathy, and fever. CBC, serum chemistry, and serology (IFA and SNAP® 4Dx®) were conducted between days 0 and 60. DNA was isolated and quantitative PCR performed. Inflammatory cytokine aberrations were evaluated using serum samples on the Luminex multi-analyte platform.

The most prominent clinical signs observed were generalized lymphadenopathy and scleral injection, with one dog (98E4 strain in neutrophils) developing fever lasting 4 days (103.9F–104.1F). Biochemistry parameters remained normal throughout the infection, with no changes in hepatic or renal functions. Most notable changes occurred in leukocyte and platelet counts, with prominent and sustained leukopenia and thrombocytopenia occurring among all dogs. *Anaplasma* morulae were noted in blood smear preparations between days 10–11. All dogs seroconverted by day 10–15 (IFA), and by day 17–21 (SNAP® 4Dx®), and all dogs developed infection (positive through and including day 60) with *A. phagocytophilum* as determined by qPCR using the *msp2* gene target. Cytokine analysis revealed a 10-fold increase in IL-2, IL-18, and TNFα in the dog infected with *A. phagocytophilum* 98E4 strain in neutrophils.

There is substantial evidence to demonstrate that all strains produced infection, although canine *A. phagocytophilum* 98E4 reproduced clinical signs, hematologic changes, and inflammatory cytokine elevations most consistent with granulocytic anaplasmosis; therefore this strain will be used in future studies of *A. phagocytophilum* infection in dogs. Since animal numbers were small, additional studies are currently being carried out for definitive interpretation of pilot study results.

ABSTRACT #268

EXPERIMENTAL *E. CANIS* INFECTION IN DOGS: ANTIBODY RESPONSES PRE- AND POST-TREATMENT. B.A. Stillman[1], K. Caterina-DeBisceglie[1], J. Bradley[2], J. Saucier[1], E.B. Breitschwerdt[2], S.D. Gaunt[3], T. O'Connor[1], R. Chandrashekar[1]. [1]IDEXX Laboratories, Westbrook, ME. [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC. [3]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

In an effort to better understand serological changes in dogs following *Ehrlichia canis* infection and treatment, we monitored

antibody levels in two groups of dogs experimentally infected with *E. canis*, before and after treatment with doxycycline. Twelve dogs were infected with *E. canis*; six dogs were subsequently treated at 28 days post-infection, while six dogs remained untreated. Serum samples obtained throughout the course of infection and during and following treatment were evaluated by microtiter plate-format ELISAs to detect antibodies against two different major immunoreactive *E. canis* proteins (p16 and p30), by IFA, and by SNAP® 4Dx® (IDEXX Laboratories). For the majority of dogs (7 of 12), the p16 and IFA assays detected antibodies by day 14, while the p30 and SNAP 4Dx assays became positive around day 21. In doxycycline-treated dogs (10 mg/kg, SID, 28 days), titers of antibodies peaked between post-infection days 20 and 80, and p16 antibodies decreased following treatment more quickly than p30 antibodies over time. IFA titers and SNAP 4Dx assay spot intensity values also decreased in the treated group. In untreated dogs, antibody levels remained high in all assays out to 200 days post-infection (no decrease from early post-infection levels). These studies detail the antibody response to p16, p30 and *E. canis* whole cell antigens during pre- and post-treatment phases of canine ehrlichiosis. These data, together with other data derived in this study, indicate that antibody levels should decrease progressively following successful treatment of canine ehrlichiosis.

ABSTRACT #269
SEROSURVEY OF ANTIBODIES AGAINST *B. BURGDORFERI, E. CANIS, E. EWINGII, E. CHAFFEENSIS,* AND *ANAPLASMA* IN DOGS FROM THE UNITED STATES. D. Daniluk, B. Thatcher, J. Saucier, R. Krah, M. Beall, T. O'Connor, R. Chandrashekar, B.A. Stillman. IDEXX Laboratories, Westbrook, ME.

Tick-borne diseases in dogs can be produced by a number of infectious agents, including *Anaplasma sps*., *Borrelia burgdorferi, Ehrlichia canis, Ehrlichia chaffeensis*, and *Ehrlichia ewingii*. In an effort to understand the organism-specific infection rates in dogs, we examined over 4000 serum samples, obtained from a network of diagnostic laboratories, from dogs suspected of having a tick-borne illness. Serum samples were tested on a prototype in-clinic ELISA (IDEXX Laboratories), using genus- and species-specific markers for the presence of antibodies to the above agents. The data obtained from this evaluation are summarized below.

Species	% Incidence in dogs suspected of tick-borne illness	Number of samples with single infection	Number of samples with co-infection found (2 or more)
Anaplasma sps.	11.7	232	234
Borrelia burgdorferi	46.3	1647	233
Ehrlichia canis	13.2	464	65
Ehrlichia chaffeensis	0.9	26	12
Ehrlichia ewingii	3.6	75	70

Of the 4000 samples tested, 46.3% had antibodies to *B. burgdorferi*; 11.7% to *Anaplasma*, while 13.2%, 0.9%, and 3.6% had antibodies to *E. canis*, *E. chaffeensis*, and *E. ewingii* analytes, respectively. The highest incidence of co-infection was for *Anaplasma*/*Borrelia* dual infection (21.5% of samples) followed by *E. canis*/*E. ewingii* (1.1%). The demographics of the data, despite the limitation of samples being sourced from discrete laboratory locations, reveal endemic areas of both borreliosis and anaplasmosis in the Northeast, upper Midwest, and Pacific coast regions. The data also show widespread incidence of *E. canis* across the country, with endemic pockets of *E. chaffeensis* and *E. ewingii*.

ABSTRACT #270
ASSOCIATION OF VECTORIAL INFECTIOUS DISEASES WITH IMMUNE-MEDIATED ANEMIA IN THE DOG: 40 CASES. M Pastor[1], E Videmont[1], HJ Boulouis[2], L Chabanne[1]. [1]Ecole Nationale Vétérinaire de Lyon, Marcy l'Etoile, France, [2]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.

Immune-mediated anemia (IMA) in the dog is usually identified as primary (i.e., idiopathic), because etiology is not known in most cases. Infectious diseases, especially those with vectorial transmission, are frequently quoted as probable causes of IMA. The purpose of this study is to evaluate the incidence of vectorial infectious diseases, particularly tick-transmitted diseases, among potential causes of IMA.

Forty French dogs were included between September 2005 and April 2007. Inclusion criteria were the existence of an anemia (hemoglobin < 12 g/dL), and the identification of the immune origin of this anemia: positive direct Coombs' test (DCT) (threshold of 1/8) and/or in-saline agglutination and/or marked spherocytosis. The following analyses were performed: complete hemogram, DCT at 37 °C (N = 35) and at 4 °C (N = 17), antinuclear antibodies (N = 6) and myelogram in case of persistent nonregenerative anemia (N = 12). For each dog, presence of or exposure to several tick-transmitted pathogens was evaluated: *Ehrlichia canis* (serology and PCR), *Anaplasma phagocytophilum* (serology and PCR), *Rickettsia* spp. (PCR), *Bartonella* spp. (PCR) and *Babesia* spp. (blood smears). Exposure to the following infectious agents was assessed in accordance with diagnostic suspicion: *Leishmania infantum*, *Leptospira interrogans*, *Borrelia burgdorferi* and *Neospora caninum*. Moreover, diagnostic imaging and biochemical analyses were performed if anomalies were detected during physical examination.

Anemia was generally moderate to severe, and nonregenerative in 16 cases. Thrombocytopenia was present in 22 dogs, and leukocytosis in 21 dogs. Direct identification and serology allowed identification of vectorial infectious diseases in 11 cases (27.5%): 7 babesiosis, 4 monocytic ehrlichiosis, 1 granulocytic anaplasmosis and 1 leishmaniosis (2 cases of co-infections: babesiosis and monocytic ehrlichiosis, monocytic ehrlichiosis and granulocytic anaplasmosis). No pathogen was identified using myelogram. All PCR assays were negative. Antinuclear antibodies were positive in 2 cases. Leptospirosis was identified in 2 cases and non-infectious disease in 3 last cases (hemangiosarcoma, malignant histiocytosis and bullous pemphigoid). No concomitant affection was identified in 24 dogs (60%).

This study confirms the predominance of idiopathic immune anemia and the relevance of the direct Coombs' test for their diagnostic. Unlike others, who find that neoplasia is the most common cause of secondary IMA, this study shows that infectious disease, especially tick-transmitted disease, is the first type of concomitant affection with IMA. Geographical location could be one of the explanations. Nevertheless, routine assays to evaluate exposure to vectorial diseases are useful for treatment. This study also points out the complementarity of direct and indirect diagnostic techniques for infectious origin, especially in cases of early anti-infectious treatments.

ABSTRACT #271
SEROLOGICAL SURVEY OF LEISHMANIA INFECTIONS IN CATS FROM NORTH GREECE. J. Huebner, E. Müller, I. Langbein-Detsch, T. Naucke. Laboklin, Bad Kissingen, Germany.

Leishmaniosis was first described in dogs and cats from Syria in 1756 by Russel and around 1900 sand flies (*Phlebotominae*) became known as the transmitting vector. After World War II when dichloro-diphenyl-trichloroethane (DDT) was used in an eradication program for *Anopheles,* leishmaniosis in animals became rare because the sand fly was decimated too. Then in 1980 Morsy et al. showed a prevalence of 20.5% in cats in Jordania and since that time leishmaniosis became an issue especially in animals from the Mediterranean area. In cats cutaneous leishmaniosis seems to be the most frequent form. Typical signs include nodular to ulcerous lesions on the nose, lips, ears, eyelids and alopecia. Recent reports confirmed *Leishmania infantum* infections in cats serologically as well as by PCR. In the last years animal welfare and rescue organisations started to import dogs and cats from Southern Europe to Germany and leishmaniosis became a topic in German veterinary practices. Leishmaniosis is known to cause either a persistent disease or a life long carrier state. To determine the prevalence and therefore the risk to import infected cats, we investigated in our study feral cats captured in a neutering program in the area of Thessaloniki for antibodies of *Leishmania infantum*. All cats got a full clinical examination and were grouped regarding their health status and body conditions. An EDTA and a serum sample were taken from each cat under sedation. The serum samples were shipped on dry ice to Germany and were investigated in our laboratory by IFAT for the presents of antibodies for *Leishmania infantum*.

From 389 investigated cats, 84 samples were positive, with titres varying from 1:64 (n = 63) to 1:1024 (n = 1). These results show a prevalence of 21.6%. *Leishmania*-related clinical signs were seen in 16 (19%) of positive cats, which mean 4.1% of all investigated cats.

None of all tested cats (n = 389) was positive for FeLV and only 5.3% (n = 21) were positive for FIV. In the group of *leishmania*-antibody-positive cats 1.8% were FIV-positive.

Despite these results and many other studies, cats are still regarded as unusual *leishmania* hosts.

In comparison to our study, Papadopoulou et al. showed a higher prevalence rate of up to 45.4% in dogs from this area. One explanation might be the effect of the cats, nocturnal and twilight activity. Sand flies are also most active at dusk. But they need a host that is more or less motionless for at least 10 minutes before they bite. So cats might be simply too active in comparison to dogs at this time of day and that might explain the lower prevalence rate.

ABSTRACT #272

OCCURRENCE OF CROSS-REACTION BETWEEN CANINE VISCERAL LEISHMANIASIS AND CHAGAS DISEASE BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST (IFAT) AND IMMUNOCHROMATOGRAPHIC DIPSTICK TEST. MF Zanette[1], VMF Lima[1], MD Lauenti[2], J.P. Vides[1], D.L. Silva[1], C.N. Rossi[1], F.A. Ikeda-Garcia[1], F.A. Rosa[1], L.S.V. Sobrinho[1], D.C. Costa[1], S.H.V. Perri[1], A.A. Camacho[3], M Marcondes[1]. [1]São Paulo State University, Araçatuba, São Paulo, Brazil. [2]University of São Paulo, São Paulo, São Paulo, Brazil. [3]São Paulo State University, Jaboticabal, São Paulo,Brazil.

Visceral leishmaniasis, also known as Kalazar, is an anthropozoonosis with world distribution in tropical and subtropical areas of the world. It is caused by a protozoan placed in the order Kinetoplastida, family Trypanosomatidae and genus *Leishmania* that infects dogs and a wide variety of vertebrates. Serodiagnosis is the most valuable tool for its diagnosis. However, the specificity of the serological tests may be harmed due to the occurrence of cross-reaction among diseases caused by others Trypanosomatidae, such as *Trypanosoma cruzi*. Thus, the aim of this work was to determine the ocurrence of cross-reaction between *Trypanosoma cruzi* and *Leishmania chagasi* antigens, by ELISA, indirect immunofluorescent antibody test (IFAT) and immunocromatographic dipstick test. The ELISA and indirect immunofluorescence assay were performed using *L. chagasi* promastigote antigen while the immunocromatographic test employed rK39 antigen. The study was carried out with a group of 14 naturally and experimentally *T. cruzi* infected dogs. Of the 14 sera samples tested for visceral leishmanisis, nine (64.3%) were positive by ELISA, six (42.9%) were positive by indirect immunofluorescence assay and all of them were found to be negative by immunochromatographic test (Table 1). Based on the results of this study, among the three methods assessed, the immunocromatographic dipstick test using the rK39 antigen was the best option due to the ability to distinguish succesfully patients infected by *T. cruzi* from those infected by *L. chagasi*.

ABSTRACT #273

OCCURRENCE OF CROSS-REACTION BETWEEN CANINE VISCERAL LEISHMANIASIS AND BABESIOSIS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST (IFAT) AND IMMUNOCHROMATOGRAPHIC DIPSTICK TEST. MF Zanette[1], VMF Lima[1], MD Lauenti[2], JP Vides[1], DL Silva[1], CN Rossi[1], FA Ikeda-Garcia[1], FA Rosa[1], LSV Sobrinho[1], DC Costa[1], SHV Perri[1], MK Hagiwara[2], M Marcondes[1]. [1]São Paulo State University, Araçatuba, São Paulo, Brazil. [2]University of São Paulo, São Paulo, São Paulo, Brazil.

Dogs play an important role as reservoirs for human visceral leishmaniasis in Brazil. Therefore, according to the Ministry of Health, the local disease control program is based on the elimination of seropositive dogs. Since the disease can adopt a broad spectrum of clinical signs and the laboratory findings are frequently not conclusive, the detection of specific anti-*Leishmania* circulating antibodies by means of serologic methods is very helpful in the diagnosis of visceral leishmaniasis. However, the specificity of the serological tests may be harmed due to the occurrence of cross-reaction among diseases such as babesiosis. Thus, the aim of this work was to determine the ocurrence of cross-reaction between *Babesia sp.* and *Leishmania chagasi* antigens by ELISA, indirect immunofluorescent antibody test (IFAT) and immunocromatographic dipstick test. The ELISA and IFAT assay were performed using *L. chagasi* promastigote antigen while the immunocromatographic test employed rK39 antigen. The study was carried out with a group of 12 naturally and experimentally *Babesia sp.* infected dogs. Of the 12 sera samples tested for visceral leishmaniasis, all of them were found to be negative by ELISA, indirect immunofluorescence and immunochromatographic test. According to the results obtained in the present study it was possible to conclude that cross-reaction between *Babesia sp.* and *Leishmania chagasi* antigens by the three methods assessed doesn't occur.

ABSTRACT #274

OCCURRENCE OF CROSS-REACTION BETWEEN CANINE VISCERAL LEISHMANIASIS AND EHRLICHIOSIS BY ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA), INDIRECT IMMUNOFLUORESCENT ANTIBODY TEST (IFAT) AND IMMUNOCHROMATOGRAPHIC DIPSTICK TEST. MF Zanette[1], VMF Lima[1], MD Lauenti[2], JP Vides[1], DL Silva[1], CN Rossi[1], FA Ikeda-Garcia[1], FA Rosa[1], LSV Sobrinho[1], DC Costa[1], SHV Perri[1], MK Hagiwara[2], M Marcondes[1]. [1]São Paulo State University, Araçatuba, São Paulo, Brazil. [2]University of São Paulo, São Paulo, São Paulo, Brazil.

The diagnosis of canine leishmaniasis is a complex task for the clinician because the disease can adopt a broad spectrum of clinical features and the laboratory findings, such as renal and liver function, hematology and urinalysis are frequently not conclusive. Therefore, the detection of specific anti-*Leishmania* circulating antibodies by means of serologic methods became the most valuable tool for the diagnosis in Brazil. However, the specificity of the serological tests may be harmed due to the occurrence of cross-reaction among diseases such as ehrlichiosis. Thus, the aim of this work was to determine the ocurrence of cross-reaction between *Ehrlichia canis* and *Leishmania chagasi* antigens, by ELISA, indirect immunofluorescent antibody test (IFAT) and immunocromatographic dipstick test. The ELISA and indirect immunofluorescence assay were performed using *L. chagasi* promastigote antigen while the immunocromatographic test employed rK39 antigen. The study was carried out with a group of 13 experimentally *E. canis* infected dogs. Of the 13 sera samples tested for visceral leishmaniasis, one (7.7%) were positive by ELISA and by immunochromatographic test and all of them were found to be negative by indirect immunofluorescence test. According to the results obtained in the present study it was possible to conclude that cross- reaction between *Ehrlichia canis* and *Leishmania chagasi* antigens may occur and, among the three methods assessed, the indirect immunofluorescence test seemed to be the best choice when double infection with *E. canis* and *L. chagasi* is suspected.

ABSTRACT #275

CLINICOPATHOLOGIC FINDINGS IN DOGS WITH PROTEIN-LOSING NEPHROPATHY AND ANTI-*BORRELIA BURGDORFERI* ANTIBODIES. DJ Slade, TJ Nolan, MP Littman. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

The purpose of this retrospective study was to review findings in clinical cases at a referral teaching hospital which were positive for *Borrelia burgdorferi* (Bb) antibodies by the SNAP-3Dx or SNAP-4Dx (IDEXX) in-house tests and which demonstrated evidence of protein-losing nephropathy (Lyme+PLN). Medical records were reviewed for variables such as signalment, history, clinical signs, results of CBC, biochemical profile, urinalysis, urine protein/creatinine ratio (UPC), exposure to co-infections, renal histopathologic findings, treatments, and outcome.

From May 2001, the SNAP-3Dx (IDEXX) in-house assay was used to test for heartworm antigen and antibodies against Bb and *Ehrlichia canis*. After Nov 2006, the SNAP-4Dx assay was used to test for the above and anti-*Anaplasma phagocytophilum*/*platys* (Ap)

antibodies. Positive Bb antibody test results were found in 215/919 (23%) of healthy blood donor dogs compared with 962/3941 (24%) of hospital clinic cases during the period of May 2001–Dec 2007.

Medical records from the hospital population of Bb-seropositive dogs were studied. Within our referral population, 102/962 (10%) of Lyme-positive dogs showed evidence of protein-losing nephropathy (hypoalbuminemia, proteinuria). A preliminary review of 55 of these cases was undertaken. Breeds most frequently represented included Labrador Retrievers (16/55, 29%), Golden Retrievers (6/55, 10%), and mixed breed (15/55, 27%), with males comprising 47% (26/55). Average patient weight was 26.1 kg and average age at presentation was 6.3 years.

Common presenting complaints in 55 dogs included inappetance (89%), lethargy (89%), vomiting (72%), weight loss (69%), peripheral lymphadenopathy (33%), and polyuria/polydipsia (31%); only 9% had a history of lameness and 11% had previous Lyme vaccination. Common blood test abnormalities on admission included anemia (49/53, 92%), thrombocytopenia (42/53, 79%), azotemia (50/52, 96%), hypoalbuminemia (47/52, 90%), and hypercholesterolemia (15/52, 29%). Urinalysis and UPC in all 55 dogs confirmed proteinuria (100%) with USG <1.022 in 72%, glucosuria (27%), and bilirubinuria (27%). Hypertension during hospitalization was found in 69% (37/54). A small group of patients showed antibodies to *Ehrlichia canis* (3/55, 5%) or *Rickettsia rickettsii* (2/38, 5%). Anti-Ap antibodies were found in 14% (2/14) of Lyme+PLN dogs tested.

Patients were treated with doxycyline (42/55, 76%), ACE inhibitors (43/55, 78%), amlodipine (14/55, 25%), low-dose aspirin (18/55, 33%), and famotidine (33/55, 60%). A small group (6/55, 11%) received immunosuppressive doses of prednisone. Follow-up of 30 dogs found 60% (18/30) were euthanized or died with mean survival of 24 days post-admission.

In this preliminary study, dogs seropositive for Bb antibodies and exhibiting signs of protein-losing nephropathy had a high mortality rate in the initial period following diagnosis. Further study is warranted to investigate whether other therapeutic modalities, including immune suppression, may be of benefit to these patients.

ABSTRACT #276

DYNAMICS OF EXPOSURE TO VECTOR-BORNE ORGANISMS IN DOGS IN NORTH AMERICA: 2004–2006.

PPVP Diniz[1], M Morgado[1], BC Hegarty[1], N Cherry[1], M Sullivan[2], EB Breitschwerdt[1]. [1]Intracellular Pathogens Research Laboratory, College of Veterinary Medicine, North Carolina State University, Raleigh, NC. [2]IDEXX Laboratories, Westbrook, ME.

Vector-borne infections in dogs occur throughout the US; however, temporal trends in prevalence for most infections have been poorly described. The objective of this study was to evaluate the seroprevalence of *Anaplasma* spp., *Babesia canis, Bartonella henselae*, *Bartonella vinsonii* subsp. *berkhoffii, Borrelia burgdorferi, Dirofilaria immitis*, *Ehrlichia canis*, and *Rickettsia rickettsii* in dog blood samples submitted to the Vector-Borne Diseases Diagnostic Laboratory at NCSU from January 2004 to December 2006. From the laboratory database, 7049 accessions (2208 dogs in 2004, 2400 dogs in 2005 and 2441 dogs in 2006), for which serological results were available for at least three test organisms, were selected for analyses. An indirect immunofluorescence assay (IFA) with a cut-off of 1:64 was used to detect exposure to *B. canis B. henselae, B. v. berkhoffii, E. canis* and *R. rickettsii*. The Snap® 4Dx® was used to detect exposure to *Anaplasma* spp., *B. burgdorferi, D. immitis* and *E. canis*. Gender information was available only for 2006, with 1218 males and 1179 females. 6775 samples (96.1%) were submitted from 47 states of the US, with South and Midwest regions overrepresented (68.1% and 20.1% of US samples, respectively). 274 samples (3.9%) were submitted from Canada. Seroprevalences in 2004, 2005 and 2006 are presented below:

	Organism seroreactivity (% of total per year of tested samples)								
Year	*Anaplasma* spp.	*Babesia canis*	*Bartonella henselae*	*Bartonella v. berkhoffii*	*Borrelia burgdorferi*	*Dirofilaria immitis*	*E. canis* (by IFA)	*E. canis* (by Snap® 4Dx®)	*Rickettsia rickettsii*
2004	1.7	1.3	10.6	1.0	6.2	1.0	4.7	5.8	6.1
2005	1.8	2.7	4.4	1.9	5.4	0.5	3.9	4.9	16.0
2006	1.9	2.0	3.8	2.9	6.0	0.8	3.3	5.2	15.2
N	6452	7045	5395	7049	7018	6452	7049	6452	7049

A significant decrease in *B. henselae* seroprevalence occurred from 2004 to 2006 in northeastern states (12%, 2.7% and 4.7%, $p = 0.02$), in southern states (11.5%, 4.6% and 3.8%, $p<0.0001$), especially in the south Atlantic states (DC, DE, FL, GA, MD, NC, SC, GA, and WV) and in Canada (16.3%, 4.7% and 0%, $p = 0.0004$). A significant increase in *B. v. berkhoffii* seroprevalence occurred from 2004 to 2006 in Midwestern states (0.8%, 2.2% and 3.1%, $p = 0.027$), especially in IL, IN, MI, OH, and WI, and in southern states (1.2%, 1.9% and 2.8%, $p = 0.002$), especially in south Atlantic states. There was a significant increase in *R. rickettsii* seroprevalence from 2004 to 2006 in midwestern states (2.2%, 6.9%, and 9.2%, $p<0.0001$) and in southern states (6.7%, 19.7%, and 19.2%, $p<0.0001$), especially in south Atlantic states. *Ehrlichia canis* exposure defined by IFA and Snap® 4Dx® test results were similar in 96.7% of the samples (Kappa: 0.641, CI: 0.596–0.686). Monitoring vector-borne exposures in pets over years is critical for establishing trends and future actions, not only in veterinary but also in human medicine.

ABSTRACT #277

MOLECULAR AND SEROLOGICAL PREVALENCES OF VECTOR-BORNE DISEASES IN CATS FROM MADRID, SPAIN.

T Ayllón[1], PPVP Diniz[2], A Sainz[1], A Villaescusa[1], EB Breitschwerdt[2]. [1]Complutense University of Madrid, Spain. [2]College of Veterinary Medicine, Intracellular Pathogens Research Laboratory, North Carolina State University, Raleigh, NC.

Anaplasma phagocytophilum (*Aph*), *Bartonella henselae* (*Bh*) and *Ehrlichia canis* (*Ec*) are considered emerging or re-emerging diseases in human and veterinary medicine worldwide. In Spain, human bartonellosis, ehrlichiosis and, recently, anaplasmosis have been reported. Classically, non-specific clinical and laboratory abnormalities are induced by infection with these organisms in animals and human patients, which leads to misdiagnosis and artificially low estimates of disease prevalence. Furthermore, limited epidemiological data is available for these organisms in cats from Spain.

The aim of this study was to determine the molecular and serological prevalences of *Aph*, *Bh* and *Ec* in 155 cats examined at the Veterinary Teaching Hospital in Madrid, Spain. Between September, 2005 and May, 2006, blood samples obtained from cats for any diagnostic purpose were entered into the study. Epidemiological data recorded for each cat included: breed, gender, age, access to outdoor environment, contact with other animals, arthropod-exposure history, endoparasite treatments, previous anti-rickettsial treatments and travel history. Antibody reactivity against *Aph*, *Bh* and *Ec* antigens, was determined using an indirect immunofluorescence antibody (IFA) test with cut-off titers of 1:40 for *Aph* and *Ec*, and 1:64 for *Bh*. Using PCR, *Aph* and *Ec* DNA was amplified targeting the 16S rRNA and groESL genes. *Bh* DNA was amplified targeting the intergenic transcribed spacer (ITS) region.

Seroprevalences were *Aph*: 13.5% (21 cats), and *Bh*: 5.2% (8 cats) and *Ec*: 11% (17 cats). Two cats were *Aph* and *Ec* seroreactive, two cats were *Aph* and *Bh* seroreactive and one cat was *Bh* and *Ec* seroreactive. Neither *Anaplasma* spp. nor *Ehrlichia* spp. DNA was amplified from any sample. *Bartonella* spp. DNA was amplified, cloned and sequenced from one sample. When consensual sequences were compared with other GenBank sequences (Nov/2007), the Spanish cat ITS sequence was 100% homologous to *B. henselae* Houston-1 (BX897699). With the exception of an association between the *Aph* seroreactivity and pure breed cats ($p = 0.024$), there were no statistical associations between *Aph*, *Bh*, or *Ec* seroreactivity and epidemiological parameters. These results indicate that a portion of the cat population examined at a Veterinary Teaching Hospital in Madrid, Spain, has been exposed to the vector-borne organisms evaluated in this study. Although seroreactivity to *Anaplasma* spp. and *Ehrlichia* spp. was detected in some cats, there was no molecular evidence of active infection with either genus. Surprisingly, the *B. henselae* serological and molecular prevalence was comparatively low in this cat population. Demographics of the study population, including the majority of subjects being client-owned cats, and changes in vector distribution may justify disagreement with prevalences encountered in other locations in Spain. Since this hospital population does not represent the regional or national feline population, these data cannot be extrapolated to other cat populations or other regions of Spain.

ABSTRACT #278

PREVALENCE OF *BARTONELLA* SPP., *RICKETTSIA FELIS*, AND HAEMOPLASMAS IN THE BLOOD OF CATS AND *CTENOCEPHALIDES FELIS* IN EASTERN AUSTRALIA. VR Barrs[1], JA Beatty[1], JR Hawley[2], N Evans[3], R Baral[4], R Gowan[5], MR Lappin[2]. [1]Faculty of Veterinary Science, The University of Sydney, NSW, Australia. [2]Department of Clinical Sciences, Colorado State University, Ft. Collins, CO. [3]Creek Rd Cat Clinic, Brisbane, QLD, Australia. [4]Paddington Cat Hospital, Sydney, NSW, Australia. [5]The Cat Clinic, Melbourne, VIC, Australia.

The *Ctenocephalides felis*-associated pathogens *Bartonella* spp., *Rickettsia felis*, *Mycoplasma haemofelis* (Mhf), '*Candidatus* M. haemominutum' (Mhm), and '*Candidatus* M. turicensis' (CMt) are common in cats and fleas around the world. *R. felis* is the cause of flea-borne spotted fever, an emerging infectious disease of humans. The objective of this study was to define the prevalence of these organisms in cats and fleas in Sydney, Melbourne and Brisbane, Australia.

Veterinarians in participating clinics collected blood (EDTA), serum, and fleas (at least 2 fleas per cat) from 111 cats with *C. felis* infestations. The samples were stored at −20 °C until shipped to Colorado State University. DNA was extracted from the blood and fleas for performance of previously reported conventional PCR assays that amplify the DNA of *Bartonella* spp., Mhf, Mhm, CMt, and *Rickettsia* spp. Band size was used to determine *Bartonella* spp. Samples giving a band appropriate for Mhf or CMt in the hemoplasma PCR assay and all *Rickettsia* spp. positive samples with adequate DNA were sequenced. Sera were assayed for *Bartonella* spp. IgG by ELISA.

From the 111 cats, DNA of Mhf, CMt, and Mhm was amplified from one cat (0.9%), one cat (0.9%), and 17 cats (15.3%), respectively. Of the 111 flea sets, DNA of Mhm (62 sets; 55.9%) but not Mhm or CMt was amplified. *Bartonella* spp. IgG was detected in 42 cats (37.8%), 11 of these cats (26.2%) were positive for *Bartonella* spp. DNA in blood. Overall, the prevalence rates for *Bartonella* spp. DNA in the cats and the flea sets was 15.3% (17 cats) and 28.8% (32 flea sets), respectively. Some cats and flea sets were infected with *B. henselae* alone, *B. clarridgeiae* alone, or both *B. henselae* and *B. clarridgeiae*. *Rickettsia felis* DNA was amplified from 22 of the flea sets (19.8%), but none of the cat blood samples. Overall, DNA of one or more of the organisms was amplified from 27% (30 of 111) of the cats and 67.6% (75 of 111) of the flea sets.

This is the first study to determine *R. felis* prevalence rates in *C. felis* and the cats from which they were collected in Australia. Results of this study document that *C. felis*-associated infectious agents are common in cats and fleas in Eastern Australia and support the recommendation that stringent flea control be maintained on cats.

ABSTRACT #279

ASSOCIATION BETWEEN FELINE PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATION AND THE PRESENCE OF SERUM ANTIBODIES AGAINST *TOXOPLASMA GONDII* AND *BARTONELLA* SPP. DB Bayliss[1], AK Morris[1], JR Hawley[1], MM Brewer[1], SV Radecki[1], JM Steiner[2], JS Sucholdolski[2] MR Lappin[1]. [1]Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado, CO. [2]Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Feline pancreatitis is commonly diagnosed; however the cause is frequently unknown. It is possible that some cases may be caused by *Toxoplasma gondii* or *Bartonella* spp. infection. Feline pancreatic lipase immunoreactivity (fPLI) is a non-invasive test for pancreatitis. The purpose of this study was to examine the association between fPLI concentration and *T. gondii* or *Bartonella* spp. antibodies in serum from cats.

Serum samples from 464 cats in which fPLI concentrations had been determined were assayed for *T. gondii* IgG, *T. gondii* IgM, and *Bartonella* spp. IgG using ELISA. Logistic regression analysis was used to evaluate the relationship between fPLI concentration and *T. gondii* or *Bartonella* spp. antibodies, with fPLI considered as both a binomial and continuous variable.

Of 179 (38.6%) cats with a serum fPLI concentration suggestive of pancreatitis ($\geq$ 12 µg/L), 15 (8.4%), 13 (7.3%), and 34 (19%) were seropositive for *T. gondii* IgG, *T. gondii* IgM, or *Bartonella* spp. IgG, respectively. Of 285 cats with a serum fPLI concentration $<$ 12 µg/L, 19 (6.7%), 20 (7.0%), and 54 (18.9%) were seropositive for *T. gondii* IgG, *T. gondii* IgM, or *Bartonella* spp. IgG, respectively. Cats with fPLI $\geq$ 12 µg/L were no more likely to be seropositive for *T. gondii* or *Bartonella* spp. than cats with fPLI $<$ 12 µg/L ($P > 0.05$). Additionally, the correlation between fPLI concentration and *T. gondii* or *Bartonella* spp antibodies was not significant ($P > 0.05$).

These results suggest that serological tests for these organisms may not be useful in all cases of feline pancreatitis.

ABSTRACT #280

COMPARISON OF CLONAL RELATEDNESS OF FECAL *ESCHERICHIA COLI* ISOLATES FROM DOGS AND THEIR OWNERS AND EPIDEMIOLOGICAL ANALYSIS OF WITHIN HOUSEHOLD SHARING OF BACTERIA. Stenske KA, Gillespie BE, Oliver SP, Bemis DA, Matteson KJ, Draughon FA, Bartges JW. University of Tennessee, Knoxville, TN.

With the increasing human animal bond, cross-species bacterial transmission has become a concern among pet owners, veterinarians, and public health officials. Improved epidemiologic understanding of bacterial sharing may help minimize this risk. The goals of this study were to determine the prevalence of fecal *E. coli* sharing between dogs and their owners and to analyze potential epidemiological risk factors involved in inter-host transfer.

Fecal swabs and a survey were collected from 61 healthy dog and human owner pairs and a control group. Three representative *E. coli* colonies were isolated from each fecal sample. Pulse field gel electrophoresis (PFGE) using restriction endonuclease XbaI was performed on DNA from each isolate, and similarity matrices were used to compare profiles within households. Surveys questioned frequency of behaviors including sleeping in the same bed, kissing on the face, washing hands after petting and before feeding, disposal of feces, drinking out of toilets, and time spent awake together. Chi-square and Mann-Whitney analysis were used to compare fingerprint and survey results.

A wide array of *E. coli* PFGE profiles was observed in all groups. Fecal *E. coli* isolates from only one dog-owner pair had identical profiles. Isolates from twelve other dog-owner pairs had $>$ 90% fingerprint similarity. No behaviors surveyed were found statistically more often in households with $>$ 90% fecal *E. coli* similarity than households without similar strains.

Although sharing of genetically similar fecal *E. coli* is uncommon between dogs and their owners, proper hygiene should be encouraged to minimize potential transmission.

ABSTRACT #281

COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF FECAL *ESCHERICHIA COLI* FROM HEALTHY DOGS AND THEIR OWNERS. Stenske KA, Bemis DA, Matteson KJ, Draughon FA, Bartges JW. University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

Routine contact between human beings and companion animals may allow transmission of pathogenic and antimicrobial resistant bacteria between species. The goal of this study was to determine antimicrobial susceptibilities of *E. coli* isolates from healthy dogs and their owners.

Fecal swabs were collected from 61 healthy dog and human owner pairs and a control group of non-dog-owners. Volunteers were excluded if they had received antimicrobial therapy within 2 weeks. Three representative *E. coli* colonies were isolated from each sample. Susceptibilities were determined using a disc diffusion method for 17 antimicrobials included in the National Antimicrobial Resistance Monitoring System.

In all groups, lowest percent susceptibility was to cephalothin (48% susceptibility in dogs, 59% in owners, 60% in controls), ampicillin (67% in dogs, 61% in owners, 50% in controls), and amoxicillin-clavulanic acid (80% in dogs, 87% in owners, 80% in controls). Imipenem was the only antimicrobial to which all isolates were susceptible. Susceptibility patterns of isolates from dog owners and controls, and from paired dog owners and dogs, were compared using Chi-square and McNemar Chi-square analyses, respectively,

and no significant differences were found between groups or within households. Multiple drug resistance (MDR), defined as resistance to 3 or more antimicrobial agents, was seen in 4/61 (7%) dogs, 10/61 (16%) owners, and 5/30 (17%) controls; differences between groups were not significant ($P = 0.10$).

In conclusion, antimicrobial resistance, including MDR, was common among fecal *E. coli* isolates from healthy dogs and human beings. Dog ownership did not increase risk of harboring resistant fecal *E. coli*.

ABSTRACT #282

MULTI-DRUG RESISTANCE IN FECAL *ESCHERICHIA COLI* FOLLOWING ROUTINE ENROFLOXACIN BUT NOT AMOXICILLIN THERAPY IN DOGS. N Debavalya[1], DM Boothe[1], and T Hathcock[2]. [1]Department of Anatomy, Physiology and Pharmacology and [2]Department of Pathobiology, Auburn University, Auburn, AL.

Increased prevalence of fluoroquinolones (FQs) therapy-induced FQ resistant microbes has become a major concern in both human and veterinary medicine. Antimicrobial resistance has emerged in pathogenic organisms, as well as commensal organisms. This includes the gastrointestinal tract, with *Escherichia coli* among the organisms for which MDR has emerged. Based upon our pervious work, resistance to FQs rapidly appears as part of MDR expressed in clinical *E. coli* isolates cultured from patients receiving FQs.

The purpose of this study was to demonstrate and characterize, emerging resistance, using fecal *E. coli* as sentinel organisms, after routine use of two popular antimicrobials. Purpose-bred drug free hound dogs (n = 8 per group) maintained on a standard diet were studied. Dogs were dosed with either amoxicillin (G1, 10 mg/kg every 12 hr), enrofloxacin (G2, 5 mg/kg every 24 hr) or not dosed (control; G3) until the resistance to the treatment drug emerged. Dogs were studied for a minimum of 7 and a maximum of 21 d. Fecal samples were collected per rectum at baseline (day that drug administration began; B), and every 3 days until resistance ($\geq$ 75% of CFU resistant; T). Drug was then discontinued and monitoring continued weekly up to 4 weeks, or until the resistance resolved ($\leq$25% of CFU resistant; E), whichever came first. Husbandry was implemented to minimize mechanical transmission. Outcome measures, determined at 3 time points (B, T and E), included: Total coliform counts, total *E. coli* counts, percent *E. coli* and percent *E. coli* resistant to either drug, MIC_{90} (using Etest®), and the presence of MDR (yes or no) based on a commercial 13 drug panel antibiogram.

No resistance was present in any dog at time B. Resistance to amoxicillin developed in both G1 (6 d; n = 8) and G2 (9d; n = 4), but not G3. Resistance to enrofloxacin emerged in G2 (D 9; n = 4), but in neither G1 nor G3. MIC_{90} for resistant isolates exceeded the breakpoint MIC for either drug by at least 8 fold. MDR did not develop in association with amoxicillin resistance. In contrast, all isolates resistant to enrofloxacin exhibited MDR. Amoxicillin resistance generally resolved by 2 weeks after completion of amoxicillin therapy in both G1 and G2, whereas enrofloxacin resistance resolved in varied times from 1 to more than 4 weeks in G2. Interestingly, *E. coli* was undetectable by day 9 and remained undetectable in 4 G2 dogs. These data indicate that both antimicrobial therapies facilitated the emergence of high-level resistance using recommended dosing regimens, and that resistance to enrofloxacin tends to be associated with MDR. This may reflect the synthetic nature of FQs: baseline resistance is likely to be low because isolates are not exposed to FQs under natural conditions, but once exposed, resistance involves complex mechanisms leading to MDR. This study suggests that use of FQ might be used judiciously due to its apparent facilitation of emergent MDR in *E. coli*.

ABSTRACT #283

PHENOTYPIC AND GENOTYPIC EXPRESSION OF ANTIMICROBIAL RESISTANT FECAL *ESCHERICHIA COLI* IN DOGS FOLLOWING ROUTINE ANTIMICROBIAL THERAPY. N Debavalya[1], S Suh[2], O. Oyarzabal[3], and DM Boothe[1]. [1]Department of Anatomy, Physiology and Pharmacology, [2]Department of Biological Sciences, and [3]Department of Poultry Science, Auburn University, Auburn, AL.

E. coli increasingly is emerging as an organism expressing multidrug resistance (MDR) in dogs or cats. We have demonstrated that administration of enrofloxacin at routine doses results in the emergence of *E. coli* isolates exhibiting MDR, whereas amoxicillin at routine doses does not lead to MDR. The purpose of this study is to further characterize the phenotypic and genotypic expression of resistance in isolates in which non-MDR versus MDR has emerged following treatment with either amoxicillin at 10 mg/kg orally twice daily (n = 8 dogs; no MDR detected) or enrofloxacin at 5 mg/kg orally once daily (n = 8 dogs; only MDR detected; see sister abstract and poster). Ten *E. coli* isolates were randomly selected from the population of resistant isolates recovered from each dog receiving either drug. Isolate phenotype status was determined by a commercial antibiogram (Vitek®; Amoxicillin = A, Amoxicillin/clavulanic acid = X, Ceftiofur = C, Tetracycline = T, Enrofloxacin = E, Gentamicin = G, Sulfa/trimethoprim = S). Isolates collected from dogs treated with the same drug were subgrouped based on phenotype. For either drug, all resistant isolates cultured from the same dog were phenotypically similar. For amoxicillin treated dogs, 3 phenotypes were present among the 8 dogs studied: AC (G1), ACT (G2) and AT (G3). For enrofloxacin treated dogs, 2 MDR phenotypes were present among the 8 dogs: ATE (G4) and AXTEGS (G5) respectively.

Three representative isolates from each group were subjected to pulse-field gel electrophoresis (PFGE) with *XbaI* restriction for genotype analysis. For G1 and G2, genotypes were the same (based on 90% homology) despite differing phenotypes. For G3 (AT phenotype, representing 3 amoxicillin treated dogs), 3 distinct genotypes emerged, one in each dog. For enrofloxacin treated dogs, each phenotype was genetically distinct. These data support clonal expansion of resistant isolates (e.g. only one phenotype/genotype emerged in each dog) for both drugs. However, amoxicillin resistance tended to be genotypically similar among dogs despite phenotypic differences in dogs, whereas enrofloxacin resistance tended to be both genotypically and phenotypically different among dogs. These data suggest that routine antimicrobial use impacts on the emergence of antimicrobial resistance, with clonal expansion supportive of selection pressure as a mechanism of emergent resistance. Further, genotypes may not be related to phenotypes for some drugs (e.g. enrofloxacin). The differences in genotypes for enrofloxacin may contribute to the MDR.

ABSTRACT #284

THE ASSOCIATION OF CLASS 1 INTEGRONS IN CANINE AND FELINE *ESCHERICHIA COLI* ISOLATES EXPRESSING MULTIDRUG-RESISTANT PHENOTYPES. BW Shaheen[1], DM Boothe[1], OA Oyarzabal[2], T Smaha[1]. [1]Department of Anatomy, Physiology, and Pharmacology, [2]Department of Poultry Science, Auburn University, Auburn, AL.

Resistant *E. coli* is emerging as an organism that develops multidrug resistance (MDR) in the face of antimicrobial therapy, as we have documented previously in dogs and cats with spontaneous infections (ACVIM 2007). Integrons associated with plasmids may carry genes that impart MDR. Mechanisms conferred by intergrons include altered cell permeability drug efflux or an increased rate of mutation that confers other mechanisms of resistance. The purpose of this study was to investigate the potential role of class 1 integrons in the emergence of MDR in canine and feline *E. coli* clinical isolates collected from 4 different regions in US. A total of 377 *E. coli* isolates associated with spontaneous (presumed) infection were collected from dogs or cats between May and September 2005. Susceptibility to 7 drugs was determined using the E-test® according to CLSI guidelines and interpretive standards: amoxicillin, amoxicillin-clavulanic acid, cefpodoxime, doxycycline, enrofloxacin, gentamicin, trimethoprim-sulfamethoxazole. A total of 32 *E. coli* isolates representing 7 different phenotypes were randomly selected to determine the presence of class 1 integrons, and, in order to determine the location of the integron, plasmids. Phenotypes included those resistant to no drug (n = 10), resistance to one drug (SDR; n = 8) or resistance to more than one drug class (MDR; n = 14, with n = 12 for all 7 drugs tested). Class 1 integrons were characterized for the 5′ and 3′ conserved segments (CS) by polymerase chain reaction (PCR) and direct sequencing. PCR analysis revealed that 59.4% (19/32) of the isolates contained class 1 integrons. MIC_{90} for integron-positive isolates were more than four fold higher than the break

point for all drugs tested. This is in contrast to integron-negative isolates for which MIC were below the breakpoints for doxycycline, enrofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. Integron- positive isolates were also more likely to be multi-resistant 73.7% (14/19); only 1 of the MDR isolates did not have a class I integrons. This is in contrast with integron-negative isolates for which 7.7% (1/13) were MDR. However, three isolates with no resistance also carried the integrons. The majority 94.7% (18/19) of classes 1 integrons were detected in the plasmids of the same integron-positive isolates. This association indicates that integrons may confer high MIC level, multidrug resistance. However, the presence of the integron is not necessarily associated with phenotypic manifestation of the genetically-directed resistance. Class 1 integrons might play an important role in contributing to the horizontal transfer of antimicrobial resistance.

ABSTRACT #285
CHARACTERIZATION OF CLINICAL *ESCHERICHIA COLI* ISOLATES EXPRESSING MULTIDRUG RESISTANCE RECOVERED FROM CANINE AND FELINE WITH SPONTANEOUS DISEASE. BW Shaheen[1], DM Boothe[1], OA Oyarzabal[2], T Smaha[1]. [1]Department of Anatomy, Physiology, and Pharmacology, [2]Department of Poultry Science, Auburn University, Auburn, AL.

The incidence of multidrug resistant *E. coli* as a cause of infection in companion animals is increasing. The purpose of this study was to phenotypically and genotypicically describe a sample population of *E. coli* associated with spontaneous disease in dogs and cats. A total of 377 isolates studied between May and September 2005. Isolates were collected from 4 different regions: West, South, Midwest, and Northeast. The isolates were phenotypically characterized based on susceptibility to 7 antimicrobial agents by E-test® according to CLSI guidelines and interpretive standards: amoxicillin (A), amoxicillin-clavulanic acid (X), cefpodoxime (P), doxycycline, enrofloxacin, gentamicin, trimethoprim-sulfamethoxazole. Isolates from each phenotype were randomly selected for genotypical characterization using pulse-field gel electrophoresis (PFGE) using *XbaI* restriction enzyme; dendrograms were generated using the Dice correlation coefficient and the unweighted pair group mathematical average clustering algorithm of BionumericsTM. Of the 377, 183 were susceptible to all drugs, with the proportion greatest in the west (68%; 49/79) and least in the south (37.5%; 50/133). Isolates collected from the ear were characterized by the greatest proportion of susceptibility (70%; 19/27) whereas the skin had the least number of isolates susceptible to all drugs (41%, 11/27). Of the 194 isolates expressing resistance, 85 expressed single drug resistance (SDR), with resistance to beta-lactams (any combination of A, X or P) predominating (83%; n = 71/85 [30% A, 28% X, and 24% AXP]. Only two isolates expressed SDR to E. The remaining 109 isolates expressed MDR (resistance to more than one drug class), with resistance to all 7 drugs (Z) representing the largest proportion (18.3%; 20/109). The remaining MDR were represented by 34 different phenotypes. The drugs most commonly involved in MDR (n = 109) were A (96.3%), X (85%) and E (61%). Of isolates expressing resistance, MDR was greater in the south region (67.9%; 57/84 MDR) and least in the west, (46.6%, 14/30 MDR). Among infection sites, isolates cultured from the skin were characterized by the highest percent of MDR (12/16; 75%). A total of 82 PFGE patterns were generated among the 90 isolates for which PFGE was determined. Dendrograms revealed 5 profiles (based on $\geq$ 90% similarity), representing 14 isolates, 6 tissues, and 2 regions. These results indicating extensive genetic diversity across recovered *E. coli* isolates, regardless of resistance phenotype, and phenotypes and genotypes are not related. The south appears to have a greater incidence of resistance, including MDR, and the west the least for both. MDR most commonly involves amoxicillin with or without clavulanic acid, and enrofloxacin.

ABSTRACT #286
COMPARISON OF MULTI-LOCUS VARIABLE NUMBER TANDEM REPEAT ANALYSIS TO PCR-RIBOTYPING FOR THE CLASSIFICATION OF *CLOSTRIDIUM DIFFICILE* ISOLATES. C.E. Medina-Torres[1], J.W. Marsh[2], H. Staempfli[1], L.G. Arroyo[1], H. Martin[1], A. Rodriguez[1], J.S. Weese[1]. [1]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Infectious Diseases Epidemiology Research Unit, University of Pittsburgh School of Medicine and Graduate School of Public Health, Pittsburgh, PA.

Clostridium difficile is an important enteropathogen in humans and various domestic animal species. Concern has been expressed about the potential for interspecies transmission of *C. difficile*. The use of molecular typing methods is critical for elucidating the potential for interspecies transmission, however there is no consensus regarding optimal methods. PCR-ribotyping is widely used, but it may not have optimal discriminatory power and inter-laboratory comparisons remain difficult. Other molecular typing methods are labor-intensive, subjective, or lack the discriminatory power to differentiate between closely related strains. Multi-locus Variable-Number Tandem-Repeat Analysis (MLVA) is an attractive alternative, which is objective, can be standardized to have good inter-laboratory reproducibility, and is amenable to automation. The objectives of the present study were to use MLVA to assess genetic relatedness between *C. difficile* ribotypes of human, environmental, and animal origin.

DNA from 102 previously ribotyped isolates from humans (n = 39), dogs (16), horses (15), cattle (11), pigs (8), cats (2), sheep (2), elk (1) and the environment (8) were used. PCR-MLVA was performed using 7 selected *C. difficile* repeat (CDR) loci. The number of copies at each of the seven CDR loci were concatenated to generate an MLVA type for each isolate, and these were clustered according to MLVA results to establish their genetic relationship. A minimum-spanning tree (MST) was generated using the Bionumerics 4.01 software (Applied Maths, Austin, TX).

Eight clusters of MLVA types with summed tandem repeat difference (STRD) $\leq$ 10 were generated by the MST. The largest cluster consisted of 18 isolates of varying species and ribotypes. A second closely related cluster (n = 11) was comprised of multiple ribotypes from a majority of human isolates, several canine isolates, 1 environmental and 1 equine isolate. The third cluster consisted of 2 closely related isolates with STRD = 3. Both of these isolates were recovered from calves and belonged to different ribotypes. Cluster 4 (n = 8), was comprised of a variety of species and ribotypes. Five isolates from 4 species within this cluster had the same MLVA type. Cluster 5 (n = 13) consisted of non-toxigenic isolates from different species. Cluster 6 contained 1 human and 1 equine isolate belonging to the hypervirulent 027/BI/NAP1 clone. Cluster 7 consisted of 1 canine and 1 equine isolate, and cluster 8 contained 2 porcine isolates with identical MLVA type.

The study demonstrated that PCR-MLVA might provide useful information to sole or combined molecular typing of *C. difficile*. The finding of isolates from multiple species within the same MLVA cluster supports the concerns for interspecies transmission of this bacterium.

ABSTRACT #287
SENSITIVITY AND SPECIFICITY OF THE MICROSCOPIC AGGLUTINATION TEST FOR THE DIAGNOSIS OF LEPTOSPIROSIS IN DOGS. MD Miller[1], KM Annis[1], MR Lappin[1], M Gill[2], KF Lunn[1]. [1]Department of Clinical Sciences, Veterinary Medical Center, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. [2]Fort Dodge Animal Health, Fort Dodge, IA.

Leptospirosis in dogs can be diagnosed by detecting the organism by PCR or culture, or by demonstrating a fourfold rise in MAT titers. A single MAT titer of $\geq$ 1:800 with compatible clinical signs and laboratory abnormalities is also considered diagnostic of leptospirosis. The purpose of this study was to determine the sensitivity and specificity of an initial MAT titer of $\geq$ 1:400 or $\geq$ 1:800 at different laboratories for diagnosing leptospirosis.

Initial MAT titers were measured at 1 to 5 laboratories in 10 dogs with leptospirosis confirmed via a fourfold rise in MAT titers or positive PCR, in 18 SPF dogs, and in 16 dogs with clinical signs and laboratory abnormalities consistent with leptospirosis in which an alternate diagnosis was confirmed. Sensitivity and specificity at titers of $\geq$ 1:400 and $\geq$ 1:800 for any serogroup were calculated for each laboratory.

Sensitivity of an initial titer of $\geq$ 1:400 ranged from 50% to 67%. Sensitivity of an initial titer of $\geq$ 1:800 ranged from 22% to 67%.

Specificity at an initial titer of ≥ 1:400 or ≥ 1:800 was 100% at all laboratories for identifying SPF dogs. Specificity at a titer of ≥ 1:400 ranged from 69% to 93% and at an initial titer of ≥ 1:800 ranged from 69% to 100% in ill dogs without leptospirosis.

Sensitivity of an initial MAT titer varied between laboratories and at different cutoffs. False positives did not occur in SPF dogs but occurred in sick dogs that do not have leptospirosis.

ABSTRACT #288
FREQUENCY OF ADMINISTRATION OF SPECIFIC ANTIGENS TO CATS BY VETERINARY SURGEONS IN THE UNITED KINGDOM. R Dean[1], D Pfeiffer[2], D Mellor[3], V Adams[1]. [1]Animal Health Trust, Newmarket. UK. [2]Royal Veterinary College, Hertfordshire. UK. [3]Faculty of Veterinary Medicine, University of Glasgow, Glasgow, UK.

The antigens commonly used to vaccinate cats in the United Kingdom (UK) have traditionally been administered annually. Controversy regarding the frequency of vaccine administration has arisen following concerns regarding the 'over-vaccination' of companion animals and vaccine safety.

A questionnaire concerning vaccination protocols was distributed to 366 practices in the UK as part of an epidemiology study about feline injection site sarcomas. One of the questions asked how frequently each practice recommends vaccinating cats against each of the commonly used antigens: feline herpes/calicivirus (FHV/FCV), feline panleukopenia (FPV), feline leukaemia (FELV), *Chlamydophila* (CHL) and rabies (RAB).

The response rate was 60% (219/366) after 2 reminders. Not all practices provided an answer for every antigen. 96.8% (212/219) of practices administer FHV/FCV annually. 89.5% (196/219) of practices administer FPV vaccines annually, 3.7% (8/219) biannually and 3.7% (8/219) tri-annually. 84% (184/219) of practices administer FELV vaccines annually, 0.5% bi-annually and 14.2% decide to vaccinate against FELV on an individual cat basis. 20.3% (44/217) of practices administer CHL vaccines annually, 31.8% never use CHL vaccines, 11.1% use the vaccine in breeding households only, and 21.7% decide to vaccinate against CHL on an individual cat basis. 2.8% (6/218) of practices administer RAB vaccines annually, 8.3% (18/218) bi-annually and 22% (48/218) tri-annually. The majority of practices only use RAB vaccines if the patient is going to travel abroad.

These data suggest that the majority of practices in the UK still commonly administer FHV/FCV, FPV and FELV vaccines annually RAB and CHL vaccines are used less frequently.

ABSTRACT #289
SYNDROMIC SURVEILLANCE FOR HOSPITAL-ASSOCIATED INFECTIONS IN A SMALL ANIMAL REFERRAL HOSPITAL. J McDonell[1], S Lefebvre[2], P Morley[3], JS Weese[4]. [1]Veterinary Emergency Clinic, Toronto, Ontario, [2]University of Guelph, Guelph, Ontario, Canada, [3]Colorado State University, Fort Collins, CO.

Hospital-associated (HA) infections are an inherent risk of hospitalization. While the field has been studied extensively in human medicine, comparatively little veterinary information is available. One limitation with monitoring of HA infections is rapid and easy identification of potential problems. Syndromic surveillance, which evaluates general clinical syndromes as opposed to specific diseases, is easy, cost-effective, and possible to incorporate into electronic medical record systems. This study evaluated the use of syndromic surveillance to determine the frequency of selected syndromes at a small animal referral hospital and to evaluate factors associated with those syndromes.

All animals that were hospitalized for greater than 1 calendar day between Oct 1, 2006 and Mar 31, 2007 at a private referral and emergency hospital were included. A standardized checklist was posted with each animal's medical record and animals that developed signs consistent with following syndromes were identified prospectively: intravenous (IV) catheter site inflammation, urinary tract infection (UTI), surgical site infection (SSI), acute respiratory or gastrointestinal infection, fever of unknown origin (FUO), and septicemia. Associations between factors and syndromes were evaluated using various statistical tests.

Ninety-two dogs and 33 cats were eligible for inclusion. Mean age of all animals was 8.1 years (SD 4.5, range 0.6–20). Mean duration of hospitalization for the 112 animals for which data were recorded was 6.0 days (SD 5.0, range 1–34). One or more syndrome was identified in 24% of animals. Of these animals, IV catheter inflammation/infection was recorded for 9.8%, UTI for 7.1%, acute gastrointestinal illness for 8.1%, FUO for 5.4%, SSI for 3.6%, acute respiratory infection for 1.8%, and sepsis for 0.9%. Cats were 37.1 times as likely to develop FUO as dogs were ($P < 0.001$). No other differences between species were detected. Animals that developed a FUO were 8.2 times as likely to have received a surgical implant as other animals were($P = 0.016$). Those that developed a UTI were 104 times as likely to have received a urinary catheter as the others were ($P < 0.001$). Animals that developed SSIs were 9.5 times as likely to have received perioperative antimicrobials as other animals ($P = 0.043$). Duration of hospitalization was significantly ($P = 0.026$) longer for animals that developed any syndrome; however, it is unclear whether that association represents cause or effect. Similarly, animals with IV catheter inflammation or UTIs were hospitalized longer than others were ($P = 0.01$ and 0.001, respectively).

Syndromic surveillance was easily performed and provided useful baseline information. While syndromic surveillance cannot confirm the origin of infection or even that infection had occurred, it can be used to monitor syndromic rates over time to more rapidly identify potential infectious disease outbreaks and facilitate comparisons between different hospitals or services.

ABSTRACT #290
EFFICACY OF A SINGLE DOSE OF AN OTIC IVERMECTION PREPARATION OR SELAMECTIN FOR THE TREATMENT OF *OTODECTES CYNOTIS* INFESTATION IN NATURALLY INFECTED CATS. L Nunn-Brooks, R Michael, L Ravitz, MR Lappin. Department of Clinical Sciences, Colorado State University, Ft.Collins, CO.

Otodectes cynotis infestation is common in kittens housed in crowded environments. Safe, inexpensive, and rapid acting treatments should be used to potentially lessen spread. It is unknown how rapidly *O. cynotis* is killed within the first 72 hours of treatment. The objective of this study was to describe the speed to kill of a two compounds with known activity against *O. cynotis*.

Kittens > 4 weeks of age that were shown to have live *O. cynotis* AU were accepted into the study. Each kitten was administered 0.5 ml of 0.01% Ivermectin otic suspension (Acarexx®, IDEXX Pharmaceuticals), once, AU or selamectin (Revolution®, Pfizer Animal Health) once, on the skin following the manufacturers' instructions. Repeat cytologic examination was performed on individual ears based on a randomization schedule at 6, 12, 24, 36, 48, or 72 hours after treatment. A drug was considered effective if there were dead mites or no evidence of mites on repeat examination.

Dead mites were detected as early as 12 hours after administration of either drug (ivermectin, 2 of 7 ears; selamectin, 2 of 8 ears).

Percentage of ears with dead mites during combined recheck times:

Combined recheck times	Ivermectin	Selamectin
6–24 hours	22.2% (4/18)	9.5% (2/21)
36–72 hours	66.7% (12/18)	42.9% (9/21)

Results between groups were not statistically different by Fischer's exact test.

There was no evidence of toxicity noted with either drug. The results suggest that both drugs have an effect against *O. cynotis* as early as 12 hours after administration with an increasing effect over time.

ABSTRACT #291
CELLULAR AND MOLECULAR ANALYSIS OF HORMONE PRODUCTION AND GENE EXPRESSION IN A FELINE INSULINOMA. TC Jackson[1], B DeBey[1], S Lindbloom-Hawley[1],

B Jones[2], T. Schermerhorn[1]. [1]Kansas State University, College of Veterinary Medicine, Manhattan, KS. [2]The Animal Clinic, Hastings, NE.

Feline insulinoma is a rarely reported functional endocrine pancreatic tumor that produces hypoglycemia via excessive insulin secretion. Clinical signs, laboratory findings, and tumor histopathology in reported feline insulinoma cases are similar to those recognized in dogs with insulinoma. However, little is known about pathways responsible for the functional activity of these tumors. While some information is available about the pattern of hormone production exhibited by feline insulinomas, the molecular mechanisms that underlie the exaggerated insulin secretory response by the tumor have not been investigated. This study undertook analysis of a naturally-occurring feline insulinoma with these objectives: 1) determine the pattern of hormone expression exhibited by the tumor; 2) characterize tumor expression of select genes with important roles in glucose recognition and insulin secretion.

A pancreatic tumor obtained at the time of surgical resection from a cat with clinical signs and laboratory evidence of hypoglycemia and hyperinsulinemia was used for these studies. The clinical diagnosis of insulinoma was confirmed by histologic examination of the tumor. Normal (non-neoplastic) pancreatic tissue from the same cat served as control tissue for gene expression studies. Expression of select pancreatic peptide hormones was determined using immuno-histochemistry (IHC). Expression of *GLUT2* (glucose transporter isoform 2), *INS* (insulin), *HK1* (hexokinase 1), and *GCK* (glucokinase) genes was determined in tumor and control pancreas by routine RT-PCR and quantitative RT-PCR (qRT-PCR).

The tumor stained positive for chromogranin A and insulin. About 5% of tumor cells stained positive for somatostatin. Tumor staining for glucagon and pancreatic polypeptide was negative. *GLUT2*, *INS*, *HK1*, and *GCK* expression was detected by RT-PCR in tumor and in control pancreas. Quantitative expression analysis using qRT-PCR showed that tumor *GCK* expression was ~20-fold higher than in normal pancreas, while *HK1* expression was equivalent in both tissues. The relative ratio of *GCK* to *HK1* (*GCK*:*HK1* ratio) was > 20-fold higher in tumor than in normal pancreas.

In conclusion, the study results add to current understanding of feline insulinoma and offer new insights into the functional activity of these tumors. The most important finding is that *GCK*, a key gene involved in glucose recognition, is overexpressed in feline insulinoma. *GCK* overexpression is predicted to enhance glucose sensitivity in tumor cells, which may partly explain persistent insulin secretion despite hypoglycemia in cats with insulinoma.

ABSTRACT #292

RESTING AND STIMULATED CORTISOL CONCENTRATIONS IN DOGS WITH HYPOGLYCEMIA ASSOCIATED WITH INSULINOMA. AM Boag[1], AK Boag[2], LM Freeman[1], OM Mahony[1], EA Rozanski[1]. [1]Tufts University Cummings School of Veterinary Medicine, North Grafton, MA; [2]Queen Mother Hospital for Animals, Department of Veterinary Clinical Sciences, Royal Veterinary College, Hatfield, UK.

Hypoglycemia is a potent physiological stimulus for cortisol release; both insulin-producing tumors and hypoadrenocorticism can result in moderate to severe hypoglycemia. A recent study reported that hypoadrenocorticism may be practically excluded, without an ACTH-stimulated cortisol concentration, if the resting cortisol concentration was > 2 μg/dl. (Lennon et al, JAVMA 2007 231:413–416) The purpose of this study was to evaluate the resting and ACTH-stimulated cortisol concentrations in hypoglycemic dogs that were subsequently established to have an insulinoma as the cause of their hypoglycemia. The hypothesis was that severe hypoglycemia due to insulinoma would result in a marked increase in basal cortisol concentrations and that resting plasma cortisol concentration > 2 μg/dl would preclude the need for ACTH stimulation testing.

The medical records of the Foster Hospital for Small Animals (FHSA) and the Queen Mother Hospital for Animals (QMHA) were searched for dogs with a confirmed diagnosis, by both histopathology and a positive insulin/glucose ratio, of insulinoma.

Eight dogs were identified with insulinoma that also had an ACTH stimulation test performed during diagnostic testing. The dogs were a variety of breeds with a mean age of 8.9 ± 1.9 years. The mean glucose value was 42.4 ± 12.3 mg/dl. The basal cortisol concentration was 4.8 ± 3 μg/dl and all dogs had a normal response to ACTH stimulation with a mean stimulated cortisol concentration of 15.3 ± 6.2 μg/dl. Two dogs (25%) had a basal cortisol of < 2 μg/dl (1.5 μg/dl and 1.8 μg/dl). There was no correlation between blood glucose and basal cortisol.

Dogs with insulinoma have a normal, not supraphysiologic, basal cortisol concentration, and ACTH-stimulated cortisol responses are appropriate. A basal cortisol level < 2 μg/dl is not sufficient to identify inadequate adrenal function in dogs with hypoglycemia. Therefore an ACTH stimulation test is warranted in dogs with unexplained hypoglycemia, especially when both hypoadrenocorticism and insulinoma remain possible diagnoses.

ABSTRACT #293

INSULIN SECRETION IS PRESERVED IN CANINE INSULINOMA CELLS MAINTAINED IN SHORT-TERM CULTURE. T Schermerhorn, O Suwitheechon. Kansas State University College of Veterinary Medicine, Manhattan, KS.

Canine insulinoma is a functional endocrine pancreatic tumor characterized by autonomous insulin secretion that is not inhibited by hypoglycemia. The highly functional nature of canine insulinoma suggests that this tumor could be useful model for investigating cellular and molecular aspects of insulin secretion. The study objectives were to develop techniques for isolation and culture of canine insulinoma cells and to assess the capacity for *in vitro* insulin secretion.

Tissue from three dogs undergoing pancreatic tumor resection was used to establish *in vitro* cultures of insulinoma cells. For cell isolation, tumor tissue was finely minced with a razor and passed through a steel mesh to further disrupt tissue. Disrupted tissue was collected by centrifugation, re-suspended into RPMI media (10% FBS), and plated in multiwell plastic plates. Cells were maintained in 95% air/5% CO2 at 37C with media changes every 3 days.

All tumors studied had a histological diagnosis of insulinoma. Light microscopy performed immediately after plating revealed the technique yielded a mixture of single cells and small clusters (2–20 cells). Cytospin preparations of cultured cells performed 1 and 10-weeks after isolation stained (+) for insulin. ELISA performed on culture media was (+) for insulin up to 10 weeks after establishment in culture. At 10-weeks, insulinoma cells showed an increase in insulin secretion after stimulation with 20 mM glucose.

In conclusion, canine insulinoma cells were successfully maintained in short term culture. Cultured insulinoma cells were functional as evidenced by cellular insulin content, secretion of insulin into the culture media, and preserved glucose responsiveness.

ABSTRACT #294

STRUCTURAL CHARACTERIZATION AND PARTIAL SEQUENCE OF THE FELINE GLUCOKINASE GENE. V. Vandersande, S. Lindbloom-Hawley, M. LeCluyse, T. Schermerhorn. Dept. Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS.

The glucokinase gene (*GCK*) is primarily expressed in hepatocytes and pancreatic islets, where it is needed for normal glucose metabolism. Genetic polymorphisms and mutations in *GCK* influence glucose tolerance and can produce a diabetic phenotype. Knowledge of the *GCK* gene will facilitate molecular and genetic studies of feline glucose metabolism, nutrition, and diabetes. Previous work in our laboratory has elucidated the coding sequence of feline pancreatic GCK cDNA (GenBank #EF121813) but feline *GCK* has not been studied at the genomic level. The study objective was to obtain the genomic DNA sequence of feline *GCK*. DNA isolated from peripheral leukocytes from a normal cat and routine PCR and sequencing methods were used to obtain the *GCK* sequence.

Alignment of overlapping genomic DNA clones (each sequenced 2–4×) generated by PCR using feline-specific primers produced a 3522 bp partial *GCK* sequence. The feline genomic *GCK* sequence contained 9 putative exons, which were identified by homology with the known feline pancreatic *GCK* cDNA sequence, and intervening

intronic sequences. Feline exon sequences and lengths were highly conserved compared with human *GCK* (overall nucleotide identity was 93%); intronic sequences were less conserved. The linear arrangement of exons 2–10 in feline *GCK* is also found in rodent and human *GCK*, indicating that gene structure is conserved in this region. In humans and rodents, expression of hepatic- and pancreatic-specific *GCK* mRNAs is accomplished via use of separate exon 1 isoforms. The existence of multiple exon 1 isoforms was not confirmed by the current study but preliminary analysis shows that feline *GCK* has a homologue to the human pancreatic specific exon 1B.

In conclusion, the primary nucleotide sequence and organization of the feline *GCK* gene appears similar to other species. The feline *GCK* sequence that we have elucidated can serve as a reference sequence for future studies of GCK polymorphisms or mutations within the feline population.

ABSTRACT #295
Abstract withdrawn.

ABSTRACT #296
EVALUATION OF A CONVENTIONAL URINE GLUCOSE TEST STRIP METHOD FOR DETECTION OF GLUCOSURIA IN DOGS AND CATS. EN Behrend[1], J Tapia[1], EG Welles[2], J Suddeth[2]. [1]Department of Clinical Sciences, [2]Department of Pathobiology, Auburn University, College of Veterinary Medicine, Auburn AL.

Measurement of urine glucose concentration can be important for disease detection as well as for monitoring disease control, e.g. diabetes mellitus. Point-of-care testing such as the Bayer Multistix™ is often used; however, the overall accuracy of the strips in canine and feline urine has not been evaluated. Therefore, the purpose of this study was to assess the sensitivity, specificity and positive and negative predictive values of the Bayer Multistix™ for detection of glucosuria in canine and feline urine.

Urine samples submitted to the Auburn University Clinical Pathology Laboratory for urinalysis were collected over a 3-month period; 258 and 98 canine and feline samples, respectively, were used. The Multistix reagent strips were used according to the manufacturer's instructions. Analyte reactions were determined by means of a Clinitek 50 Analyzer (Bayer). The glucose concentration in all samples was quantified with a Hitachi 911 Chemistry Analyzer (Boehringer Mannheim Corp.) and considered to be the true concentration (gold-standard). Reference intervals that correlate with the colors on the test strip were devised based on the package insert (negative = $\leq$75 mg/dL; trace = 76–175 mg/dL; 1+ = 176–375 mg/dL; 2+ = 376–750 mg/dL; 3+ = > 750 mg/dL). All samples with concentrations $\geq$76 mg/dL were deemed positive.

Sensitivity for the conventional test strip for glucosuria in canine and feline samples was 15% and 73%, respectively. Specificity for the conventional test strip for glucosuria in canine and feline samples was 99% and 97%, respectively. The positive predictive values for detection of glucose were 90% and 73% and the negative predictive values were 59% and 97% for dogs and cats, respectively. Overall, the accuracy of the test strip for classifying the concentration of a sample in the correct interval was 59% in dogs and 91% in cats. Of the misclassifications, 101/106 (95%) and 5/9 (56%) were underestimations in dogs and cats, respectively.

Therefore, the strips appear to be more accurate in cats than in dogs for detection and quantization of glucosuria, and inaccuracies tend to be underestimations. In dogs, the test strips have a high percentage of false negative results for detection of glucosuria.

ABSTRACT #297
EVALUATION OF INTENSIVE BLOOD GLUCOSE CONTROL USING GLARGINE IN DIABETIC CATS. K Roomp[1], JS Rand[2]. [1]Max Planck Institute for Informatics, Saarbruecken, Germany. [2]Centre for Companion Animal Health, University of Queensland, Australia.

There are no reported studies of the outcome of long-term intensive blood glucose control in diabetic cats. The aim of this study was to determine the feasibility of such an approach, and if rigorous control of hyperglycemia soon after diagnosis was associated with higher remission rates.

Fifty-five cats diagnosed with diabetes mellitus were included in the study. Cats diagnosed with acromegaly were excluded. Fifty cats in the cohort were initially treated with other insulins (47 with procine lente insulin) for a median of 15 weeks, but failed to achieve remission prior to switching to glargine, and 43 of these cats were exclusively fed a low carbohydrate wet food diet.

Data were obtained from owners who joined the online German Diabetes-Katzen Forum and followed an intensive blood glucose regulation protocol using glargine. The aim was to achieve euglycemia (50–100 mg/dL as measured using a portable blood glucose monitor) using twice daily insulin dosing and a low carbohydrate wet food diet. Owners performed an average of 5 ± 2 blood glucose measurements per day, and supplied spreadsheets recording daily insulin dosages, blood glucose concentration and clinical information.

Thirty-five cats (64%) in the cohort achieved remission. The median time to remission was 59 days (1.9 months) after beginning the intensive protocol (range = 6 days to 10.2 months). Twenty-six cats (74% of remission cats) remained off insulin and the median duration of remission was 10.8 months (range = 2.8 months to 3.0 years). Nine (26% of remission cats) relapsed and required insulin again. Two of these relapsed cats achieved a second remission.

Twenty cats (36%) of the cohort required insulin throughout the study to control blood glucose concentrations and did not achieve remission. The median length of time on the protocol was 12.7 months (range = 2.6 months–2.1 years). The majority (70%; 14/20) of long-term diabetics were considered well regulated with a median blood glucose concentration of $\leq$150 mg/dL, 15% (3/20) were moderately well regulated (blood glucose $\leq$200 mg/dL) and 15% (3/20) remained difficult to regulate, which represented 6% of all cats in the study.

Asymptomatic hypoglycemia (blood glucose concentration $<$ 50 mg/dL) was measured in most (93%) cats at least once. However symptomatic hypoglycemia was rare, with only a single event in one cat which had mild signs (restlessness). Maximum insulin doses administered to cats in the study ranged from 1.00 to 9.00 IU twice daily.

Significantly higher remission rates occurred if the protocol for intensive glycemic control was initiated soon after diagnosis. The remission rate was 84% for cats started on the protocol within 6 months of diagnosis, and 35% for cats that began more than 6 months after diagnosis ($P < 0.001$).

We conclude that intensive blood glucose control is a feasible and safe approach in cats, and that high remission rates are possible in previously treated cats if hyperglycemia is intensively managed using glargine within 6 months of diagnosis.

ABSTRACT #298
THE SOMOGYI EFFECT IS RARE IN DIABETIC CATS MANAGED USING GLARGINE AND A PROTOCOL AIMED AT TIGHT GLYCEMIC CONTROL. K Roomp[1], JS Rand[2]. [1]Max Planck Institute for Informatics, Saarbruecken, Germany. [2]Centre for Companion Animal Health, University of Queensland, Australia.

The Somogyi effect is defined as hyperglycemia caused by the release of counter-regulatory hormones in response to insulin-induced hypoglycemia, and it is widely believed to exist in diabetic cats. However, studies in human diabetic patients over the last quarter century have rejected the common occurrence of the Somogyi phenomenon. The aim of this study was to determine the frequency of the Somogyi effect in diabetic cats treated with glargine.

Fifty-five cats diagnosed with diabetes mellitus were included in the study. Data were collected over 20 months, and were obtained from owners who joined the online German Diabetes-Katzen Forum, and followed a protocol of intensive blood glucose regulation using glargine. The aim was to achieve euglycemia (50–100 mg/dL as measured using a portable blood glucose monitor) using twice

daily insulin dosing. Owners performed an average of 5 ± 2 blood glucose measurements per day, and supplied spreadsheets recording all blood glucose concentrations.

The Somogyi effect was defined differently in cats that had good glycemic control compared to those that were not well controlled. For cats that were well controlled and had their nadir concentrations in the normal range (50–80 mg/dL) on an almost daily basis for ≥ 2 weeks, a Somogyi effect was defined as a glucose concentration of ≤40 mg/dL, followed by a fast, steep rise in blood glucose concentration to ≥ 400 mg/dL, and/or concentrations that were at least 150 mg/dL above the usually measured higher concentrations. The two subsequent insulin doses showed almost no effect, and the glucose concentration remained elevated for ≥ 24 hours. In cats that had never had a glucose concentration in the normal range or only had nadir glucose concentrations in the normal range for several days, a Somogyi was defined as a blood glucose concentration of ≤70 mg/dL, followed by a fast, steep rise in glucose concentration to ≥400 mg/dL and/or concentrations that were at least 200 mg/dL above the usually measured higher concentrations for that cat. The two subsequent insulin doses showed almost no glucose lowering effect, and the glucose concentrations remained elevated for ≥24 hours.

Asymptotic or biochemical hypoglycemia was common and most cats (93%) had blood glucose concentrations ≥ 40 to < 50 mg/dL measured at some point during the study, 84% had glucose concentrations ≥ 30 to <40 mg/dL, and 51% had some glucose concentrations ≥ 20 to < 30 mg/dL.

Although biochemical hypoglycemia was common, the Somogyi effect was very rare in this cohort of diabetic cats. Based on the criteria, only 4 single events were identified in 4 different cats.

We conclude that that the Somogyi effect is rare in cats treated with glargine on a protocol aimed at tight glycemic control, despite the frequent occurrence of biochemical hypoglycemia.

ABSTRACT #299

FACTORS PREDICTIVE OF NON-INSULIN DEPENDENCE IN DIABETIC CATS INITIALLY TREATED WITH INSULIN. K Roomp[1], JS Rand[2]. [1]Max Planck Institute for Informatics, Saarbruecken, Germany. [2]Centre for Companion Animal Health, University of Queensland, Australia.

Diabetic cats treated with insulin are frequently able to maintain euglycemia without insulin therapy within weeks to months of beginning treatment, often termed diabetic remission. The aim of this study was to determine the factors which might predict non-insulin dependence in insulin-treated diabetic cats.

Fifty-five cats diagnosed with diabetes whose owners followed an intensive blood glucose regulation protocol using glargine and fed a low carbohydrate diet were studied. Cats diagnosed with acromegaly were excluded. Fifty cats in the cohort were initially treated with other insulins (47 with porcine lente insulin) for a median of 15 weeks, but failed to achieve remission prior to switching to glargine and an intensive blood glucose regulation protocol.

Thirty-five cats (64%) in the cohort achieved remission. Cats treated with corticosteroid in the 6 months prior to being diagnosed with diabetes were more likely to go into remission than cats without prior corticosteroid treatment (P = 0.0014, Fisher's exact test, 95% CI 2.45, Inf). Cats which displayed a plantigrade stance at diagnosis or milder signs of peripheral neuropathy such as a difficulty climbing stairs, were significantly less likely to go into remission (P = 0.0036, Fisher's exact test, 95% CI 0.018, 0.606). However, when cats with only a plantigrade stance were examined, that is, cats with milder forms of peripheral neuropathy were excluded, the results were no longer significant. There was a significant difference in mean maximum insulin dose between cats which became non-insulin dependent during the study (0.43 IU/kg BID) and cats which remained insulin dependent (0.66 IU/kg BID) (P = 0.016, Wilcoxon rank sum test with continuity correction). Cats that started with intensive blood glucose control within 180 days (6 months) of diagnosis of diabetes were more likely to achieve non-insulin dependence than cats that were put on the protocol later than 180 days after diagnosis (P < 0.001, Fisher's exact test, 95% CI 2.42, 45.48).

Other factors which were examined but were not predictors of remission were age at diagnosis, gender, weight at diagnosis, evidence of diabetic ketoacidosis at diagnosis, development of azotemia during therapy, hyperthyroidism and frequency of asymptotic hypoglycemia. Obesity was not negatively correlated with remission.

We conclude that prior corticosteroid treatment, peripheral neuropathy, lower maximum insulin dose and intensively managed blood glucose concentrations using glargine within 6 months of diagnosis are associated with higher rates of non-insulin dependence in diabetic cats.

ABSTRACT #300

EFFICACY AND SAFETY OF PORCINE INSULIN ZINC SUSPENSION (IZS-P) FOR REDUCING HYPERGLYCEMIA AND ASSOCIATED CLINICAL SIGNS IN CATS WITH DIABETES MELLITUS. P Brianceau[1], T Chester[1], A Smith[1], L Horspool[2], D Laxton[1]. [1]Intervet Inc. Millsboro, DE. [2]Intervet International bv, Boxmeer, The Netherlands.

An open, unmasked study was conducted to provide substantial evidence of the clinical effectiveness and safety of porcine insulin zinc suspension (IZS-P, Vetsulin®) for reducing hyperglycemia and its associated signs in cats with diabetes mellitus. The study was conducted in the US in accordance with the current FDA, CVM and VICH Guidance on Good Clinical Practice.

Cats were enrolled based on elevated fasting blood glucose (> 250 mg/dL on two occasions) with concurrent glycosuria and clinical signs of diabetes mellitus. Cats that met specified criteria on prior treatment or with coexisting primary diseases were excluded. A baseline blood glucose curve (Day 0) was completed (Accu-Chek® Advantage®) and IZS-P therapy (approximately 1 to 2 IU per subcutaneous injection q 12 h) initiated. Glucose curves and clinical signs were re-evaluated on Days 7, 14, 30, 60 and 180 and the dose was adjusted accordingly. Overall response to treatment in each cat was recorded on a visual analogue scale (VAS). Additional examinations to assess the long-term safety were carried out at 90, 120 and 150 days. Complete blood count (CBC) and serum chemistry were performed during the course of the study and urinalysis prior to enrollment.

Mean VAS score decreased from 93.8 mm on Day 0 to 31.2 mm and 25.0 mm on Days 60 and 180 , respectively (p < 0.001 both days). This was mirrored by a substantial reduction in polyuria, polydipsia and polyphagia. Mean blood glucose decreased from 394.1 mg/dL on Day 0 (n = 77) to 216.7 mg/dL (n = 76) and 219.5 mg/dL (n = 73) on Days 60 and 180, respectively (p < 0.001 both days). Mean glucose nadir decreased from 343.3 mg/dL on Day 0 to 145.7 mg/dL and 155.9 mg/dL on Days 60 and Day 180, respectively (p < 0.001 both days). Blood glucose was < 300 mg/dL in 5.2%, 75.0% and 71.2% of the cats and blood glucose nadir < 200 mg/dL in 0%, 72.4% and 69.9% of the cats on Days 0, 60 and 180, respectively. Mean fructosamine concentration decreased from 604.3 µmol/L on Day 0 to 451.3 µmol/L and 448.4 µmol/L on Days 60 and 180, respectively (p < 0.001 both days). Diabetes remission occurred in four cats. Less than 3% (2/77) of the cats failed to respond to treatment.

Hypoglycemia (blood glucose concentration < 50 mg/dl) was reported as an adverse event associated with clinical signs (lethargy, diarrhea, vomiting, and/or hypothermia) in 13/78 cats and without clinical signs in 17/78 cats, 11 of the latter received oral glucose replacement. Five cats experienced more than one hypoglycemic event. Polyneuropathy was documented in 5.1% (4/78) of the cats. Two injection site reactions were reported (mild bruising at the injection site, mildly thickened subcutis).

These results confirm that IZS-P is effective and safe for reducing hyperglycemia and its associated clinical signs in cats with diabetes mellitus.

ABSTRACT #301

ADIPONECTIN CONCENTRATION DOES NOT CORRELATE WITH OBESITY-ASSOCIATED CHANGES IN FASTING INSULIN SENSITIVITY IN DOGS. Kurt R Verkest[1], Linda M Fleeman[1], John M Morton[1], Jacquie S Rand[1], Felicity J Rose[1], Ayanthi A Richards[1], Jonathan P Whitehead[1], Katsumi Ishioka[2]. [1]The University of Queensland, Brisbane, Australia. [2]Nippon Veterinary and Life Science University, Tokyo, Japan.

Decreased circulating adiponectin concentrations are associated with obesity-related changes in insulin sensitivity in all species tested to date, including laboratory rodents and human beings. A recent study in Japan reported significantly lower adiponectin concentrations in overweight and obese dogs. The aim of the current study was to assess whether adiponectin is associated with obesity-associated changes in fasting insulin sensitivity in spontaneously overweight and obese dogs.

We recruited 123 client-owned lean, overweight, and obese dogs in Australia. Age, gender, breed, and body weight were recorded. Dogs were fasted for 24 hours prior to blood collection. Body condition score was assessed on a 9-point scale by one veterinarian (KRV). Plasma total adiponectin was measured by commercial ELISA, validated in the previous Japanese study, and by Western blotting. Fasting insulin sensitivity was estimated as the product of fasting plasma insulin and glucose concentrations (HOMA). Adiponectin measured by ELISA and Western blotting were compared using Pearson's correlation coefficient. Linear regression models were used to assess effects of adiposity on total plasma adiponectin concentration and HOMA, adiponectin on HOMA, and to check for confounding of the association between adiposity and adiponectin due to age, sex, neuter status, gender, breed genetic background, body weight, and estimated lean weight. Raw data from a previous study of 71 dogs from 4 Japanese veterinary clinics was also obtained and the observed association between obesity and adiponectin concentrations was examined for confounding to assess potential reasons for the disparity between the results of the two studies.

There was close correlation between plasma adiponectin concentrations measured by ELISA and estimated by Western blot (correlation coef. 0.92, $p < 0.01$). There was a strong association between adiposity and HOMA (ß-coef. 0.78; $p < 0.01$) which did not change when adiponectin was accounted for. There was no significant association between adiposity and plasma total adiponectin concentration (ß-coef. −1.2, $p = 0.38$). There was no association between total adiponectin concentration and fasting insulin sensitivity (ß-coef. −0.006, $p = 0.57$). Accounting for age, sex, neuter status, gender, breed genetics, and body weight did not substantially alter the observed weak association between adiposity and adiponectin from the Australian study or the observed strong negative association from the Japanese study.

We conclude that (1) total adiponectin concentration was not strongly associated with adiposity in Australian dogs with naturally occurring obesity, and (2) total adiponectin concentration does not appear to mediate obesity-associated fasting insulin resistance in dogs.

ABSTRACT #302
LACK OF CORRELATION BETWEEN ADIPONECTIN MULTIMERS AND OBESITY-RELATED CHANGES IN INSULIN SENSITIVITY IN DOGS. Kurt R Verkest[1], JacquieS Rand[1], LindaM Fleeman[1], JohnM Morton[1], FelicityJ Rose[2], Ayanthi A Richards[2], Jonathan P Whitehead[2]. [1]Centre for Companion Animal Health, and [2]Diamantina Institute, The University of Queensland, Brisbane, Australia.

In human beings, adiponectin is implicated in the development of obesity-induced insulin resistance and of type 2 diabetes mellitus. Obese dogs also develop insulin resistance, but type 2 diabetes is not well documented in dogs. Adiponectin circulates as low- and high-molecular weight (HMW) multimers. Changes in HMW adiponectin account for most of the variation in human total adiponectin concentrations. This study aimed to examine whether total and/or HMW adiponectin are associated with obesity-related insulin resistance in dogs.

Twelve client-owned dogs were recruited. Six obese dogs (body condition score (BCS) 8-9/9) and six lean dogs (BCS 4-5/9) were matched for age and gender. Insulin sensitivity was measured using a frequently-sampled glucose tolerance test with Bergman's Minimal Model analysis. We measured the proportion of adiponectin that was HMW multimers (S_A) using velocity centrifugation on sucrose gradients followed by Western blotting. Total adiponectin was measured by ELISA. Absolute HMW adiponectin concentration was calculated as S_A multiplied by total adiponectin concentration. Linear regression was used to assess effects of obesity on each of insulin sensitivity, total adiponectin, angular-transformed S_A, and absolute HMW adiponectin concentration and the effects of total adiponectin, S_A, and absolute HMW adiponectin on insulin sensitivity. Results are reported as mean ± SEM or regression coefficient ±SE.

Obese dogs were half as insulin sensitive as lean dogs (3.0 ± 0.8 versus $6.5 \pm 1.5 \times 10^{-4}\,L\,mU^{-1}\,min^{-1}$, respectively; $p = 0.04$). S_A did not vary with obesity (obese 0.76 ± 0.05 versus lean 0.76 ± 0.06, $p = 0.98$). Total and absolute HMW adiponectin were numerically lower in obese dogs than lean dogs, but the associations were not statistically significant (total adiponectin: obese 15 ± 2 versus lean $25 \pm 9\,\mu g/mL$, $p = 0.26$; absolute HMW adiponectin: obese 11.9 ± 2.2 versus lean $20.8 \pm 8.5\,\mu g/mL$, $p = 0.29$). Insulin sensitivity did not vary significantly with total adiponectin (ß-coef. -0.009 ± 0.081; $p = 0.91$), S_A (ß-coef. -6.8 ± 8.2; $p = 0.37$), or absolute HMW adiponectin (ß-coef. -0.025 ± 0.085; $p = 0.78$); similar results were observed after accounting for adiposity in the regression analysis. When one glucose intolerant dog was excluded a higher S_A was associated with lower insulin sensitivity (ß-coef. -23 ± 7.2, $p = 0.01$) but neither total (ß-coef. -0.3 ± 0.08, $p = 0.70$) nor absolute HMW adiponectin (0.06 ± 0.08, $p = 0.52$) varied with insulin sensitivity.

We conclude that (1) unlike human beings, in dogs the proportion of HMW adiponectin (S_A) is not decreased with obesity, and (2) S_A does not appear to mediate obesity-induced changes in insulin sensitivity in dogs. The differences between species in adiponectin physiology might help to explain differences in susceptibility to type 2 diabetes mellitus.

ABSTRACT #303
ASSOCIATION OF ADIPONECTIN MULTIMERS WITH DIETARY NUTRIENT COMPOSITION, BODY WEIGHT GAIN, MEAL FEEDING, AND INSULIN SENSITIVITY IN CATS. HY Tan[1], JS Rand[1], JM Morton[1], LM Fleeman[1], M Coradini[1], PJ Armstrong[2], KR Verkest[1], K Ishioka[3], F Rose[1], A Richards[1], JM. Rawlings[4], JP Whitehead[1]. [1]The University of Queensland, Australia, [2]University of Minnesota, MN, [3]Nippon Veterinary & Life Science University, Japan, and [4]WALTHAM Centre for Pet Nutrition, UK.

Adiponectin has been investigated widely due to its association with adiposity and the metabolic syndrome in human beings. Adiponectin circulates as low- (LMW) and high-molecular weight (HMW) multimers and the latter are the more bioactive forms. There are no reports of the relative proportion (distribution) of adiponectin multimers in feline plasma. The aim of this study was to assess the association of dietary nutrient composition, body weight gain, meal feeding, and insulin sensitivity with HMW adiponectin concentration and adiponectin multimer distribution in cats.

Healthy, neutered, young adult, mixed-breed cats ($n = 32$) with ideal body weight were matched by gender, insulin sensitivity, and body weight, and were randomly allocated to either a low carbohydrate (19% metabolisable energy [ME]) or high carbohydrate (52% ME) diet. Cats were fed for 4 weeks at maintenance energy requirements, followed by 8 weeks of *ad libitum* feeding. The distribution of adiponectin multimers in plasma (S_A = HMW/(HMW+LMW)) was measured in a random sample of 16 of these cats (4 males and 4 females from each dietary group) after 3 and 8 weeks of feeding using velocity centrifugation on sucrose gradients followed by Western Blotting. The concentration of HMW adiponectin was obtained by multiplying the S_A with the total adiponectin concentration measured by a commercial ELISA. The relationship of dietary nutrient composition, body weight gain, and insulin sensitivity with adiponectin multimer distribution and concentration were assessed using generalised linear models and linear regression. Paired t-tests assessed the effect of meal feeding on adiponectin profile before and 6 hours after eating.

Cats fed the high carbohydrate diet had higher fasting concentrations of HMW adiponectin than cats fed the low carbohydrate diet ($4.6 \pm 2.2\,\mu g/ml$ and $2.2 \pm 1.6\,\mu g/ml$, $p = 0.015$), but there was no significant difference between diets 6 hours after eating, and the relative proportion of HMW to LMW adiponectin was not affected by diet. Neither HMW concentration nor distribution differed significantly at 6 hours after eating from fasting values ($p > 0.447$). Mean percent body weight gain at 8 weeks was $22 \pm 14\%$ (range 10% to

50%), and this was not associated with a corresponding change in adiponectin distribution or concentration of HMW adiponectin ($p > 0.632$). Insulin sensitivity was not significantly associated with HMW concentration or with adiponectin distribution ($p > 0.140$).

In conclusion, a high carbohydrate diet fed at maintenance energy requirements is associated with higher fasting but not postprandial concentrations of HMW adiponectin in cats, whereas insulin sensitivity, body weight gain, and meal feeding were not strongly associated with circulating HMW adiponectin concentration or adiponectin multimer distribution.

ABSTRACT #304
OXIDATIVE STRESS AND NEUTROPHIL FUNCTION IN CATS WITH DIABETES MELLITUS COMPARED TO CONTROLS: ASSESSING THE IMPACT OF NUTRITION. LB Falkowski and CB Webb. Colorado State University, Fort Collins, CO.

Oxidative stress is a key component in the pathophysiology of Type 2 diabetes mellitus (DM) in humans, and an estimated 85% of cats with DM are Type 2 diabetics. Accordingly, the use of high protein-low carbohydrate diets in diabetic cats may lead to a significant reduction in oxidative stress as the production of free radicals is driven by the metabolism of excess carbohydrates. Neutrophil function may be impaired by oxidative stress in patients with DM, resulting in a decreased ability to prevent or eliminate infections. This study was designed to test the hypothesis that cats with DM have increased oxidative stress and decreased neutrophil function that will improve with consumption of a diabetes-specific diet.

Body weight, serum fructosamine level, complete blood count, and parameters of oxidative stress and neutrophil (PMN) function were measured in 15 cats with DM and in 20 healthy control cats before and after being fed a commercially available diet designed specifically for feline diabetics (Purina Veterinary Diets® DM®) for 8 weeks. Erythrocyte superoxide dismutase (SOD), blood glutathione peroxidase (GPx), malondialdehyde, and reduced glutathione:oxidized glutathione ratios were evaluated spectrophotometrically. PMN phagocytosis and subsequent respiratory burst activity was evaluated using flow cytometry. Results were compared both between groups and within groups over time.

The diabetic cats were significantly older and heavier than the control group. Prior to the diet change, cats with DM had significantly less plasma SOD (211 U/ml ± 143) than control cats (352 U/ml ± 200)($P = 0.02$). Other oxidative stress parameters and measures of neutrophil function were not significantly different between the two groups. Following 8 weeks of consuming a diabetes-specific diet, the control group gained a significant amount of weight (5.2 to 5.4 kg; $P = 0.005$), the mean fructosamine value was significantly decreased in both groups (control, 273 to 245 μmol/L, $P = 0.004$; DM, 416 to 353 μmol/L, $P = 0.03$), and GPx increased significantly in both groups (control, 37K to 68K U/L, $P < 0.005$; DM, 29K to 67K U/L, $P < 0.005$). PMN function did not change significantly in either group.

Diabetes mellitus may be associated with an increase in oxidative stress in cats. Diet appears to significantly impact some parameters of oxidative stress in both affected and healthy cats, supporting the continued development and evaluation of targeted antioxidant dietary supplementation. A diabetes-specific diet may be beneficial in cats predisposed to developing DM, although the potential for weight gain needs to be considered.

ABSTRACT #305
EVALUATION OF STRESS DURING HOSPITAL VISITS IN RETIRED RACING GREYHOUNDS WITH AND WITHOUT DIARRHEA. L Miller, CG Couto, CA Buffington, N Westendorf, MC Iazbik, LM Marín, P Vilar Saavedra. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Anecdotal evidence and our clinical experience suggest that acute large bowel diarrhea is common in a subset of healthy Greyhounds presented to veterinary hospitals. In this study, we examined the influence of activation of the stress response system on the development of diarrhea by evaluating both the hypothalamic-pituitary-adrenocortical (HPA) axis (i.e., increased cortisol concentrations in plasma and saliva) and the sympatho-adrenomedullary (SAM) axis (i.e., increased epinephrine and norepinephrine concentrations in plasma). By evaluating salivary cortisol concentrations (SAL CORT), we compared the stress response in the hospital to the baseline level of "stress" detected in the home environment in order to better understand the cause of the acute diarrhea in Greyhounds. We hypothesized that most Greyhounds would have a higher SAL CORT in the hospital than at home, and that Greyhounds with high salivary and plasma cortisol concentrations would be more likely to develop diarrhea at the hospital.

Two groups of Greyhounds were evaluated, including 36 healthy blood donor Greyhounds (group 1) and 32 recently retired racing Greyhounds being housed at the hospital for one week to be spayed and neutered (group 2). In group 1, saliva samples were taken at home by the owners prior to arrival at the hospital and at least 48 hours after their hospital visit; in group 2, saliva samples were taken at arrival and at least 2 days after surgery. For both groups, stool, saliva, and blood samples were obtained in the hospital. Stool samples were scored to determine if the dog had diarrhea or formed feces; salivary cortisol and plasma epinephrine, norepinephrine, and cortisol concentrations were also determined.

There was a strong correlation ($p < 0.001$) between salivary and plasma cortisol concentrations. For group 1, the SAL CORT in the samples obtained by owners in the home environment were significantly lower ($p < 0.001$ for "pre-visitation" and $p < 0.01$ for "post-visitation") than those obtained during the hospital visit. For group 2, there was no significant difference between initial cortisol samples and those obtained post-surgery. During their first day in the hospital, 8 (11.8% of total) Greyhounds developed diarrhea, and 13 (19.1% of total) Greyhounds developed diarrhea within that week. Greyhounds with diarrhea had significantly higher plasma cortisol concentrations ($p < 0.001$) than those without diarrhea; there were no significant differences in the concentrations of epinephrine or norepinephrine between Greyhounds with and without diarrhea.

As suggested by the high plasma cortisol concentrations in the affected dogs, the HPA axis is likely involved in the development of acute diarrhea in Greyhounds arriving at the hospital. Since SAL CORT corresponded well with plasma cortisol concentrations, this allows for a non-invasive measurement of stress that can be utilized in the home environment, as a baseline for subsequent samples, and promotes increased accuracy by avoiding the "white coat effect". The Greyhounds in group 1 appeared to return to a normal level of "stress" within 2 days of returning home, and the dogs in group 2 retained high cortisol concentrations (corresponding well with the prolonged hospital stay and their recent surgeries).

ABSTRACT #306
EVALUATION OF THE EFFECT OF TWO DOSES OF ACTH ON SERUM FREE CORTISOL CONCENTRATIONS IN CLINICALLY HEALTHY DOGS. LG Martin[1], EN Behrend[2], N Graf[1], HP Lee[2]. [1]Washington State University, College of Veterinary Medicine, Pullman, WA. [2]Auburn University, College of Veterinary Medicine, Auburn, AL.

The adrenocortical response of healthy dogs to synthetic ACTH (cosyntropin) has been reported using several doses of intravenous ACTH. Both 0.5 and 5 μg/kg doses cause maximal stimulation with regard to serum total cortisol concentration. The effect of these doses on serum free cortisol concentration, however, has not been evaluated. The purpose of this study was to determine adrenal response to 0.5 and 5 μg/kg ACTH with respect to free cortisol in clinically healthy dogs.

Two dose-response trials were performed in 10 clinically healthy dogs. Each dog was given 0.5 or 5 μg/kg of cosyntropin (Cortrosyn) IV with a 2-week wash out period between doses. Blood samples were obtained before and 60 min after ACTH administration. Samples were centrifuged after clotting, and the serum was separated and stored at −80 °C until analysis. Data were analyzed using repeated measures ANOVA on ranks; significance was set at the $p < 0.05$ level.

For measurement of serum free cortisol concentration, samples were assayed by a modified ultrafiltration technique. For validation, free cortisol % and concentration were determined in 53 samples obtained from another population of healthy dogs before and after administration of either 1 μg cortrosyn/dog or 5 μg/kg body weight. A significant ($p < 0.0001$) linear relationship between total serum and % free cortisol was found by linear regression; the regression equation was similar to that found when free cortisol was measured in canine plasma by a centrifugal ultrafiltration-dialysis technique (Kemppainen RJ et al, AJVR, 1991). In addition, a single basal sample from a dog was divided into 6 equal aliquots and the free cortisol concentration measured in all 6 ultrafiltrate portions obtained. The coefficient of variation was 3.2%.

Mean total and free serum cortisol concentration increased significantly after administration of both dosages when compared to baseline ($p < 0.0001$). However, mean post-ACTH total and free cortisol concentration did not differ between doses. For all basal and stimulated serum cortisol concentrations ($n = 20$ each), mean % free cortisol was 5.8% (range 1.1–14.5) and 12.7% (range 8.1–18.0), respectively.

In conclusion, cosyntropin administered at 0.5 and 5 μg/kg intravenously significantly increases total and free serum cortisol concentrations in clinically healthy dogs. These results can be used in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in healthy and critically ill dogs.

ABSTRACT #307

VALIDATION OF AN ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF ACTH PRECURSORS (PRO-OPIOMELANOCORTIN AND PRO-ADRENOCORTICOTROPIN) IN FELINE PLASMA. G. Benchekroun[1], P. de Fornel Thibaud[1], M. Dubord[2], M. Le Chevoir[1], C. Petit[3], O. Dossin[4], F. Fracassi[5], C. Maurey-Guenec[1] and D. Rosenberg[1]. [1]Internal Medicine Unit, National Veterinary School of Alfort, France. [2]Veterinary Biochemistry Laboratory, National Veterinary School of Alfort, France. [3]Parasitology-Dermatology Unit, National Veterinary School of Toulouse, France. [4]Internal Medicine Unit, National Veterinary School of Toulouse, France. [5]Veterinary Clinical Department, Faculty of Veterinary Medicine of Bologna, Italy.

Feline hyperadrenocorticism is a rare condition in cats; approximately 80% of cats have an autonomously functioning pituitary tumor. Diagnosis of FH is quite difficult. Clinical signs are non specific, except for the non systematic feline skin fragility syndrome. The specificity of all the tests validated in that species is questionable as well. An ACTH precursors (POMC/pro-ACTH) assay (OCTEIA POMC kit, Immunodiagnostic System, UK) has been validated in canine species (J Vet Intern Med 2005; 19:23–28). High ACTH precursors plasma concentrations have been measured in dogs with large corticotropic tumors whereas ACTH precursors are not detectable in healthy dogs or dogs with other pathological conditions (unpublished data). The aim of this study was to validate OCTEIA POMC assay in cats according to current recommendations for bioanalytical method validation in order to further evaluate its usefulness for diagnosis of feline pituitary-dependent hyperadrenocorticism (FPDH).

The calibration curve was prepared by spiking blank feline plasma with six different concentrations of human POMC/pro-ACTH. Blank plasma was collected from 10 healthy cats, 4 hours after IV administration of dexamethasone phosphate at 1 mg/kg of body weight and pooled.

Sensitivity, defined as the concentration corresponding to the mean plus 2 standard deviations of replicate analyses of blank plasma, was 26 pmol/L. Accuracy, calculated with 3 known concentrations, measured five times each was 111%. Linearity, assessed by diluting a sample with blank feline plasma (1 in 1 to 1 in 16) was 104%. Intra-assay and inter-assay coefficient of variations determined on three samples, measured five times each, were 8%, 10%, 16% and 13%, 10%, 15% respectively.

This work validates the use of OCTEIA POMC kit on feline plasma samples. Its usefulness in FPDH is currently investigated with encouraging preliminary results.

ABSTRACT #308

CHROMATOGRAPHIC ANALYSIS OF THE LIPID FRACTIONS IN OBESE DOGS AND DOGS WITH HYPERADRENOCORTICISM. MM Jericó[1], FC Chiquito[1], TM Ferrarias[1], K Kajihara[1], MAB Moreira[1], VS Nunes[2], ER Nakandakare[2]. [1]Veterinary Hospital, Anhembi Morumbi University, SP, Brazil. [2]Laboratory of Lipids LIM 10, School of Medicine of the USP, Brazil.

Obesity and endogenous hyperadrenocorticism in dogs are common clinical conditions and both present clinical and laboratory similarities, such as weight gain and dyslipidemia. The hyperlipidemic conditions are usually associated to a variety of clinical problems, which include gastrointestinal alterations, hepatic lipidosis, atherosclerosis and metabolic syndrome. The aim of the study was to characterize the lipid profile and lipoprotein fractions in normal dogs ($n = 10$) and compare them with obese dogs ($n = 10$) and dogs with hyperadrenocorticism ($n = 6$), selected from a veterinary hospital environment. The lipoproteins were separated by liquid chromatography in high-resolution gel filtration in the FPLC (fast protein liquid chromatography) system and the plasma concentrations of total cholesterol (TC) and total triglycerides (TG) were measured by enzymatic methods. In the normal group, the TC means and SD were 193 ± 44 mg/dL (percentage distribution of the fractions: VLDL-COL 2.34%, LDL-COL 15.79%, HDL-COL 81.86%); TG values were 50 ± 15 mg/dL (percentage distribution of the fractions: VLDL-TG 39.17%, LDL-TG 35.9%, HDL-TG 24.94%). In the group of obese dogs, TC means and SD were 240 ± 63 mg/dL (VLDL-COL 3.983%, LDL-COL 21.21%, HDL-COL 74.81%) whereas the TG values were 93 ± 52 mg/dL (VLDL-TG 59.79%, LDL-TG 26.62%, HDL-TG 13.68%). In the group of dogs with hyperadrenocorticism the TC means and SD values were 348 ± 105 mg/dL (VLDL-COL 15.11%, LDL-COL 28.63%, HDL-COL 56.26%) whereas TG values were 300 ± 227 mg/dL (VLDL-TG 64.07%, LDL-TG 29.65%, HDL-TG 6.231%). When compared to the normal and obese dogs, the animals with hyperadrenocorticism presented a significant increase ($p < 0.01$) in the total triglycerides and cholesterol levels and a higher distribution in the VLDL-CT fraction. Additionally, the distribution of the HDL-TC and HDL-TG fractions was significantly lower in dogs with hyperadrenocorticism when compared to normal dogs. When compared to the normal dogs, the obese dogs did not show significant alterations, although they presented proportionally higher levels of TC, TG and LDL-TC and LDL-TG as well as lower HDL-TC and HDL-TG levels. It was concluded that, regarding the group of studied animals, the dogs with hyperadrenocorticism present a significant difference when compared to normal and obese dogs in relation to cholesterol and triglyceride metabolism and their VLDL and HDL fractions, which favors the differential diagnosis between these diseases and suggests a higher risk of metabolic and atherosclerotic complications in dogs with hyperadrenocorticism.

ABSTRACT #309

EFFECT OF GLUCOCORTICOID ADMINISTRATION ON SERUM ALDOSTERONE CONCENTRATION. Corrigan AM[1], Behrend EN[1], Martin LG[1], Kemppainen RJ[2]. [1]Department of Clinical Sciences and [2]Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL.

ACTH has a role in aldosterone synthesis and secretion, so ACTH deficiency could diminish serum aldosterone. However, to our knowledge, the effect of glucocorticoid administration and resultant suppression of ACTH secretion on serum aldosterone concentration has not been evaluated. The purpose of this study was to assess the effect of anti-inflammatory doses of prednisone administered over 4 weeks on aldosterone secretion in clinically healthy dogs.

Ten healthy adult American Fox Hounds were randomly assigned to placebo or prednisone (0.55 mg/kg PO q12 h) groups. Serum sodium (Na), chloride (Cl), potassium (K) and bicarbonate (HCO_3) concentrations were measured and ACTH stimulation tests were performed weekly beginning before (wk 0) and ending 2 weeks after cessation of drug administration (wk 6). For the stimulation test, ACTH (Cortrosyn) was administered (1.0 mcg/kg IV) and

blood samples were taken before and at 20 and 60 min post. Aldosterone concentrations were measured in the pre and 20 min samples and cortisol in the pre and 60 min samples using previously validated radioimmunoassays. Repeated measures ANOVA on ranks was performed to assess changes over time within a group; significance was set at $p < 0.05$.

For the treated dogs, compared to baseline, serum Na concentration at weeks 2–4 was significantly lower and at week 5 (i.e. one week after stopping the prednisone) significantly higher, serum Cl and corrected Cl concentrations at weeks 1–4 were significantly lower, and serum HCO_3 concentration at weeks 2–4 was significantly higher. As expected, pre- and post-ACTH cortisol concentrations were significantly lower in dogs during prednisone therapy. Post-ACTH aldosterone concentrations were significantly lower than baseline on week 5.

Therefore, administration of anti-inflammatory doses of prednisone causes significant changes in clinically healthy dogs in serum Na, Cl, HCO_3, cortisol and aldosterone concentrations. Although no change in aldosterone concentration was noted during glucocorticoid administration, interestingly, serum ACTH-stimulated aldosterone concentration decreases after cessation of glucocorticoid administration but quickly returns to normal.

ABSTRACT #310

THYROID GLAND AND PITUITARY GLAND HISTOLOGIC CHANGES ACCOMPANYING SULFAMETHOXAZOLE-INDUCED HYPOTHYROIDISM IN DOGS. A. Gal, TK Graves, T Keel, KL Campbell. University of Illinois College of Veterinary Medicine, Urbana, IL.

Archived, thyroid and pituitary biopsies from dogs with sulfamethoxazole (SMZ)-induced hypothyroidism were examined for changes in histologic morphology. Dogs were treated alternately with SMZ (30 mg/kg BID) and placebo for 6-week periods in a crossover design.

Random fields were examined on thyroid sections under microscopy. Morphometric analysis was done using MetaMorph® software. Relative proportions of follicular epithelial cells compared with colloid-filled lumina were estimated by subtracting cumulative cross-sectional area of colloid-filled follicles from total area of each field. In pituitary samples, thyrotrophs were detected by immunohistochemical staining, using an anti-canine TSH antibody. TSH-positive pituitary cells were counted in 6 random fields on each slide.

Colloid-containing follicles were markedly smaller and numbers of epithelial cells were greater in dogs following SMZ ($N = 6$) vs. placebo ($N = 6$). The median and interquartile range (IQR) of the percentage of thyroid tissue populated by epithelial cells in SMZ-treated dogs was 77.9 (IQR 64.5–91.3) vs. 32.0 (IQR 24.9–39.1) in placebo-treated dogs. The difference between the groups was significant ($P = 0.014$). SMZ-induced changes were reversed following 6 weeks of placebo. The median number of TSH-positive pituitary cells per field was significantly greater ($P < 0.001$) on biopsies from SMZ-treated (median 28.5; IQR 12.5–44.5; $N = 3$) vs. placebo-treated dogs (median 5.0; IQR 0.0–12.25; $N = 3$).

Our results demonstrate that SMZ-induced hypothyroidism is characterized by reversible thyroid follicular epithelial hyperplasia and colloid collapse. An increased population of pituitary thyrotrophs in SMZ-treated dogs supports recent reports of transdifferentiation of pituitary cells in response to hormonal needs.

ABSTRACT #311

PRELIMINARY INVESTIGATION OF ATYPICAL CONGENITAL HYPOTHYROIDISM IN A FELINE COLONY. J Morrison[1], AJ Fales-Williams[2], MD Winter[1], JM Clemans[1], GJ McLellan[4], NM Ellinwood[3]. [1]Dept. of Veterinary Clinical Sciences, and [2]Dept. of Veterinary Pathology, College of Veterinary Medicine, and [3]Dept. of Animal Science, College of Agriculture and Life Sciences, Iowa State University, Ames, IA; [4]Dept. of Surgical Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI.

Phenotypic characteristics consistent with congenital hypothyroidism were identified in a feline colony used to study an inherited congenital form of glaucoma. The characteristics included: short stature, decreased growth rate, delayed dental eruption, and thyroid enlargement.

Dysmorphia and stunted growth were noted in a 5 week old kitten from a mixed breed colony (Siamese and DSH). Total cholesterol was elevated at 6 weeks of age. Total T4 (TT4) at 7 weeks was below the limit of detection but by 10 weeks was within the normal adult range (< 0.5 μg/dl and 2.3 μg/dl respectively, normal 4–8 μg/dl). At 10 weeks, TSH was $\sim 5 \times$ normal at 1.37 ng/ml. Thyroid ultrasound was performed at 15 weeks, and thyroid scintigraphy was performed at 16 weeks. Sonographically, both lobes of the thyroid gland showed normal, homogeneous echogenicity and echotexture; however, overall thickness (0.41 cm) was increased compared to a phenotypically normal littermate (0.27 cm). Scintigraphic evaluation of the thyroid lobes showed bilaterally symmetric, uniformly increased activity, with T:S markedly greater than 1.0. Levothyroxine supplementation was initiated at this time.

Dysmorphia was noted in a closely related 3 week old female DSH. At 5 weeks TT4 was < 0.5 μg/dl and TSH was > 12 ng/ml ($\sim$250 times that of a clinically normal littermate). Levothyroxine supplementation was initiated at 7 weeks of age.

A mating of the affected cats produced 3 kittens. Kitten 1 died at 10 days of age of unknown causes. Of the 2 remaining kittens, dysmorphia was noted at 4 weeks and both kittens had low TT4 measured at 6 weeks (Kitten 2 = 0.52 μg/dl, Kitten 3 = 0.7 μg/dl). Kitten 3 was euthanized at 10 weeks due to complications of glaucoma. Kitten 2 remains alive and is currently not on levothyroxine supplementation so as to fully document the un-supplemented clinical condition.

Histopathology on Kitten 1 showed bilateral, severe, thyroid hypoplasia and edema. Thyroid histopathology on Kitten 3 showed severe, diffuse, bilateral adenomatous hyperplasia. No lesions were noted in pituitary or hypothalamic tissues.

Pedigree analysis suggests autosomal recessive inheritance. Breeding trials continue. To date, all affected cats have congenital glaucoma but not *vice versa*. It is unclear if these conditions are independent or co-segregating, or if the hypothyroid trait is dependent on expression of the glaucoma trait. Syndromic genetic diseases comprising hypothyroidism and glaucoma have been described in species other than the cat. The described hypothyroid cats are unlike reports of hypothyroidism due to dyshormonogenesis. This model could prove important in the implicated breed (Siamese), and as a useful model for understanding thyroid gland development and function.

ABSTRACT #312

A LONGITUDINAL STUDY OF THE EFFECTS OF CHRONIC EXPERIMENTAL HYPOTHYROIDISM ON CANINE SKELETAL MUSCLE. JH Rossmeisl[1], KD Inzana[1], DL Panciera[1], GD Shelton[2]. [1]Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, and [2]Comparative Neuromuscular Laboratory, Department of Pathology, University of California at San Diego, La Jolla, CA.

The purpose of this study was to evaluate the effects of chronic hypothyroidism on canine skeletal muscle and carnitine metabolism. It was hypothesized that carnitine metabolic derangements occur in hypothyroid dogs and may participate in the pathogenesis of hypothyroid myopathy, as thyroid hormone dependent alterations in gene expression of carnitine palmitoyltransferase have been demonstrated in other species.

Nine clinically normal, euthyroid, mixed breed females were studied. Hypothyroidism was induced by ^{131}iodine irradiation in six dogs; three served as untreated controls. Clinical, electrophysiologic, muscle histopathologic, histomorphometric and ultrastructural examinations, plasma biochemical analyses, and plasma, urine, and muscle carnitine fractional quantifications were performed at baseline and 6, 12, and 18 months after induction of hypothyroidism. Data were analyzed using repeated measures ANOVA.

At baseline, no differences between groups were detected for any variable. All animals remained asymptomatic for neuromuscular disease throughout the study. Hypothyroid dogs developed electromyographic, and significant biochemical, histopathologic, and histomorphometric evidence of myopathy by 6 months, which persisted throughout the study. Hypothyroid dogs developed increases in plasma CK, AST, and LDH activities, increased urinary excretion of carnitine, and depletion of skeletal muscle carnitine. Histomorphologically, the myopathy was characterized by nemaline rod inclusions, temporally progressive Type I myofiber predominance and Type II myofiber atrophy, and subsarcolemmal accumulations of abnormal mitochondria.

This canine model resulted in reproducible subclinical myopathic alterations similar to those described in dogs with spontaneous hypothyroidism. Further studies are needed to determine if observed carnitine metabolic alterations contribute to the pathogenesis of hypothyroid myopathy or occur as epiphenomena.

ABSTRACT #313

PREVALENCE OF AND RISK FACTORS FOR FELINE HYPERTHYROIDISM IN HONG KONG. Cornelia S De Wet[1], Carmel T Mooney[3], Peter N Thompson[2], Johan P Schoeman[1]. [1]Department of Companion Animal Clinical Studies, [2]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa. [3]University Veterinary Hospital, University College, Dublin, Ireland.

Feline hyperthyroidism is an important disorder in middle-aged and older cats. The cause and pathogenesis are still unknown and there are few published incidence rates or prevalence estimates.

A descriptive, cross-sectional study was undertaken to determine the prevalence of and potential risk factors for feline hyperthyroidism in Hong Kong. Serum total thyroxine (T_4) concentrations were measured in 305 aged cats that presented at various veterinary clinics in Hong Kong between June 2006 and August 2007. Data was collected about the health of the cats as well as their vaccination history, internal and external parasite control, diet and environment. Serum T_4 concentration was determined by use of a commercially available radioimmunoassay kit (Coat-a-count®, DPC®). For T_4 the reference interval was 12.8–50 nmol/L (1.0–3.9 ug/dL). All cats with serum T_4 concentrations greater than 50 nmol/L were classified as hyperthyroid. Serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were measured in all cases. Prevalence of hyperthyroidism with exact binomial 95% confidence intervals was calculated for all cats combined, for cats classified as healthy and for cats classified as sick. Univariable associations between potential risk factors, clinical signs, raised ALT and raised ALP activities and hyperthyroidism were assessed using a two-tailed Fisher's exact test. A multiple logistic regression model was used to estimate the effect of the risk factors on the development of hyperthyroidism. The fit of the final logistic regression model for risk factors was assessed using the Hosmer-Lemeshow goodness-of-fit test.

The prevalence of hyperthyroidism in Hong Kong was 3.93% (95% CI: 2.05–6.77) and there was no significant difference in prevalence between healthy (3.16%) and sick (4.37%) cats ($P = 0.76$). There was no statistically significant relationship between sex, vaccination status, parasite control, indoor environment or the consumption of canned food and the development of hyperthyroidism. Domestic shorthair cats were less likely to be diagnosed with hyperthyroidism and the two older age groups of cats (15–19 years old and $\geq$20 years old) were more likely to be diagnosed with hyperthyroidism (OR = 2.77; 95% CI = 0.82–9.4 and 11.88; 95% CI = 1.06–133.7, respectively). There were no characteristic clinical features amongst the cats that had hyperthyroidism. The presence of polyphagia, diarrhea, palpable thyroid nodule and raised ALT and ALP activities was significantly associated with hyperthyroidism.

This study concluded that the prevalence in Hong Kong is less than in most other parts of the world, despite the presence of previously identified risk factors.

ABSTRACT #314

EFFECTS OF RECOMBINANT HUMAN THYROID STIMULATING HORMONE ON THYROID UPTAKE OF RADIO ACTIVE IODINE AND SERUM TOTAL T4 CONCENTRATION IN HYPERTHYROID CATS: A PRELIMINARY STUDY. I van Hoek, S Daminet, E Vandermeulen, A Dobbeleir, L Duchateau, K Peremans. Ghent University, Ghent, Belgium.

Administration of recombinant human thyroid stimulating hormone (rhTSH) increases the radio active iodine uptake (RAIU) and allows a reduction of therapeutic radioiodine (^{131}I) dose in humans with nodular goiter (Nieuwlaat et al., JCEM 2003;88:3121-3129). A similar dose reduction of ^{131}I for treatment of hyperthyroidism in the cat could possibly be achieved after rhTSH administration, thereby respecting the ALARA (as low as reasonably achievable) principle. The objective of this study was to investigate the effect of rhTSH on RAIU and serum total T4 (TT4) concentration in hyperthyroid cats.

Inclusion criteria were clinical signs compatible with hyperthyroidism and increased serum TT4 concentration. On day 1, a bloodsample for measurement of TT4 (serum $TT4_0$) was taken by jugular venipuncture and 25 µg rhTSH was injected IV. One hour later a dose of 11.4 ± 4.1 (mean ± sd) MBq ^{123}I was injected IV. Bloodsamples for TT4 measurement were taken and RAIU was measured 6, 12 and 24 hours after rhTSH injection (RAIU-rhTSH). Blood was centrifuged and stored for 3 days (−20 °C) until analysed for serum TT4 concentration. A static planar ventral image of 200.000 counts using a low energy high resolution (LEHR) collimator was made with the cat in sternal recumbency under general anesthesia. A syringe with a known amount of radioactivity (2.5 ± 1.6 MBq) was located next to the animal. This was used as the standard activity necessary to calculate RAIU. On day 9, the study from day 1 was repeated with injection of 2 ml NaCl 0.9% instead of rhTSH (RAIU-blanco). Results are expressed as mean ± sd.

Five hyperthyroid cats were included (age 13 ± 2 years, 3.3 ± 0.4 kg bodyweight). Percentages of RAIU-rhTSH (and RAIU-blanco) at 6, 12 and 24 hours after administration of rhTSH were 34 ± 18 (31 ± 21), 46 ± 20 (38 ± 18) and 47 ± 15 (36 ± 14), respectively. There was an overall statistically significant effect of rhTSH administration on RAIU ($P = 0.043$) with an overall mean difference of 7.33% between RAIU-rhTSH and RAIU-blanco. There was no statistically significant difference in effect between the timepoints after rhTSH administration ($P = 0.070$). There was no statistically significant effect of rhTSH on serum TT4 concentration 6 hours ($P = 0.250$), 12 hours ($P = 0.313$) and 24 hours $P = 0.313$) after administration. Baseline serum $TT4_0$ concentration influenced significantly RAIU-rhTSH at 6 hours ($P = 0.037$) but not at 12 hours ($P = 0.074$) nor at 24 hours ($P = 0.522$) after administration of rhTSH. No statistically significant effect of baseline serum $TT4_0$ was found on RAIU-blanco at 6 hours ($P = 0.052$), at 12 hours ($P = 0.079$) or at 24 hours ($P = 0.464$) after administration of NaCl 0.9%.

This preliminary study shows promising results with an increased RAIU being observed after rhTSH administration. Future studies are needed to optimize the dose and timing of rhTSH.

ABSTRACT #315

SERUM MAGNESIUM CONCENTRATIONS IN ASSOCIATION WITH CANINE CALCIUM METABOLIC DISORDERS. PA Schenck. Diagnostic Center for Population and Animal Health, Endocrine Diagnostic Section, Michigan State University, Lansing, MI.

Parathyroid hormone (PTH) production is influenced by serum ionized magnesium (iMg) concentration, and either a deficiency or excess may suppress PTH production. Cell membrane receptors also may have decreased sensitivity to serum ionized calcium (iCa) in the presence of low serum iMg concentrations. Many dogs with hypoparathyroidism have been shown to have low serum iMg, but iMg concentrations have not been studied in other calcium metabolic disorders. The objective was to determine the serum iMg status in dogs with calcium metabolic disorders. A total of 19,871 canine cases submitted for PTH and iCa measurement were reviewed, and sorted by diagnosis into the following categories: normal, borderline hypercalcemia, secondary hyperparathyroid, primary

hyperparathyroid, parathyroid independent hypercalcemia, humoral hypercalcemia of malignancy (with elevated parathyroid hormone related protein; PTHrP), and hypoparathyroid. In addition, those dogs with a history of chronic renal failure but without secondary hyperparathyroidism were identified for comparison to those with secondary hyperparathyroidism. The mean iMg concentration in each group was within the canine reference range (0.41–0.61 mmol/L); however, there were significant differences in the mean values, and the distribution of concentrations was not the same in each group. Hypoparathyroid dogs (n = 794) had the lowest mean iMg concentration (0.46 mmol/L), and 32% of hypoparathyroid dogs had an iMg concentration below the reference range. Dogs with elevated PTHrP (n = 1170), parathyroid independent hypercalcemia (n = 4379), or primary hyperparathyroidism (n = 4761) had a significantly higher mean iMg (0.49, 0.50 and 0.51 mmol/L respectively), with 16%, 16%, and 11% below the reference range respectively. Dogs with secondary hyperparathyroidism (n = 2112) had significantly higher iMg concentrations (mean 0.53 mmol/L, median 0.51 mmol/L, 15% below the reference range, 20% above the reference range) than the previous groups, but still significantly lower than normal or borderline hypercalcemic dogs. Mean concentrations of iMg were similar in dogs with normal PTH and iCa concentrations (n = 2933, mean 0.54 mmol/L), dogs with borderline hypercalcemia (n = 1816, mean 0.53 mmol/L), and in those with CRF but not secondary hyperparathyroidism (n = 243, mean 0.53 mmol/L). Approximately 9% of dogs with CRF but not secondary hyperparathyroidism had iMg concentrations below the reference range, as compared to 5% of those with borderline hypercalcemia, and 6% of those with normal PTH and iCa. These data suggest that differences in serum iMg concentrations do occur in association with calcium metabolic disorders.

ABSTRACT #316
SERUM MAGNESIUM CONCENTRATIONS IN ASSOCIATION WITH FELINE CALCIUM METABOLIC DISORDERS. PA Schenck. Diagnostic Center for Population and Animal Health, Endocrine Diagnostic Section, Michigan State University, Lansing, MI.

Serum ionized magnesium (iMg) concentration can influence parathyroid hormone (PTH), and either a deficiency or excess may suppress PTH production. With low serum iMg concentrations, receptors have decreased sensitivity to serum ionized calcium (iCa). In general, low iMg concentrations are associated with significant illness or hypoparathyroidism. However, serum iMg concentrations have not been studied in other calcium metabolic disorders. The objective was to determine the serum iMg status in cats with calcium metabolic disorders. A total of 11,320 feline cases submitted for PTH and iCa measurement were reviewed, and sorted by diagnosis into the following categories: normal, borderline hypercalcemia, secondary hyperparathyroid, primary hyperparathyroid, parathyroid independent hypercalcemia, humoral hypercalcemia of malignancy (with elevated parathyroid hormone related protein; PTHrP), and hypoparathyroid. In addition, those cats with a history of chronic renal failure but without secondary hyperparathyroidism were identified for comparison to those with secondary hyperparathyroidism. The mean iMg concentration in each group (except for hypoparathyroid cats) was within the feline reference range (0.54–0.70 mmol/L); however, there were significant differences in the mean values, and the distribution of iMg concentrations was not the same in each group. Hypoparathyroid cats (n = 80) had the lowest mean iMg concentration (0.46 mmol/L), with 85% below the reference range. Cats with secondary hyperparathyroidism (n = 1491) or CRF without secondary hyperparathyroidism (n = 394) had significantly higher iMg concentrations (mean 0.55 mmol/L in both groups) as compared to hypoparathyroid cats, but these means were still significantly lower than cats with other disorders. In cats with secondary hyperparathyroidism, 49% had iMg values below and 9% had iMg values above the reference range, and in those with CRF but not secondary hyperparathyroidism, 44% of values were below and 6% of values were above the reference range. Cats with normal PTH and iCa (n = 2229), or borderline hypercalcemia (n = 749) had significantly higher mean iMg concentrations (0.58 and 0.57 mmol/L, respectively) as compared to previous groups. Approximately 33% of cats with normal PTH and iCa and borderline hypercalcemia had iMg concentrations below the reference range. Cats with primary hyperparathyroidism (n = 434), parathyroid independent hypercalcemia (n = 5847), or humoral hypercalcemia of malignancy with elevated PTHrP (n = 184) had the highest mean iMg concentrations (0.62, 0.59, and 0.60 mmol/L respectively). In primary hyperparathyroidism, iMg concentrations showed a wide spread, with 28% of iMg values below, and 27% above the reference range. Approximately 25% of cats with elevated PTHrP and 31% of cats with parathyroid independent hypercalcemia had iMg concentrations below the reference range. These data suggest that difference in serum iMg concentrations do occur in association with calcium metabolic disorders, and that cats are more likely to have serum iMg concentrations below the reference range as compared to dogs.

ABSTRACT #317
BREED-DEPENDENCY OF BASAL PLASMA CREATININE: A CAT IS NOT A CAT. BS Reynolds, C. Germain, K Boudet, T Daste, JP Braun, HP Lefebvree. Department of Clinical Sciences, National Veterinary School, Toulouse, France.

Plasma creatinine (P-creatinine) is considered as the best indirect indicator of renal function and used for staging of chronic kidney disease. Nevertheless, to our knowledge, no rational definition of reference interval for P-creatinine has been published in the feline species. The objective of the present study was to determine the reference interval of P-creatinine in 4 major feline breeds.

Blood was collected on heparinised tubes in fasted clinically healthy owned cats. Blood was immediately centrifuged. Plasma was harvested and stored at −20 °C until assay by the enzymatic method using an analyzer (Vitros 250 chemistry system, Ortho-Clinical Diagnostics, Raritan, NJ). Data (without or with logarithmic transformation) were tested for normality by Kolmogorov-Smirnov test. Effect of breed, body weight (BW), age and gender was assessed using a general linear model.

Birman (n = 137), Chartreux (n = 134), Maine Coon (n = 143), and Persian (n = 141) cats were sampled. The corresponding reference intervals (RI) were 1.0–2.8 mg/dL, 0.7–2.3 mg/dL, 0.7–2.2 mg/dL, and 0.8–1.9 mg/dL, respectively. A higher P-creatinine ($P < 0.001$) was observed in Birman breed compared to the others. P-creatinine increased ($P < 0.05$) with age in all breeds, and with BW in Birman, Chartreux and Maine Coon. P-creatinine was higher ($P < 0.05$) in female than male Birman cats.

In conclusion, breed, age, and BW should be considered when interpreting basal P-creatinine in cats.

ABSTRACT #318
PLASMA CLEARANCE OF EXOGENOUS CREATININE, EXO-IOHEXOL AND ENDO-IOHEXOL IN HEALTHY CATS, CATS WITH HYPERTHYROIDISM AND CATS WITH CHRONIC KIDNEY DISEASE. I van Hoek[1], HP Lefebvre[2], S Croubels[1], S Daminet[1]. [1]Ghent University, Ghent, Belgium. [2]École Nationale Vétérinaire de Toulouse, Toulouse, France.

Measurement of glomerular filtration rate (GFR) in cats allows detection of a decreased kidney function in an early stage of kidney disease. The plasma clearance of exogenous creatinine (PECCT) seems to be a promising alternative to the more complicated methods for GFR measurement in cats. The objective of this study was to compare PECCT, exo-iohexol (Pex-ICT) and endo-iohexol (Pen-ICT) for discriminating healthy cats (H), hyperthyroid cats (HT) and cats with chronic kidney disease (CKD) suspected to have respectively normal, high and low GFR values.

Inclusion criteria for the H cats were no clinically significant abnormalities on clinical exam and blood and urine analysis. Cats with HT were included with clinical signs compatible with hyperthyroidism, increased total thyroxin serum concentration and increased thyroidal uptake of $^{99m}TcO_4^-$. Antithyroid drugs had been discontinued at least 3 weeks prior to inclusion. Cats with CKD were included based on symptoms of CKD and azotemia (IRIS stage II or higher). The PECCT, Pex-ICT and Pen-ICT were per-

formed in a combined manner (described by van Hoek et al., JVIM 2007;21:950-958). A linear mixed model was used for statistical analysis. Results are expressed as mean ± sd.

The study included 6 H cats (age 7–12 months, BW 4.3–5.6 kg), 6 HT cats (age 8–16 years, BW 2.6–6.2 kg) and 4 CKD cats (age 10–13 years, BW 4.5–6.6 kg). Plasma clearance values (ml/min/kg) for the Pex-ICT, Pen-ICT and PECCT respectively are 1.8 ± 0.3, 3.1 ± 0.6 and 2.8 ± 0.5 in the H cats, 4.1 ± 1.2, 4.3 ± 1.2 and 4.7 ± 1.2 in the HT cats, and 0.9 ± 0.2, 0.9 ± 0.1 and 1.0 ± 0.1 in the cats with CKD. There was a significant difference between Pex-ICT and Pen-ICT ($P < 0.001$) and PECCT ($P < 0.001$) but not between Pen-ICT and PECCT ($P = 0.210$) in H cats. However, there was no significant difference between GFR methods in cats with CKD ($P = 0.386$) or HT cats ($P = 0.185$). There was a significant difference between CKD cats and HT cats for Pex-ICT ($P < 0.001$), Pen-ICT ($P < 0.001$) and PECCT ($P < 0.001$), between H and HT cats for Pex-ICT ($P = 0.001$), PECCT ($P = 0.005$) but not for Pen-ICT ($P = 0.067$), and between CKD and H cats for Pen-ICT ($P = 0.004$), PECCT ($P = 0.018$) but not for Pex-ICT ($P = 0.280$).

The Pex-ICT, Pen-ICT and PECCT do not differ between eachother in cats with low or high GFR values. However, there is a significant difference in cats with normal GFR values. The Pex-ICT and Pen-ICT can detect the large difference between low and high GFR values. However, Pex-ICT cannot detect the smaller difference between normal and low GFR values, and Pen-ICT cannot detect the smaller difference between normal and high GFR values. The PECCT is able to detect a significant difference in GFR between H, HT and CKD cats. This supports the hypothesis that the PECCT is valuable to detect a decreased kidney function.

ABSTRACT #319

EVALUATION OF MINERAL METABOLISM IN DOGS AT DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE: PRELIMINARY RESULTS.

O. Cortadellas[1], M[a] J. Fernández del Palacio[2], J. Talavera[2]. [1]Clinica Veterinaria Germanías, Gandía - Valencia. [2]Departamento de Medicina y Cirugía Animal, Universidad de Murcia, Spain.

Disorders of mineral metabolism (phosphate, ionized calcium (iCa), 1,25-$(OH)_2D_3$ (calcitriol) and PTH) occur early in the course of chronic kidney disease (CKD), both in humans and animals. These abnormalities lead to the development of secondary hyperparathyroidism which induces deleterious effects in many organ systems and a negative impact in CKD progression. In dogs is still unknown at what stage of CKD these changes occur. An early recognition of these disorders would allow appropriate therapeutics and potentially, a better prognosis for patients with CKD. The aim of this study was to evaluate, prospectively, the mineral metabolism in a population of dogs at the different stages of naturally occurring CKD.

Twenty-four dogs with CKD and 15 healthy control dogs have been enrolled in the study. Laboratory evaluation included blood count, biochemistry, urinalysis (specific gravity, dipstick, sediment examination and urine protein/creatinine ratio (UPC)) and determination of PTH, calcitriol and iCa concentrations. Serum creatinine concentration (SCr) and UPC were used to stage the severity of CKD (IRIS staging scheme). Results of laboratory evaluation (mean ± SD) are shown at the following table (*denotes statistically significant differences).

Dogs/Stage CKD[‡]	Phosphate (mg/dL)	Ca (mg/dL)	iCa (mg/dL)	Calcitriol (ng/mL)	PTH (ng/mL)
Control (n = 15)	4.6 ± 0.6	10.7 ± 0.8	4.4 ± 0.7	67.9 ± 12.1	17.8 ± 11.2
Stage 1 (n = 4)	4.3 ± 0.8	10.4 ± 0.7	4.9 ± 0.6	52.6 ± 4.2	29.4 ± 16
Stage 2 (n = 4)	5.5 ± 1.9	10.9 ± 0.6	4.5 ± 0.4	39.9 ± 7.1*	46.3 ± 21.6
Stage 3(n = 12)	6.6 ± 1.2	11 ± 0.8	4.7 ± 0.6	34.6 ± 4.4*	86.8 ± 57
Stage 4 (n = 4)	15.8 ± 5.4*	10.7 ± 2.5	3.4 ± 0.4	23.7 ± 15.7*	307.2 ± 193*

[‡]Stage 1:SCr < 1.4 mg/dL; UPC > 0.5; stage 2 SCr:1.4 to 2 mg/dL; stage 3:Cr: 2.1 to 5 mg/dL; stage 4: SCr > 5 mg/dL).

These preliminary results have shown that serum phosphate and PTH concentrations start to increase at stage 2 of CKD. However, statistically significant differences with healthy dogs are only reached at stage 4 of CKD. In contrast, a progressive and statistically significant decline in calcitriol concentrations was already present by stage 2 of CKD. To properly validate these results, it would be convenient to include a significant amount of animals in each stage of CKD.

ABSTRACT #320

EFFICACY AND SAFETY OF LANTHARENOL® ON PHOSPHORUS METABOLISM IN CATS WITH CHRONIC KIDNEY DISEASE.

BH Schmidt[1], U Spiecker-Hauser[1], M Murphy[2]. [1]Bayer Healthcare AG, Monheim, Germany. [2]Charles River Laboratories, Ballina, Ireland.

The objective of this study was to assess the efficacy and safety of Lantharenol®, a novel intestinal phosphate binder, in cats with renal impairment.

There was a single study group consisting of 10 cats (6 male and 4 female cats) that had successfully undergone a two-stage partial renal ablation procedure. The cats were normophosphataemic and clinically normal except for the renal impairment. Each animal was individually housed and offered Lantharenol once daily mixed with wet cat feed at the following concentrations: 0–0.3–1.0–3.0–0 g Lantharenol kg^{-1} feed original substance, each dose being administered for two weeks. During the last three days of each dose period, urine and feces were quantitatively collected from each animal, and the quantity of diet consumed was measured. Quantitative P excretion in urine and feces, and the derived apparent digestibility and intestinal absorption of P served as efficacy parameters. Renal function was assessed by monitoring urea, creatinine, calcium and inorganic P levels in serum as well as specific gravity and creatinine levels in urine. Safety was determined by measuring the P balance as well as feed intake and body weight, repeated health status examinations and the observation of the daily tolerance of Lantharenol.

Lantharenol was well accepted, tolerated and effective as an intestinal phosphate binder in the dose range of 0.3 to 3 g kg^{-1} wet feed. In the specific feed used for the present study, this range corresponded to 1.77 to 17.7 g kg^{-1} complete feed. The highest dose tested mediated the greatest reduction in absorption and apparent digestibility of P, along with a significant increase in fecal P excretion. The concomitant decrease in P balance is explained by an increase in urinary P excretion in Lantharenol-treated animals. Renal function and the defined safety parameters were not compromised in any manner potentially related to test item administration.

In conclusion, feed supplementation with Lantharenol results in a safe, dose-dependent, statistically significant and clinically relevant reduction of P availability, thus ending up in a relief of kidney burden to excrete excess P. Longer-term studies will be required to assess potential effects on serum inorganic P levels especially in chronic kidney disease patients afflicted with hyperphosphataemia.

ABSTRACT #321

EVALUATION OF URINARY PROTEIN ELECTROPHORESIS AND ALBUMINURIA IN DOGS WITH HYPERADRENOCORTICISM, AND RELATIONSHIP TO SYSTEMIC ARTERIAL BLOOD PRESSURE.

CZ Cavalcante[1], MM Kogika[1], ML Santoro[2], A Bacic[1], SI Miyashiro[1], JP Sault[1], DMN Simões[1], CS Prosser[1]. [1]School of Veterinary Medicine – University of São Paulo, Brazil. [2]Institute Butantan, São Paulo, Brazil.

Hyperadrenocorticism (HAC) is a common endocrinopathy in dogs; chronic hypercortisolemia may promote several complications including glomerulonephritis (GN) and systemic arterial hypertension, and this latter *per se* may also cause secondary GN or worsening of previous glomerular disease. Loss of albumin in urine is characteristic of glomerular diseases. The aim of the current study was to investigate proteinuria, qualitatively and quantitatively, and whether hypertension is associated with increased albuminuria, in dogs with naturally occurring HAC. The qualitative study of proteinuria was performed by sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) stained with Comassie blue, and for the quantitative evaluation urine protein-to-creatinine (UPC) ratio, normalized albumin (nUAlb) concentra-

tion, and urinary albumin-to-creatinine (UAC) ratio (using a competitive canine albumin capture ELISA) were determined.

Thirty dogs of various breeds (6 male, 24 female) with documented HAC, ranging in age from 5 to 13 y-old were subdivided in two groups as follows: group I composed of 13 dogs with hypertension (systolic pressure $\geq$ 180 mmHg) and group II consisted of 17 normotensive dogs. None of the dogs had received anti-hypertensive drugs. Results were compared with data of 30 clinically healthy dogs (controls). All data of HAC dogs (groups I and II) compared with controls were statistically different. Hypertension in dogs with HAC did not affect the magnitudes of proteinuria or albuminuria when compared to normotensive HAC dogs (mean $\pm$ SEM, respectively), concerning UPC ratio (3.68 ± 1.21 *vs* 2.22 ± 0.76; $P > 0.05$), nUAlb concentration (0.13 ± 0.03 *vs* 0.11 ± 0.05 mg/mL; $P > 0.05$) and UAC ratio (0.92 ± 0.31 *vs* 1.03 ± 0.52; $P > 0.05$). Also the comparison of molecular weights of urinary proteins of HAC dogs of groups I and II showed no difference; low molecular weight was considered for bands < 60 kDa (LMW - region B) and high molecular weight proteins for bands ≥ 60 kDa (HMW- region A, which albumin is included). UPC ratio determinations for each region, LMW - region B (1.10 ± 0.51 *vs* 0.47 ± 0.15; $P > 0.05$) and HMW – region A (2.58 ± 0.85 *vs* 1.74 ± 0.63; $P > 0.05$), also demonstrated no significant difference.

In conclusion, dogs with hyperadrenocorticism showed tubular lesions detected by increased number of LMW proteins bands (tubular proteinuria) as well as glomerular lesions characterized by the presence of albuminuria and HMW proteins bands in urine, independently of systemic arterial hypertension, suggesting that blood pressure did not influence consistently the intensity of urinary protein loss or glomerular damage, and chronic hypercortisolemia is likely to be the main cause of the development of kidney disease.

ABSTRACT #322

WITHIN-DAY AND BETWEEN-DAY VARIABILITY OF BLOOD PRESSURE MESUREMENT IN HEALTHY CONSCIOUS DOGS. EP Rattez[1], BS Reynolds[1], CJ Layssol Lamour[1], MM Ségalen[1], HP Lefebvre[1]. [1]Department of Clinical Sciences, National Veterinary School, Toulouse, France.

Diagnosis and management of systemic arterial hypertension in dogs is based on repeated blood pressure measurement (BPM). The objective was here to assess the within- and between-day variability of BPM using a new device (petMAP, Ramsey Medical, Inc., Tampa, FL).

The study was blinded and randomized. Five healthy conscious adult Beagles were used. They were not familiar with BPM procedure and the three investigators. Each investigator performed two testing on each dog on Day 1. The cuff was placed on the forelimb. For each testing (ie 8 consecutive BPM), the investigator could discard up to 3 outlier BPM and then averaged the values. Similarly, BPM was performed again on Day 2. Coefficients of variation (CV) and standard deviations (SD) for within- and between-day variability were calculated for each investigator using a general linear model.

Four hundred and eighty BPM were made on the two consecutive days. Average systolic (SAP) and diastolic (DAP) blood pressure ranges were [134–209 mmHg] and [66–126 mmHg], respectively. The CV ranges were [9.0–10.1%] and [12.8–16.4%] for within-day and between-day variability of SAP, respectively. The corresponding SD values were [14.7–16.6 mmHg] and [21.0–27.1 mmHg], respectively. The within-day and between-day variability CVs for DAP were [10.3–14.4%] and [14.2–24.9%].

In conclusion, the within-day and between-day variability need to be documented for validation and relevant clinical interpretation of BPM.

ABSTRACT #323

IDENTIFICATION OF POLYMORPHISMS IN GENES INVOLVED IN ALDOSTERONE SYNTHESIS AND ACTIVITY IN FELINE HYPERTENSIVE CHRONIC KIDNEY DISEASE. SJ Keele, AM Murphy, M Binns, J Elliott, HM Syme. Royal Veterinary College, University of London, London, UK.

Systemic hypertension is a common disorder of aged cats. It is most often diagnosed in cats with chronic kidney disease (CKD) with around one-third of such cats being hypertensive; however, cats that are normotensive at the time of CKD diagnosis rarely develop hypertension subsequently. Hypertensive cats have significantly lower plasma potassium than normotensive cats with CKD, possibly due to hyperaldosteronism, which could in turn be due to polymorphisms in relevant encoding genes. Polymorphisms in genes involved in aldosterone synthesis (11-beta hydroxylase, aldosterone synthase) and activity (amiloride-sensitive epithelial sodium channel; ENaC) are associated with altered risk of developing essential hypertension in humans and were investigated in this study.

Cats with naturally-occurring CKD (plasma creatinine > 2.0 mg/dL and with inappropriately low urine specific gravity < 1.035) were selected for this study. Hypertension was diagnosed if systolic blood pressure measured by Doppler was > 175 mmHg on at least two occasions or on one occasion if hypertensive ocular lesions were present. Genomic DNA was extracted by silica-gel membrane adsorption (QIAamp® DNA blood kit; QIAgen) from buffy coat-enriched packed cells collected into potassium EDTA tubes. Sequencing primers were designed from contigs built from the *Felis catus* whole genome sequence trace archive (NCBI). Sections of genes encoding 11-beta hydroxylase, aldosterone synthase and the three subunits of ENaC were amplified by polymerase chain reaction and sequenced in three normotensive and three hypertensive cats. Single nucleotide polymorphisms (SNPs) were identified. Selected SNPs were genotyped by single base extension (SNaPshot™, Applied Biosystems) in a further 24 normotensive and 24 hypertensive cats to establish allele frequencies.

Genes for aldosterone synthase and 11-beta hydroxylase were largely monomorphic (minor-allele frequencies of SNPs: 0.03–0.09). Genes for the alpha, beta and gamma ENaC subunits respectively contained two SNPs with minor-allele frequencies 0.38 and 0.49, four SNPs with minor-allele frequencies 0.20–0.44, and five SNPs with minor-allele frequencies 0.11–0.49.

This study has identified numerous polymorphisms that can now be evaluated to determine if they are associated with hypertension in the cat. Work to genotype 250 more cats with CKD and known blood pressure status is currently ongoing.

ABSTRACT #324

DETERMINATION OF PLASMA UROTENSIN-II IN CATS WITH CHRONIC KIDNEY DISEASE. J Elliott and MJ Kleinz. Royal Veterinary College, London, UK.

High blood pressure is common in cats with chronic kidney disease (CKD) and if untreated can lead to severe end organ damage and further promote kidney damage. This complex link between hypertension and CRF suggests that therapies targeting a potential pathophysiological mechanism linking these two processes may be superior in treating feline renal hypertension. The aim of our study therefore was to validate a urotensin assay for cat plasma and determine plasma levels of urotensin-II, a potent vasoconstrictor peptide produced in the kidney, in cats with renal hypertension and in healthy controls.

Systolic blood pressure (SBP) measurements were obtained and plasma creatinine levels determined in 60 cats (xmale, xfemale, average age: 12.7 ± 4.4 years). EDTA plasma was collected for analysis using a commercial urotensin-II (U-II) enzyme-linked immunoassay (EK-071-05, Phoenix Pharmaceuticals, Belmont, CA, USA). Assay validation examined assay specificity in cat plasma (dilutional parallelism and recovery). Cats were grouped according to their blood pressure (hypertension defined as repeated SBP measurements > 170 mmHg or a single measurement combined with characteristic ocular lesions) and renal status (CKD defined as plasma creatinine persistently > 177 µmol/L associated with urine specific gravity < 1.035) and U-II levels compared between groups using Mann-Whitney-U or Kruskall-Wallis test ($p < 0.05$ indicating statistical significance). Hypertensive cats with no evidence of CKD were categorised as idiopathic hypertensives (iHT).

Dilution curves of feline samples were parallel to the standard curve and recovery was greater than 70%. U-II levels were significantly lower in hypertensive cats compared to normal controls

(median (25–75 percentile): 0.27 ng/ml (0.23–0.61) vs. 0.35 ng/ml (0.27–0.74), p = 0.044). However, U-II levels were not different between normotensive and hypertensive cats with CKD (non-hypertensive and non-azotemic: 0.41 ng/ml (0.24–0.65); non-hypertensive and azotemic: 0.3 ng/ml (0.23–0.54); hypertensive and non-azotemic/iHT: 0.26 ng/ml (0.18–0.44); hypertensive and azotemic: 0.29 ng/ml (0.23–0.36); p = 0.23) and no correlation observed between urotensin-II, creatinine and SBP (p = 0.15/0.19 respectively).

In this study we report for the first time plasma U-II levels in the cat. Despite the reported upregulation of plasma U-II in experimental renal failure in the rat and in human patients with high blood pressure, our findings, showing strong overlap of plasma urotensin-II levels in cats with hypertension compared to normotensive controls animals, and demonstrating no significant difference in U-II between normotensive and hypertensive cats with CRF, suggest that changes in plasma levels of this vasoactive renal peptide hormone do not play a significant role in the pathogenesis of feline renal hypertension. Indeed, our results suggest urotensin production may be physiologically down-regulated in hypertensive cats.

ABSTRACT #325

PERITONEAL DIALYSIS IN CATS WITH ACUTE KIDNEY INJURY. Cooper RL[1] and Labato MA[2]. [1]Wheat Ridge Animal Hospital, Wheat Ridge CO. [2]Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA.

Peritoneal dialysis (PD) is most frequently used in the management of acute kidney injury (AKI) refractory to fluid therapy. The purpose of this study is to examine indications, effectiveness, outcomes and complications associated with the use of PD in cats with AKI.

A database search of all cats receiving PD for AKI at Tufts University Cummings School of Veterinary Medicine from 2001 to 2006 was performed. The following data was collected from the 22 cats who qualified: signalment, weight, indication for PD, number of PD cycles, number of days of PD, outcome (discharge, euthanasia or death), days from the start of PD to endpoint (discharge vs. euthanasia or death), mean survival time (MST) from onset of PD, and method of catheter placement (surgical placement with omentectomy vs. non-surgical placement). Complications were recorded from each patient's daily record. Pre- and post-PD values were obtained for the following variables: blood urea nitrogen, creatinine, phosphorus, sodium, potassium, chloride, total protein, albumin, and urine output.

Indications for PD consisted of acute-on-chronic renal failure (7/22), urolithiasis (5/22), nephrotoxins (4/22), spay complications (3/22), unknown reasons (3/22). 45.5% of cats were discharged from the hospital after PD. 31.8% of cats were euthanized before discharge from the hospital. 22.7% of cats died in the hospital while receiving PD. There was a significant difference (p = 0.034) in MST depending on the indication for PD. 100% (3/3) of cats with spay complications were discharged from the hospital. The MST for these cats from onset of PD was 1423 days (984–1867). 60% (3/5) of cats with urolithiasis-induced AKI were discharged from the hospital. The MST for this group was 332.6 (0–866). 42.8% (3/7) of cats with acute-on-chronic renal failure were discharged from the hospital. The MST for this group was 175.7 days (0–839). Of cats presenting with toxicities, there were no survivors (0%, 0/4). The MST for cats with toxicity was 2.5 days (1–4).

Common complications of PD included dialysate retention and sequestration of dialysate under the skin. Less common complications included clogged PD catheters, leakage of fluid from catheter sites, painful abdomen, peritonitis, dehiscence of surgical incision and pleural effusion. Hypoproteinemia and hypoalbuminemia were the most common bloodwork abnormalities noted. Technical complications were present in both methods of placement of PD catheters, but the complication rate was lower in cats with surgically placed PD catheters with partial omentectomy compared to ER placement of catheters.

PD is an important therapeutic tool for reducing uremia and giving kidneys time to recover in cats with AKI when conventional therapy is no longer effective.

ABSTRACT #387

INFLUENCE OF AN ORALLY ADMINISTERED GLYCOSAMINOGLYCAN PREPARATION ON URINARY SATURATION FOR CALCIUM OXALATE AND STRUVITE IN HEALTHY CATS. B Young, J Bartges, C Kirk, S Cox, M Mustillo, T Moyers, H Byrd. The University of Tennessee, Knoxville, TN.

Glycosaminoglycans are recommended commonly for treatment of osteoarthritis and feline idiopathic cystitis; however, they may also have a role in urolith formation. Glycosaminoglycans inhibit crystal and urolith formation, and decreased urinary levels of glycosaminoglycans are associated with increased risk of calcium oxalate urolith formation in human beings. During urolith formation and growth, glycosaminoglycans are incorporated into the urolith matrix; therefore, they may also promote urolith formation and growth. We hypothesized that oral administration of a commercially available preparation containing chondroitin sulfate and glucosamine, commonly used for treating osteoarthritis in dogs and cats, to healthy adult female cats would be associated with increased urinary relative supersaturation for calcium oxalate and struvite. Our objectives were to evaluate administration of a commercially available compound containing chondroitin sulfate and glucosamine used for management of osteoarthritis and idiopathic cystitis in cats on urinary excretion of electrolytes and minerals, and urinary saturation for calcium oxalate.

Six healthy, spayed female cats, aged 4–6 years, and weighing 3.5–6.5 kg, were evaluated. Twenty-four urine samples were collected before and after administration of an oral glycosaminoglycan preparation at recommended dosage for management of osteoarthritis (Cosequin, Nutramax Laboratories) for two weeks. Twenty-four hour urine samples were collected using a modified litter box. A dry, adult maintenance food (SportMix, Midwestern Pet Foods) was fed to maintain body weight and condition. Twenty-four hour urine samples were mixed, the volume recorded, and pH, sodium, potassium, chloride, calcium, magnesium, phosphorous, citrate, oxalate, and ammonia concentrations were determined. Molar concentrations of these analytes were entered into a computer program (EQUIL 89d, University of Florida) for determination of relative supersaturation for calcium oxalate monohydrate and dihydrate, and struvite. Data were analyzed using 2-tailed, paired t-tests; $p \leq 0.05$ was significant.

Body weight did not change between the start and finish of the treatment period. Significant differences were not found for 24-hour urine pH, 24-hour urine volume, 24-hour urinary excretion of minerals or electrolytes, or for urinary relative supersaturation of calcium oxalate or struvite.

Oral administration of a commercially available glycosaminoglycan preparation at recommended dosages for two weeks did not increase or decrease urinary saturation for calcium oxalate or struvite in healthy adult female cats.

ABSTRACT #326

PRELIMINARY EVALUATION OF ESOPHAGEAL STENTING FOR RECURRENT BENIGN ESOPHAGEAL STRICTURES IN 1 FERRET AND 2 DOGS. C Weisse[1], A Berent[1], J Kaae[2], S Murphy[2], K Richter[3]. [1]Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA. [2]University of Wisconsin School of Veterinary Medicine, Madison, WI. [3]Veterinary Specialty Hospital of San Diego, San Diego, CA.

Benign esophageal strictures are uncommon in animals and can occur following gastroesophageal reflux, esophagitis, and foreign body ingestion. Most animals improve with balloon dilatation, however some recur even after numerous treatments. The purpose of this study was to evaluate the use of self-expanding mesh stents for the treatment of benign esophageal strictures after balloon dilatation was either unsuccessful or declined.

Owner consent was required and follow-up information collected. All stents were placed per os using both fluoroscopy and endoscopy.

Three cases were consecutively performed at three different institutions. All strictures were believed to have occurred from gastroesophageal reflux and esophagitis following a recent anesthetic episode. Balloon dilatation was performed in all cases before stenting (1 to 7 times) and following stenting (0 to 2 times). The ferret received an uncovered nitinol stent and required one additional

balloon dilatation for stricture recurrence. The ferret is currently eating normal food 16 months post-stenting. Dog #1 received a covered nitinol stent to reduce stricture recurrence and permit stent removal following stricture resolution. Several hours after placement the stent migrated into the stomach and was endoscopically retrieved. The dog continues to do well 10 months later. Dog #2 received a bioabsorbable polydioxanone stent in order to permit stent absorption following stricture resolution. Endoscopic suturing was performed to prevent migration. This dog continues to do well 2 months post-stenting.

Preliminary experience suggests that stenting for benign esophageal strictures is feasible but can be associated with migration and stricture recurrence in certain circumstances.

ABSTRACT #327

CHRONIC GASTRIC DISEASE IN DOGS: A RETROSPECTIVE STUDY OF 73 CASES (2002–2007). JA Lidbury, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Gastric disease in dogs is poorly characterized. Chronic gastric disease without involvement of other parts of the gastrointestinal (GI) tract is believed to be uncommon in the dog. The objectives of this retrospective study were to test the hypothesis that intestinal pathology often occurs concurrently with chronic gastric disease in dogs and to characterize the historical, physical, clinicopathological, imaging, endoscopic, and histological findings in dogs with chronic gastric disease.

Medical records from 159 dogs that underwent gastroscopy, gastroduodenoscopy, or gastrotomy were reviewed retrospectively. Dogs were included in the study if abnormal histopathological findings were identified upon evaluation of gastric biopsies. The history, clinical examination findings, results of diagnostic testing, diagnoses, treatments, and outcome were recorded for each case. Cases were then divided into 3 groups: group 1 was comprised of dogs with disease primarily involving the stomach, group 2 was comprised of dogs with clinically significant disease of the stomach and other parts of the GI system, and group 3 was comprised of dogs with disease primarily involving the extra-gastric GI system with only minimal gastric involvement. Categorical data were analyzed using a χ^2 test.

Seventy-three dogs with gastric pathology were included in this study. A range of breeds and ages were represented. Group 1 accounted for 41.1% (n = 30) of all dogs included in the study, group 2 for 28.8% (n = 21), and group 3 for 30.1% (n = 22). The most frequent clinical sign recorded was vomiting, which was recorded in 57.5% (n = 42) of cases. The most common biochemical abnormality was hypoalbuminemia, occurring in 39.7% (n = 29) of cases. Hypoalbuminemia occurred in 13.3% (n = 4) of dogs in group 1, 38.1% (n = 8) of dogs in group 2, and 77.3% (n = 17) of the dogs in group 3; ($p<0.01$). Lymphoplasmacytic gastritis (LPG) was the most frequent histopathological diagnosis, recorded in 52.1% (n = 38) of cases. Of the dogs with LPG, 13.2% (n = 5) were classified as having minimal and 55.3% (n = 21) were classified as having mild LPG. Concurrent intestinal pathology was recorded in 71.9% (n = 46) of cases, where intestinal biopsies were collected. Chronic gastritis of unknown cause was the clinician's final diagnosis in 13.7% (n = 10) of cases and was more commonly diagnosed than any specific etiology of gastritis.

The results of this study suggest that chronic gastric disease occurs most commonly with concurrent intestinal pathology. However, a considerable number of dogs did not have evidence of concurrent intestinal disease. Hypoalbuminemia occurred more frequently in dogs with disease affecting the intestines than in those with disease primarily affecting the stomach. Lymphoplasmacytic gastritis was the most commonly observed gastric histopathological finding and was often of minimal or mild severity. The clinical significance of minimal or mild LPG remains uncertain. Also, the cause of chronic gastritis often remained unknown.

ABSTRACT #328

DEVELOPMENT OF CANINE INTESTINAL LYMPHOCYTE SUBSETS. N Luckschander[1,2], N Corazza[1], I Burgener[2], P Moore[3], A Zurbriggen[4], JW Blum[5], T Brunner[1]. [1]Division of Immunopathology, Institute of Pathology, and [2]Department of Clinical Veterinary Medicine, University of Bern, Switzerland, [3]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA, [4]Institute of Animal Neurology and [5]Veterinary Physiology, University of Bern, Switzerland.

Uncontrolled activation of intestinal lymphocytes has been suggested as one of the underlying reasons for inflammatory bowel disease (IBD). However, little is known about the lymphocyte populations in the neonatal and adult canine gut.

The aim of this study is the characterization of intraepithelial lymphocytes (IEL) in the canine intestinal mucosa. Differences between IEL in neonatal versus adult beagle dogs from different sections of the intestine were analyzed. Tissue samples were obtained from 15 adult (mean age 8.2 ± 2.1 years) and 6 neonatal beagle dogs (mean age 1.1 ± 1 days) in order to reduce the heterogenicity due to differences in breeds. T cell populations and distributions were phenotypically characterized by immunohistochemistry and flow cytometry using T cell-specific markers, including CD45, CD3, CD4, CD8α, CD8β, TCRαβ and TCRγδ.

Results indicate that there are significant differences between adult and neonatal dogs in the distribution of different lymphocyte subsets. In the IEL population of the small bowel (SB) of adult dogs $CD3^+TCR\alpha\beta^+$, $CD8\alpha\beta^+TCR\alpha\beta^+$, and $CD4^+TCR\alpha\beta^+$T cells predominate, whereas $CD8\alpha\beta^+TCR\gamma\delta^+$ and double negative $TCR\gamma\delta^+$ Tcells were found in increased numbers in the SB IEL population of newborn dogs. In the IEL compartment of the large bowel (LB) the same significant differences could be found.

These data obtained from healthy dogs give first insight into the developmental population of the intestinal mucosa by immune cells. They will not only allow future comparisons with intestinal lymphocyte populations in dogs suffering from IBD, but also help to develop a better understanding of the pathogenesis of IBD.

ABSTRACT #329

EFFECTS OF FIROCOXIB AND TEPOXALIN ON HEALING IN A CANINE GASTRIC MUCOSAL INJURY MODEL. L Goodman, B Torres, L Reynolds, S Budsberg. University of Georgia College of Veterinary Medicine, Athens, Georgia.

The purpose of this study was to compare the effects of therapeutically dosed firocoxib and tepoxalin on gastric mucosal healing in dogs. We hypothesized that firocoxib would alter healing of induced gastric wounds.

Gastric body and pylorus lesions were induced via endoscopic biopsy in 6 adult dogs. The dogs were then treated with tepoxalin, firocoxib, or placebo for 7 days in a three way crossover study design. Wound healing was evaluated on days 2, 4, and 7 of treatment using endoscopic mucosal scoring of lesion appearance and size. Eicosanoid concentrations in plasma and at the mucosal lesion margins were determined on days 2, 4, and 7. Repeated measures analyses were performed. All hypotheses tested were 2-sided with $P < 0.05$. Multiple comparisons were adjusted for using a Tukey's test.

Significant treatment differences were noted in the pyloric lesion area measurements with firocoxib having larger lesions than placebo or tepoxalin. Pyloric mucosal prostaglandin E_2 (PGE_2) synthesis was significantly lower in dogs administered tepoxalin than in dogs administered either firocoxib or placebo. Compared to placebo, pyloric mucosal LTB_4 levels in the tepoxalin and firocoxib groups trended to increase on day 7.

Despite larger pyloric lesions in the firocoxib treatment group, mucosal PGE_2 production did not differ significantly from placebo. Conversely, the tepoxalin treatment group had significantly lower mucosal PGE_2 production yet pyloric lesions were not significantly larger than the placebo group. In this study, suppression of PGE_2 in tepoxalin treated dogs did not alter pyloric mucosal lesion healing compared to placebo.

ABSTRACT #330

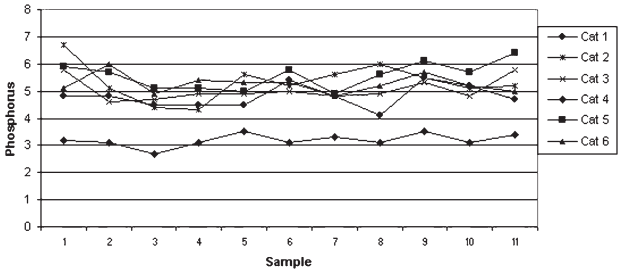
EFFECTS OF SUCRALFATE ON THE SERUM PHOSPHORUS CONCENTRATION AND URINARY FRACTIONAL EXCRETION OF PHOSPHORUS IN HEALTHY CATS. J Quimby, MR Lappin. Colorado State University, Fort Collins, CO.

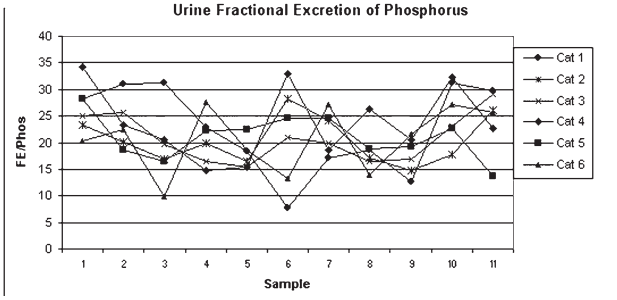
Sucralfate is currently used as a gastric protectant in cats, but may also have phosphate binding activity. In humans, this can be a deleterious side-effect of administration, occasionally leading to hypophosphatemia. The purpose of this study was to determine the effect of sucralfate administration on the serum and urine phosphorus concentrations of healthy cats.

Six healthy 1.5 year old cats of mixed sex were used. Each cat was administered 500 mg of sucralfate orally as a slurry in 1.5 milliliters of water, three times daily before food for 14 consecutive days. Blood and urine samples were collected 3 days during the week prior to sucralfate administration (samples 1–3), 6 days during the 2 weeks of sucralfate administration (samples 4–9) and 2 days during the week post-administration (samples 10 and 11). Concentrations of phosphorus, calcium, potassium, sodium and creatinine were measured on all serum and urine samples. Fractional excretions of phosphorus, calcium, potassium and sodium in urine were calculated.

No significant difference in serum phosphorus concentration or urinary excretion of phosphorus was seen between the treatment and non-treatment samples. Vomiting was noted as a side-effect 5 times out of 34 administrations.

We conclude that sucralfate does not cause hypophosphatemia in healthy cats at the administered dose.





ABSTRACT #331

LINKAGE ANALYSIS OF COBALAMIN DEFICIENCY IN CHINESE SHAR PEIS. N Grützner, MA Bishop, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Cobalamin (vitamin B_{12}) deficiency (CD) is a common disorder in the Chinese Shar Pei (Shar Pei) and is suspected to be hereditary. As the severity of clinical symptoms and the age of onset of CD vary widely, identification of a genetic marker co-segregating with CD would facilitate the development of a DNA-based test for early identification of affected dogs, facilitating further research into this condition in Shar Peis. The objective of this study was to identify genetic linkage of microsatellite markers to phenotypic CD in Shar Peis.

Whole blood and serum were collected from a total of 42 unrelated Shar Peis. Owners were asked to fill out a questionnaire regarding the current health status of each dog. Serum cobalamin concentration (reference range 252–908 ng/L) was measured for each dog and DNA was extracted from whole blood. A total of 327 microsatellite markers, 326 belonging to the canine minimal screening set 2 (MSS-2), and a single microsatellite marker not contained in the MSS-2, were amplified by polymerase chain reaction (PCR). PCR products were resolved by automated capillary electrophoresis, and sized relative to an internal standard followed by analysis of genotype data by a commercially available software package. Linkage analysis was conducted with a Fisher's exact test, and statistical significance was set at $p < 0.0001$ ($p < 1 \times 10^{-4}$).

Undetectable serum cobalamin concentrations ($<$ 150 ng/L) were observed in 14 of 42 dogs (33.3%). These dogs were considered to be cobalamin deficient. Serum cobalamin concentrations were within the reference range in the remaining 28 dogs. Allele 356 of the microsatellite marker DTR13.6 on chromosome 13 was found significantly more frequently in cobalamin-deficient Shar Peis (19 of 28 dogs or 67.9%) than in Shar Peis with normal serum cobalamin concentrations (10 of 56 dogs or 17.9%; p-value = 8×10^{-6}). Allele 315 of microsatellite REN13N11 on chromosome 13, which is not contained in the MSS-2, was found significantly more frequently in cobalamin-deficient Shar Peis (18 of 28 dogs or 64.3%) than in Shar Peis with normal serum cobalamin concentrations (10 of 56 dogs or 17.9%; p-value = 3×10^{-5}).

Both microsatellite markers on canine chromosome 13, DTR13.6 and REN13N11, showed significant linkage disequilibrium, revealing linkage of alleles 356 and 315, respectively, to serum CD in Shar Peis. This association will facilitate further evaluation of the genomic loci in close proximity to both microsatellite markers on chromosome 13 in Shar Peis, which in turn may lead to further characterization of this condition.

ABSTRACT #332

SERUM CANINE TRYPSIN-LIKE IMMUNOREACTIVITY, FOLATE, AND COBALAMIN CONCENTRATIONS IN GERMAN SHEPHERD AND WHITE SHEPHERD DOGS. N Grützner, JS Suchodolski, RM Heilmann, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

A high prevalence of exocrine pancreatic insufficiency (EPI) has been reported in German Shepherd dogs (GSD). This condition is diagnosed by a severely decreased serum canine trypsin-like immunoreactivity (cTLI) concentration. Serum cobalamin and folate concentrations are reported to be frequently altered in dogs with EPI. However, studies comparing serum cTLI, cobalamin, and folate concentrations between male and female GSD or between GSD and White Shepherd dogs (WSD), a breed that is considered to be a direct descendent of the GSD, are currently lacking. The aim of this study was to evaluate serum cTLI, cobalamin, and folate concentrations in a large group of GSD and WSD.

Serum samples, submitted for an unrelated research project, were collected from 109 GSD and 56 WSD. Owners were asked to fill out a questionnaire regarding the current health status for each dog. Serum cTLI (reference range (RR): 5.7–45.2 μg/L), cobalamin (RR: 251–908 ng/L), and folate (RR: 7.7–24.4 μg/L) concentrations were measured in each dog. A Mann-Whitney test was used to compare the median concentrations of these 3 parameters between GSD and WSD, and between sexes. A Fisher's exact test was used to compare the proportion of dogs with serum cTLI concentrations within the RR to the dogs with a cTLI below the RR ($<$5.7 μg/L) or to the dogs with a cTLI below the cut-off value for EPI ($<$2.5 μg/L). Statistical significance was set at $p<0.05$.

Serum cTLI was subnormal in 24 of 109 GSD (22.0%) and in 5 of 56 WSD (8.9%). Median serum cTLI was significantly lower in GSD (7.9 μg/L) than in WSD (10.0 μg/L; $p = 0.0004$). Median serum cTLI was also significantly lower in male GSD (6.8 μg/L) than in female GSD (8.1 μg/L; $p = 0.0262$). Median serum cTLI was not significantly different between sex groups ($p = 0.9867$) in WSD. The proportion of dogs with a serum cTLI diagnostic for EPI or a cTLI $<$ 5.7 μg/L was not significantly different between both breeds ($p = 0.2252$ and 0.0506, respectively). Serum folate was subnormal in 7 of 109 GSD (6.4%) and in 12 of 56 WSD (21.4%), and above normal in 12 of 109 GSD (11.0%) and in 3 of 56 WSD (5.4%). Median serum folate was significantly lower in GSD (12.5 μg/L) than WSD (11.0 μg/L; $p = 0.0073$). In both GSD and WSD, median serum folate was not significantly different between sex groups ($p = 0.7226$

and 0.6529, respectively). Serum cobalamin was subnormal in 14 of 109 GSD (12.8%) and in 10 of 56 WSD (17.9%), and above normal in 4 of 109 GSD (3.7%) and in 5 of 56 WSD (8.9%). Median serum cobalamin was not significantly different between GSD and WSD ($p = 0.0705$) or between sexes in GSD or WSD ($p = 0.1763$ and 0.2436, respectively).

In conclusion, in this study, GSD had significantly lower median serum cTLI and significantly higher median serum folate concentrations than WSD. However, EPI was not significantly more common in GSD than in WSD.

ABSTRACT #333

MOLECULAR ANALYSIS OF THE BACTERIAL MICROFLORA IN DUODENAL BIOPSIES FROM DOGS WITH INFLAMMATORY BOWEL DISEASE. JS Suchodolski[1], AE Jergens[2], CG Paddock[1], PG Xenoulis[1], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Department of Clinical Sciences, Iowa State University, Ames, IA.

An association between mucosa-adherent commensal bacteria and inflammatory bowel disease (IBD) has been proposed for humans. Inflammatory bowel disease is one of the most common causes of chronic diarrhea in dogs, but there are no reports characterizing the mucosa-adherent duodenal microflora in dogs using molecular methods. The aim of this study was to investigate differences in the mucosa-adherent duodenal microflora between dogs with IBD and healthy dogs.

Duodenal biopsy samples were collected from 7 dogs with a histopathological diagnosis of IBD during diagnostic gastroduodenoscopy. Duodenal biopsies were also collected from 7 healthy control dogs during endoscopy or immediately after euthanasia for unrelated studies. DNA was extracted and 16S ribosomal RNA genes (16S rDNA) were amplified using universal bacterial primers. 16S rDNA clone libraries were constructed and compared statistically between groups based on the UniFrac phylogenetic distance metric. The relative abundance of 16S rDNA clones at different phylogenetic ranks were compared using the Mann-Whitney test. Bacterial diversity indices were calculated and compared using *t*-tests.

A total of 1,035 16S rDNA clones were selected, and based on a 98% similarity criterion, 133 unique phylotypes were identified. These phylotypes belonged to 6 bacterial phyla: *Proteobacteria* (51.2%), *Firmicutes* (25.8%), *Actinobacteria* (9.0%), *Bacteroidetes* (8.3%), *Fusobacteria* (5.0%), and *Verrucomicrobia* (0.7%). There were no significant differences in bacterial diversity indices between groups. The UniFrac distance metric revealed significant differences in the relative abundance of several bacterial groups between dogs with IBD and healthy dogs ($p < 0.001$). Healthy dogs and dogs with IBD clustered according to their disease status. Dogs with IBD had a significantly higher abundance of clones belonging to *Alpha-*, *Beta-*, and *Gammaproteobacteria* ($p = 0.002$, $p = 0.02$, and $p = 0.0058$, respectively), and a significantly lower abundance of *Clostridia* ($p < 0.001$). Members of *Proteobacteria*, especially *Pseudomonadaceae* ($p = 0.003$), *Neisseriaceae* ($p = 0.009$), and *Brucellaceae* ($p = 0.033$), were significantly more likely to be present in dogs with IBD ($p = 0.006$). Members of the *Clostridiaceae* family were significantly more likely to be present in healthy dogs ($p = 0.003$).

In this study, significant differences of the mucosa-adherent duodenal microflora were observed between dogs with IBD and healthy dogs. These results warrant further investigations into the role of the intestinal microflora in the pathophysiology of canine IBD.

ABSTRACT #334

COMPARISON OF THE FECAL MICROFLORA BETWEEN HEALTHY CATS AND CATS WITH INFLAMMATORY BOWEL DISEASE USING MOLECULAR METHODS. LE Ritchie, JM Steiner, and JS Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

In human beings, inflammatory bowel disease (IBD) is associated with alterations of the intestinal bacterial flora. In fact, some studies suggest that an altered bacterial flora might be an underlying cause for IBD. In cats, IBD is associated with clinical signs such as vomiting, diarrhea, and weight loss, but little is known about the pathogenesis of this disease. Also, the fecal microflora of cats with IBD has previously not been extensively characterized. Thus, the aim of this study was to characterize and compare the fecal microflora between healthy cats and cats with IBD based on direct sequence analysis of 16S ribosomal DNA (16S rDNA).

Four healthy cats and four cats with clinical signs and a histopathological diagnosis of IBD were evaluated in this study. One fecal sample was collected from each cat and stored at $-80\,^{\circ}C$ until further analysis. Purification of the bacterial DNA was performed by phenol:chloroform:iso-amylalcohol extraction, and an amplicon of approximately 450 bp of the hypervariable region of the 16S rDNA was amplified at low PCR cycle numbers, using universal bacterial primers. For identification of bacterial 16S rDNA sequences, a clone library was constructed. Each sequence was tested for possible chimeric structures and any identified chimeras were excluded from further analysis. Obtained sequences were compared to sequences listed in the Ribosomal Database Project (RDP) and subjected to phylogenetic analysis. Differences in the relative abundance of 16S rDNA clones obtained in healthy and IBD cats were analyzed using a Fisher's exact test and a Mann-Whitney U test. Statistical significance was set at $p < 0.05$.

A total of 521 clones were analyzed. Based on a 98% similarity criterion, 47 non-redundant bacterial 16S rDNA sequences were identified. Four bacterial phyla were identified: 94.9% of identified phylotypes belonged to *Firmicutes,* 4.3% were *Actinobacteria*, and less than 1% each were *Proteobacteria* and *Bacteriodetes*. *Clostridiales* was the most abundant bacterial order in both healthy and IBD cats, representing 69.4% and 84.5% of the identified clones within each group, respectively. The proportion of clones belonging to the order *Bacillales* was significantly higher in healthy cats compared to cats with IBD ($p < 0.001$). In contrast, IBD cats had a significantly higher proportion of clones classified as *Clostridium perfringens*-like organisms (27.7% vs. 5.1%; $p < 0.001$).

These results indicate that the fecal bacterial flora of cats is comprised mainly of bacteria belonging to the phylum *Firmicutes*. Based on the results of this study, the fecal microflora of cats with IBD may be altered when compared to healthy cats. Thus, studies characterizing the fecal microflora in a larger population of cats with IBD are warranted.

ABSTRACT #335

PREVALENCE OF *TRITRICHOMONAS FOETUS* AND OTHER ENTERIC PARASITES IN AUSTRALIAN CATTERY AND SHELTER CATS. S Bissett[1], M Coccaro[1], R Malik[2], J Norris[2], C O'Brien[3], R Gowan[4], C Mansfield[5], J Nicholls[6], A Griffin[7], J Gookin[1]. [1]North Carolina State University College of Veterinary Medicine, Raleigh, NC. [2]The University of Sydney Faculty of Veterinary Science, Sydney, NSW. [3]The University of Melbourne Veterinary Clinic and Hospital, Melbourne, VIC. [4]The Cat Clinic, Prahran, VIC. [5]Murdoch University Department of Veterinary Clinical Sciences, Perth, WA. [6]Prospect Road Veterinary Hospital, Adelaide, SA. [7]Queensland Veterinary Specialists, Brisbane, QLD.

Tritrichomonas foetus (TF) is an important cause of feline diarrhea and infects up to 30% of American purebred cats. Although TF exists worldwide, feline TF has not yet been reported in Australia and many veterinarians do not specifically test cats for TF. The purpose of this study was to determine the prevalence of TF and other enteric parasites in a sample of Australian cats to help guide fecal testing.

Fresh voided fecal specimens were collected from 84 cats from 30 catteries (median 2, range 1–10 specimens per cattery) and 52 shelter cats within 5 Australian states. Where possible, fecal consistency was recorded and cat owners completed a questionnaire. Fecal examinations performed in most cats included concentration methods (65 cattery and 41 shelter cats), *Giardia* antigen detection via ELISA (65 cattery and 40 shelter cats), culture in In Pouch^{TM} TF medium (49 cattery and 52 shelter cats) and PCR amplification of TF rRNA genes using specific primers (84 cattery and 52 shelter cats).

Prevalence of TF, *Giardia* sp, Coccidia and nematodes for cattery cats (and catteries) were 1.2(3.3)%, 9.2(18.2)%, 10.8(25.9)% and 1.5(3.7)% respectively. Prevalence of TF, *Giardia* sp., Coccidia and nematodes for shelter cats were 0, 10%, 9.8% and 3.8% respective-

ly. *Giardia* sp. were mostly identified by ELISA testing, while Coccidia and nematodes were detected by faecal concentration methods. Only 1 kitten from an Ocicat cattery in Victoria was identified with TF. This kitten was in poor body condition, had diarrhea on physical examination and was co-infected with *Giardia* sp. The diagnosis of TF was made via culture and PCR and confirmed by molecular sequencing of PCR amplicons. Subsequently, fecal specimens obtained from 9 other cats within this cattery also tested positive for TF (9 via culture, 8 via PCR). Most breeders denied a history of diarrhea in their catteries, although unformed, liquid feces were documented at the time of stool collection in 7.5% of purebred cats from 20% of the catteries. Of the shelter cats, only 5.8% of fecal samples were noted to be liquid or unformed at the time of collection.

These results are the first to identify TF infection in an Australian cattery. Although the prevalence of feline TF appears to be low in Australia at this time, veterinarians should consider TF infection in cats with diarrhea where other causes are not identified. These results also indicate that Coccidia and *Giardia* sp. commonly infect Australian cats.

ABSTRACT #336

SUBCLINICAL PANCREATITIS IS MORE COMMON IN OVERWEIGHT AND OBESE DOGS IF PEAK POSTPRANDIAL TRIGLYCERIDEMIA IS > 445 MG/DL. Kurt R Verkest[1], Linda M Fleeman[1], Jacquie S Rand[1], Jan S Suchodolski[2], Jörg M Steiner[2]. [1]Centre for Companion Animal Health, The University of Queensland, Brisbane, Australia. [2]Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

In human beings, there is strong evidence that hypertriglyceridemia is associated with an increased risk for pancreatitis. Miniature Schnauzers with fasting hypertriglyceridemia have a higher risk of pancreatitis. The association of hypertriglyceridemia and pancreatitis has not been well characterized in other dogs. The aim of this study was to determine whether dogs with increased postprandial serum triglyceride concentrations are at increased risk of having serum pancreatic lipase immunoreactivity (cPLI) or trypsin-like immunoreactivity (cTLI) concentrations in the diagnostic range for exocrine pancreatic disease.

We recruited 36 client-owned, healthy (no vomiting, anorexia, abdominal pain) overweight or obese dogs (body condition score 6-9/9). Serum triglycerides were measured after a 24 hour fast and hourly for 12 hours after a meal (40% ME dietary fat) comprising 50% of daily maintenance energy requirement based on estimated lean body weight. Fasting serum cPLI (Spec cPL^{TM}) concentrations higher than 400 μg/L and cTLI lower than 2.5 μg/L were used as cut-off values for pancreatitis and exocrine pancreatic insufficiency (EPI), respectively. Low, medium, and high cut-offs for hypertriglyceridemia were set *a priori* for fasting (90, 175, and 355 mg/dL), mean postprandial (90, 265, and 530 mg/dL), and peak postprandial (135, 445, and 885 mg/dL) triglycerides. Odds of elevated cPLI were compared using Fisher's Exact tests. Results are reported as likelihood ratios (LR) - the ratios of post- to pre-test odds.

Three of the 36 dogs had a fasting cPLI above the cut-off value for pancreatitis (pre-test odds 0.09). Eight dogs had peak triglycerides higher than 445 mg/dL, of which three had a cPLI above the diagnostic cut-off for pancreatitis (LR 6.6, $p = 0.008$). Both dogs with peak postprandial serum triglycerides above 885 mg/dL had serum cPLI higher than the diagnostic cut-off for pancreatitis (LR infinite, $p = 0.005$). Dogs that exceeded 445 mg/dL were identified with sensitivity of 75%, 88%, and 88% by postprandial samples collected at 2, 3, and 4 hours, respectively. Sensitivity was 100% when two samples were evaluated at either 2 and 4, or 3 and 4 hours. No significant association was found between any of the fasting or mean postprandial triglyceride concentrations and a cPLI above the cut-off value for pancreatitis. None of the dogs had a serum cTLI concentration below the diagnostic cut-off for EPI.

The odds of having serum cPLI concentration above the diagnostic cut-off for pancreatitis are about 7 times higher in overweight and obese dogs when peak postprandial triglyceridemia is > 445 mg/dL after a standard meal with 40% ME fat fed at 50% MER. This information could allow development of a useful screening test to identify dogs that might benefit from dietary fat restriction because of increased risk of subclinical pancreatitis.

ABSTRACT #337

ASSOCIATION OF SERUM TOTAL T4 AND fPLI CONCENTRATIONS IN CATS. JM Steiner[1], M Rick[2], KM Aicher[1], JS Suchodolski[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX. [2]Veterinary Endocrinology, Michigan State University, Lansing, MI.

Hyperthyroidism is common in cats and is often associated with clinical signs of gastrointestinal disease, such as vomiting, diarrhea, weight loss, and in rare cases anorexia. Chronic pancreatitis is also common in cats and is often associated with vague clinical signs that may include some of the same clinical sings observed in cats with hyperthyroidism. The objective of this study was to explore a possible association of serum total T4 and fPLI concentrations to determine whether hyperthyroid cats may be commonly affected by concurrent pancreatitis.

Left-over serum samples from 168 cats were used for this study. Serum total T4 (TT4; reference range: 10–55 nmol/L) and feline pancreatic lipase immunoreactivity (fPLI; reference range: 2.9–6.0 μg/L) concentrations were measured in all serum samples. All datasets were evaluated for normal distribution using a D'Agostino and Pearson omnibus normality test. Median serum fPLI concentrations were compared between cats with a serum TT4 within the reference range and those with an increased serum TT4 concentration using a Mann-Whitney test. The proportion of cats with a serum fPLI concentration outside the reference range (> 6.0 μg/L) or in the diagnostic range for pancreatitis (> 12 μg/L) were compared between cats with a serum TT4 within the reference range and those with an increased serum TT4 concentration using a Fisher's exact test. Significance was set at $p < 0.05$ for all statistical analyses.

Seventy-two cats had a serum TT4 concentration within the reference range and 88 cats had an increased serum TT4 concentration (range: 56 to > 156 nmol/L). Serum fPLI concentrations in both groups of cats failed normality testing. Median serum fPLI concentration in cats with an increased serum TT4 concentration (10.2 μg/L) was not significantly different from that in cats with a serum TT4 concentration within the reference range (9.5 μg/L; p-value = 0.3874). The proportion of cats with a serum fPLI concentration above the upper limit of the reference range or above the diagnostic cut-off value for pancreatitis was not significantly different between cats with an increased serum TT4 concentration and those with a serum TT4 concentration within the reference range (p-values: 0.8119 and 0.7844, respectively).

In this study, we were unable to show a significant association of serum TT4 and fPLI concentrations, suggesting that feline pancreatitis is no more common in cats with an increased serum TT4 concentration than in those with a serum TT4 concentration within the reference range. One limitation of this study was the use of left-over serum samples, which precluded the evaluation of clinical findings and may have led to preferential inclusion of cats with clinical signs of gastrointestinal disease. Thus, further studies evaluating serum fPLI concentrations in cats with a clinical diagnosis of hyperthyroidism would be warranted.

ABSTRACT #338

DEVELOPMENT OF A PCR TEST FOR THE DETECTION OF *HETEROBILHARZIA AMERICANA* DNA IN DOG FECES. MA Bishop, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Canine schistosomiasis is an infrequently diagnosed condition caused by *Heterobilharzia americana*. Infection with this trematode causes a variety of clinical signs, including weight loss, diarrhea, hematochezia, with or without systemic signs. This disease may be under-diagnosed due to the lack of clinical suspicion, vague clinical signs, and lack of sensitive diagnostic tests. For example, eggs of this parasite rarely float during routine fecal flotation and require sodium chloride sedimentation to be visualized. Therefore, the aim of this study was to develop a PCR based method for the detection of *Heterobilharzia americana* DNA in dog feces.

Specific primers were designed and optimized to amplify a section of the variable region of the 18S ribosomal DNA gene of *Heterobilharzia americana*. PCR products were purified and analyzed by automated cycle sequencing to confirm identification of the par-

asite. *Heterobilharzia americana* eggs were extracted from the feces of a dog that was positive for the parasite on sodium chloride sedimentation and were used as a positive control. The eggs were diluted to counts of 100, 50, 25, 10, 5, 2, and 1 egg per gram of fecal extract. Feces, from a healthy dog previously negative for the parasite, were spiked with these preparations to determine the sensitivity of the PCR assay. DNA was extracted from the fecal samples and the PCR assay was repeated 10 times for each sample with the 5 lowest egg counts to ensure reproducibility. Finally, DNA was extracted from fecal samples from two dogs that were positive for *Heterobilharzia americana* based on sodium chloride sedimentation. Extracted DNA was analyzed with the PCR assay. The PCR products were purified and sequenced directly to confirm the identity of parasite DNA.

Spiking experiments of *Heterobilharzia americana* eggs revealed that the average sensitivity of the PCR assay was 1.5 eggs per gram of feces. The reproducibility experiments demonstrated that the 5 lowest egg counts could be consistently assayed 100% of the time. The two clinical cases confirmed by sodium chloride sedimentation were also positive using the PCR assay and the identification of *Heterobilharzia americana* DNA was demonstrated by direct sequencing and comparison to the published sequence.

In conclusion, the PCR assay developed was sensitive and reproducible for the detection of *Heterobilharzia americana* DNA in dog feces. Further work is in progress to compare the sensitivity and reproducibility of this PCR assay with the current gold standard for the diagnosis of *Heterobilharzia americana*, sodium chloride sedimentation, and to determine the prevalence of *Heterobilharzia americana* infection in dogs in endemic areas.

ABSTRACT #339
EVALUATION OF THE APOLIPOPROTEIN C-II GENE IN MINIATURE SCHAUZERS WITH IDIOPATHIC HYPERTRIGLYCERIDEMIA. PG Xenoulis, MA Bishop, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Miniature Schnauzers have a high prevalence of hypertriglyceridemia, reported to be 32.8% in 192 healthy Miniature Schnauzers in one study. While the actual cause of this condition has not yet been identified, this high prevalence of idiopathic hypertriglyceridemia in Miniature Schnauzers compared to other breeds suggests a possible hereditary etiology. Apolipoprotein C-II (apo C-II), a co-factor of lipoprotein lipase, plays a central role in triglyceride metabolism, and mutations of the gene encoding apo C-II have been associated with familial hypertriglyceridemia in humans. The aim of this study was to identify and compare the nucleotide sequence of the apo C-II gene of hypertriglyceridemic and non-hypertriglyceridemic Miniature Schnauzers, as well as non-hypertriglyceridemic dogs of other breeds, in order to identify polymorphisms that co-segregate with hypertriglyceridemia.

Blood samples were collected from 9 healthy Miniature Schnauzers with idiopathic hypertriglyceridemia, 6 healthy Miniature Schnauzers with a normal serum triglyceride concentration, and 2 dogs of other breeds with a normal serum triglyceride concentration. DNA was extracted from whole blood and the exonic and flanking intron sequences of the apo C-II gene were amplified. PCR amplicons were ligated into vectors and *Escherichia coli* organisms were transformed with ligation products. Recombinant organisms were grown and 4–6 colonies per sample were picked, transferred to broth, and grown for 24 hours. Plasmid DNA was extracted and sequenced and sequencing products were analyzed with an automated sequence analyzer. Obtained sequences were compared based on multiple sequence alignment and analyzed for co-segregation of polymorphisms with hypertriglyceridemia.

A total of 12 polymorphisms were initially identified in the apo C-II gene of Miniature Schnauzers. Each polymorphism was identified only in a single sequence from each dog, and none of the polymorphisms co-segregated with hypertriglyceridemia. PCRs were repeated for all dogs and exons where polymorphisms had been identified, and PCR amplicons were directly sequenced. None of the 12 initially identified polymorphisms were present in the sequences obtained after direct sequencing, suggesting that these polymorphisms were artifacts and did not represent single nucleotide polymorphisms.

In conclusion, based on the results of the present study, mutations of the apo C-II gene do not appear to be the cause of idiopathic hypertriglyceridemia in this group of Miniature Schnauzers.

ABSTRACT #340
CLINICAL SIGNIFICANCE OF SERUM HYALURONIC ACID IN CANINE HEPATIC DISEASES. M Seki, K Asano, M Sakai, N Kanno, K Teshima, K Edamura, S Tanaka. Nihon University, Kanagawa, Japan.

Hyaluronic acid (HA) is an acidic mucopolysaccharide and included in most body fluids and tissues. In human, a relatively large amount of HA is detected in synovial fluids, umbilical cords, and vitreous body of eye. In these tissues, it influences several different functions including tissue viscosity, tissue osmosis, shock, absorption, wound healing and space filling. The circulating HA is secreted from various tissues including skin, liver, and synovial membrane, and rapidly removed from blood by sinusoidal endothelial cells in liver. In human medicine, serum concentration of HA is clinically applicable as a diagnostic biomarker for some pathologic conditions including cancer, rheumatoid arthritis, and hepatic fibrosis and cirrhosis. On the other hand, clinicopathologic significance of serum HA is still unclear in small animal medicine. The purpose of this study was to demonstrate the serum level of HA in normal dogs and dogs with liver diseases.

In this study, 15 healthy Beagles (control group) and 32 dogs with liver disease [19 congenital portosystemic shunt (CPSS), 6 hepatocellular carcinoma (HCC), and 5 gallbladder mucocele (GM)] were used. Blood samples were collected from the jugular vein, and centrifuged 3,000 rpm for 10 min. The serum was separated and stored at −20 °C until the assay. Serum concentration of HA was measured by enzyme-linked immunosorbent assay. In addition, the measurement of serum HA was performed 2 and 4 weeks after the operation in the dogs with CPSS. The serum level of HA was expressed as median (range). The serum level of HA in dogs with liver diseases were statistically analyzed.

The serum levels of HA in control group, CPSS, HCC, and GM were 59.17 ng/ml (31.64–117.36 ng/ml), 308.50 ng/ml (50.55–903.66 ng/ml), 65.39 ng/ml (30.24–106.21 ng/ml), and 49.00 ng/ml (24.48–90.51 ng/ml), respectively. In dogs with CPSS, serum level of HA significantly increased compared with control group. On the other hand, serum levels of HA in dogs with HCC and GM were not significantly elevated compared with control group. In addition, postoperative level of serum HA was significantly reduced compared with the preoperative level in dogs with CPSS.

In canine CPSS, the reduction of intrahepatic portal blood flow by the shunting vessel might induce low clearance rate of HA in hepatic sinusoidal endothelial cells with increase in serum level of HA. In conclusion, serum HA is suggested to be of clinical value for evaluating the attenuation of shunting vessel and improvement of hepatic sinusoidal endothelial function in dogs with CPSS.

ABSTRACT #341
CHARACTERIZATION OF VARIOUS CANINE FOCAL LIVER LESIONS WITH SONAZOID, A NOVEL ULTRASOUND CONTRAST AGENT. H Kanemoto, K Onahno, K Nakashima, M Takahashi, Y Fujino, H Tsujimoto. Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.

Contrast-enhanced ultrasound (CEU) using microbubble-based contrast agents is a method that has begun to garner attention as a practical and useful examination in human medicine. In Japan, a new contrast agent Sonazoid was introduced in 2007. Compared to other contrast agents, Sonazoid has a greater advantage in liver CEU in that it has not only a vascular phase but also a true parenchymal phase (Kupffer phase). Kupffer phase, which is characterized by phagocytosis of the agent by the reticuloendothelial cells in the liver, enables continuous observation of enhanced images and a more accurate detection and diagnosis of liver diseases. The aim of this study was to characterize the time course enhancement by Sonazoid in the liver of healthy dogs and to evaluate the enhancement pattern of various canine focal liver lesions (FLLs) in clinical cases.

Five healthy Beagles and 15 dogs with FLL detected by conventional B-mode ultrasound were recruited for this study. FLLs were diagnosed by liver biopsy and were classified as follows: benign lesions, 4; hepatocellular carcinomas (HCCs), 8; cholangiocellular carcinoma (CC), 1; hematopoietic tumors (HTs), 2. Sonazoid 0.015 ml/kg was intravenously administered to dogs, and ultrasonographic examination was performed from immediately after the injection till 15 min after the injection. The time course enhancement of hepatic parenchyma and the portal vein was examined in healthy dogs. In the clinical study, the enhancement pattern of FLL by Sonazoid injection was examined during the arterial, portal, and parenchymal (Kupffer) phases.

In the Sonazoid CEU of healthy Beagles, sustained enhancement was observed in the liver parenchyma for more than 15 min, whereas in the portal vein, the enhancement dramatically decreased after the peak enhancement at 30 s. Based on the time-intensity curve, the arterial, portal, and parenchymal phases of Sonazoid CEU in dogs were defined as 0 to 20 sec, 20 sec to 1 min, 7 to 15 min, respectively. In HCCs, 5 lesions were hypervascular and 2 were hypovascular in the arterial phase, whereas in the parenchymal phase, irregular enhancement was observed in all cases. In CCs and HTs, we observed early enhancement washout during the vascular phase and clear defect in the parenchymal phase. In contrast to these malignant lesions, benign lesions showed sustained uniform enhancement in the parenchymal phase in 3 of 4 cases. Moreover, additional FLLs that had not been detected by conventional B-mode ultrasound were observed in some cases in CCs or HTs.

In conclusion, incomplete enhancement in the parenchymal phase was characteristic for malignant canine FLLs in Sonazoid CEU, whereas most of the benign lesions showed sustained uniform enhancement. This agent was thought to be useful in characterization and detection of canine FLLs, particularly during the parenchymal (Kupffer) phase. Further investigation of a large number of cases is required to clarify the usefulness of CEU with Sonazoid.

ABSTRACT #342

PROSPECTIVE EVALUATION FOR BACTERIAL INFECTION IN HEPATIC TISSUE AND BILE OF CATS WITH DIFFUSE HEPATOBILIARY DISEASE. Morgan M, Rankin S, Berent A, Weinstein N, Van Winkle T, Rondeau M. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

The goals of this study are to determine the prevalence of bacterial infection in cats with Feline Inflammatory Hepatobiliary Disease (FIHD), to evaluate the diagnostic utility of hepatic tissue versus bile for the identification of bacterial pathogens in cats with FIHD, and to assess the utility of real-time PCR (RT-PCR) for detection of bacterial organisms in frozen hepatic tissue and bile.

Cases in which hepatic biopsy was performed as part of a complete diagnostic evaluation of diffuse hepatobiliary disease were included. Cases were excluded for the following reasons: antibiotic therapy prior to sampling, untreated hyperthyroidism, or the presence of a hepatic mass. Liver tissue was acquired using the method desired by the attending clinician, and bile was collected via cholecystocentesis. Control samples were obtained at the time of euthanasia from normal cats that were part of a breeding colony. Aerobic and anaerobic bacterial culture was performed on all samples. RT-PCR was performed on batched, frozen samples within 6 months of their acquisition. Cases were categorized as having acute neutrophilic cholangitis (ANC), chronic neutrophilic cholangitis (CNC), acute lymphocytic cholangitis (ALC), chronic lymphocytic cholangitis (CLC), hepatic lipidosis (HL), or other hepatic disease by one pathologist (TVW). DNA was extracted from samples using the QiaAmp DNA Mini Kit, (Qiagen) as described by the manufacturer. Amplification and DNA detection were carried out in a Smart Cycler (Cepheid) using 16S rRNA primers.

Thirty cases, including 7 cats with CNC, 4 cats with CLC, 1 cat with ALC, 4 cats with HL, 6 cats with other types of hepatic disease, and 8 control cats were included. Bile culture was positive in 5 cases, including 4 cats with CNC, and 1 cat with HL and biliary carcinoma. The following bacterial organisms were isolated from bile: *E.coli* (3), α-hemolytic *Streptococcus* (1), *P. aeruginosa* (1) and an unidentified anaerobic gram-positive rod. Hepatic tissue culture was positive in 2 cases. In both cases *E.coli* was isolated from both hepatic tissue and bile. RT-PCR of the bile was positive in 4 cases, all of which had positive bile cultures. RT-PCR of hepatic tissue was negative in all cases. In 3 of the 4 cases in which RT-PCR was positive, DNA sequencing identified the same organism that had been found using traditional bacterial culture on the bile. In one case, DNA sequencing identified *B. fragilis*, which had not been identified using bacterial culture. Culture and RT-PCR revealed no evidence of bacterial infection in any control samples.

We conclude that biliary bacterial infection is more common in cats with CNC than in cats with other forms of FIHD and other types of hepatobiliary disease; bile is more useful than hepatic tissue for the purpose of bacterial identification; and RT-PCR appears to be a viable method to rapidly and accurately detect bacterial DNA in bile, but not in hepatic tissue.

ABSTRACT #343

A SURVEY OF FELINE INFLAMMATORY HEPATOBILIARY DISEASE USING THE WSAVA CLASSIFICATION SYSTEM. Morgan M[1], Rondeau M[1], Rankin S[1], Shofer F[1], Berent A[1], Van Winkle T[1], Washabau R[2]. [1]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA. [2]University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

Feline Inflammatory Hepatobiliary Disease (FIHD) has been reported to be the second most common hepatic disease of cats in the United States. The primary goals of this study were to categorize a clinical population of cats with FIHD based on the recently published WSAVA classification system, to make clinical comparisons between the separate types of FIHD, and to perform real time polymerase chain reaction (RT-PCR) on paraffin embedded hepatic tissue in attempts to isolate bacterial DNA.

Seventy-four cases were included in the study. Cases were divided into the following groups: acute neutrophilic cholangitis (ANC), chronic neutrophilic cholangitis (CNC), acute lymphocytic cholangitis (ALC), and chronic lymphocytic cholangitis (CLC) by one pathologist (TVW). Medical records were reviewed for information pertaining to signalment, presenting clinical signs, physical examination findings, clinicopathologic data, imaging findings, cytologic findings, culture results, histopathologic results, and outcome. Paraffin embedded liver tissue was obtained from cases in which it was available ($n = 40$) and from control cases with no evidence of inflammatory hepatobiliary disease ($n = 50$). DNA was extracted using the QiaAmp DNA Mini Kit, (Qiagen) as described by the manufacturer. Amplification and DNA detection were carried out in a Smart Cycler (Cepheid) using 16S rRNA primers. For categorical variables, the chi-square test was used to compare the four groups. For continuous variables, the Kruskal-Wallis test was used. $P < 0.05$ was considered significant.

Thirteen cats had ANC, 31 had CNC, 14 had ALC, and 16 had CLC. The median age for all cases included in the study was 11 years, with no significant difference between groups. 63.5% of cats in the study were male. No breed predilection was found. There were no significant differences between groups in the frequency of any clinical signs or physical examination findings. Cats with CNC were significantly more likely than cats in any other group to have increased ALT, GGT, total bilirubin, total protein, and globulin measurements. A significantly higher percentage of cats with CNC had bile duct obstructions compared to cats with other forms of cholangitis. RT-PCR was positive for the presence of bacterial DNA in 6 of the 40 cases in which paraffin embedded liver tissue was available, and in 5 of 50 control samples . Sequencing of the positive samples showed that they were all most consistent with contaminants.

Our conclusions are that (1) CNC is the most common form of inflammatory hepatobiliary disease in cats, and (2) cats with CNC are more likely to have increased ALT, GGT, total bilirubin, total solids, and globulin levels, and to have bile duct obstruction than are cats with other forms of inflammatory hepatobiliary disease. Real time PCR technology used in this study did not readily document bacterial infection in paraffin embedded liver tissue samples.

ABSTRACT #344

A PRIMARY CANINE HEPATOCYTE CULTURE MODEL FOR ANALYSIS OF CELLULAR TOXICITY AND INFLAM-

MATION. AY Au[1,2,3], JM Hasenwinkel[1], CG Frondoza[2,3]. [1]Syracuse University, Syracuse, NY. [2]Nutramax Laboratories, Inc., Edgewood, MD. [3]Johns Hopkins University, Baltimore, MD.

The liver plays a critical role in metabolism and detoxification of noxious agents. Liver tissue is capable of limited regeneration *in vivo* following injury. Hepatocytes, the major cell type in the liver, are considered an appropriate cellular model as they reflect the metabolic profiles *in vivo*. However, hepatocytes are difficult to propagate *in vitro*. They do not readily attach nor proliferate outside the liver and are phenotypically unstable. To be useful for metabolic studies *in vitro*, there is a need to extend hepatocyte survival and stabilize their phenotype. We have established a culture system that facilitates cell attachment, proliferation, and maintenance of the liver phenotype using type I collagen as substrate. Type I collagen, a major component comprising liver extracellular matrix, has been reported to support hepatocyte survival. Here we evaluated whether primary canine hepatocytes seeded on collagen films will continue to proliferate, maintain their phenotype, and respond to interleukin-1 beta (IL-1β). We also determined whether hepatocyte response to IL-1β can be modulated by the hepatoprotective agent silybin phosphatidylcholine complex (SPC). SPC has been shown to promote liver health and manage liver disorders (Marin®).

Primary canine hepatocytes were seeded onto 0.1% rat-tail type I collagen films and propagated in media fortified with growth factors. Cells were pretreated with (a) 298 ng/ml of SPC, (b) 298 ng/ml of its constituent silybin (SB), or (c) control media alone for 24 hrs. Cultures were next activated with IL-1β (10 ng/ml) for up to 72 hrs. Cell proliferation was measured by BrdU labeling. Phenotype was evaluated by albumin and cytokeratin 8 (CK8) immunostaining. Production of prostaglandin E-2 (PGE-2), as well as the chemokines (i) macrophage chemotactic protein 1 (MCP-1) and (ii) interleukin-8 (IL-8) were measured by ELISA. Statistical significance was set at $p<0.05$ using one-way ANOVA, followed by Tukey post-hoc analysis.

Hepatocytes aggregated into colonies 4 hrs after plating and formed finger-like extensions that disappeared by 48 hrs. Cells proliferated for up to 21 days and continued synthesis of albumin and CK8. Exposure to IL-1β significantly decreased hepatocyte viability to 40%. IL-1β treatment also induced significant increases in pro-inflammatory PGE-2, MCP-1, and IL-8 production. Pre-treatment with SPC and its SB constituent significantly inhibited the cytotoxic effect of IL-1β and reduced production of PGE-2, MCP-1, and IL-8. The present study demonstrates for the first time that our culture model facilitates hepatocyte proliferation and stable phenotype expression. The cell model helps maintain the ability of the hepatocytes to respond to noxious stimuli. We also show that hepatocyte response to IL-1β can be down regulated by the anti-inflammatory antioxidant compound SPC and its SB constituent. Our hepatocyte culture model serves as a useful tool to identify hepatoprotective agents and their potential mechanism of action.

ABSTRACT #345

ROLE OF PHOSPHOINOSITIDE-3 KINASE AND EPIDERMAL GROWTH FACTOR SIGNALING IN THE CYTOPROTECTIVE EFFECT OF URSODEOXYCHOLATE.

F Buckley, CRL Webster. Tufts Cummings School of Veterinary Medicine, Grafton, MA.

Cholestatic liver disorders lead to the retention of endogenous cytotoxic compounds, such as bile acids. Certain bile acids, glycochenodeoxycholate (GCDC) induce hepatocyte apoptosis, while others, tauroursodeoxycholic acid (TUDC), are cytoprotective.

The aim of this study was to determine the role of phosphoinositide-3 kinase (PI3K) and transactivation of the epidermal growth factor receptor (EGFR) in TUDC's cytoprotective effect in hepatocytes.

Primary rat hepatocyte cultures (4 hrs or 24 hrs post-isolation) were exposed to GCDC (50 or 100 uM) alone or with TUDC (50 or 100 uM) followed by GCDC treatment. Some cultures were pretreated with 20 uM LY294002, a PI3K inhibitor, or 5 uM AG1478, an inhibitor of EGFR phosphorylation, prior to bile acid treatment. Apoptosis was determined by morphological evaluation of Hoechst stained cells. EGFR activation was measured by immunoblotting with EGFR-P^{TY1173} antibodies or by direct immunoprecipitation of tyrosine phosphorylated proteins with a phosphotyrosine antibody, A4G10, followed by immunoblotting with a total EGFR antibody.

TUDC protected 4 and 24 hr cultures from GCDC induced apoptosis (80% and 60% of control values, respectively). PI3K inhibition alone induced a 3.5 and 7.5 fold increase in apoptosis in 4 hr and 24 hr hepatocytes, respectively. However, only in 24 hr hepatocyte cultures did PI3K inhibition reverse the protective effect of TUDC in GCDC mediated apoptosis. TUDC treatment increased EGFR phosphorylation in 4 hr, but not in 24 hour cultures . Inhibition of the EGFR phosphorylation had no effect on the cytoprotective effect of TUDC.

TUDC is cytoprotective in GCDC induced apoptosis in 4 hr and 24 hr hepatocyte cultures. The anti-apoptotic effect of TUDC is EGFR independent but PI3K dependent in 24 hr cultures and EGFR and PI3K independent in 4 hr cultures. Increasing time in culture promotes a greater dependent of rat hepatocytes on PI3K activation for survival.

ABSTRACT #346

MEASURING SPLENIC PULP PRESSURE IN THE EVALUATION OF PORTAL HYPERTENSION IN DOGS.

M. Sakai, Y. Sakamoto, M. Watanabe, M. Uechi. Nihon University, Kanagawa, Japan.

Portal hypertension is a complication of diseases that obstruct portal blood flow, such as primary hepatic diseases and primary vascular disorders. However, the direct measurement of portal pressure is invasive and impractical clinically. This study evaluated the adequacy of splenic pulp pressure (SPP) measurement under laparoscopy in dogs.

Six healthy dogs and 46 dogs with congenital portosystemic shunts (CPSS; n = 20) and acquired portosystemic collaterals (APSC; n = 26) were studied. An over-the-needle intravenous catheter was inserted into the spleen percutaneously under laparoscopy, and the SPP was measured through a catheter. After measuring the SPP, APSC and CPSS were identified using splenoportography, and liver samples were obtained in the dogs with APSC. The definitive diagnoses included chronic hepatitis/cirrhosis (CH; n = 16) and primary hypoplasia of the portal vein (PHPV; n = 10). In addition, mesenteric vein pressure (MVP) in healthy dogs was measured at laparotomy. There was no significant difference between the SPP (5.2 ± 0.8 mmHg) and MVP (6.0 ± 0.6 mmHg) in the healthy dogs. The SPP in dogs with APSC was 9.9 ± 3.3 mmHg (CH; 10.1 ± 3.6 mmHg, PHPV; 8.8 ± 2.4 mmHg), and was significantly elevated, as compared with healthy dogs ($p < 0.001$) and dogs with CPSS (3.5 ± 1.9 mmHg; $p < 0.001$).

The results of this study indicate that SPP measurement is a simple, minimally invasive technique for assessing portal hypertension in dogs.

ABSTRACT #347

T REGULATORY CELL SUPPRESSION OF $CD8^+$ LYMPHOCYTES DURING FIV INFECTION IS LIKELY MEDIATED BY MEMBRANE TGF-β / TGF-β RECEPTOR II INTERACTIONS.

Jonathan E. Fogle, Angela M. Mexas, Mary B. Tompkins, Wayne A. Tompkins. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Data suggest that auto-reactive $CD4^+$ and $CD8^+$ cells, as well as excessive immune responses to pathogens, are controlled by $CD4^+CD25^+$ T regulatory (Treg) cells, which regulate the duration and magnitude of immune responses. Recent data suggest that Treg cells mediate their suppressive function through TGF-β expressed on their surface. We previously reported that Treg cells are constitutively activated and suppress $CD4^+CD25^-$ T cell responses in feline immunodeficiency virus infection (FIV). Subsequently, we demonstrated that these immunosuppressive Treg cells express membrane TGF-β (mTGF-β). The following experiments explore the effect of $CD4^+CD25^+$ Treg cells on $CD8^+$ T cell responses in FIV infected cats and the possible role of mTGF-β in mediating $CD8^+$ immune suppression. SPF cats were infected with the $NCSU_1$ isolate of FIV, and peripheral lymph nodes (LN) and blood were collected at intervals during the acute and asymptomatic phases of the infection. Feline specific ELISpot assays demonstrated that Treg cells from both acutely and chronically infected cats suppressed $CD8^+$ IFN-γ production in response to immune

stimulation. LN cells and PBMCs were analyzed by flow cytometry for surface phenotype, including TGF-β and TGF-β receptor II (TGF-βRII) expression. Flow cytometric analysis revealed a low percentage of Treg cells expressed mTGF-β during the acute phase of FIV. However, analysis of cells from the same cats during the chronic phase of infection demonstrated that 40–50% of the Treg cells were mTGF-β^+. In contrast, Treg cells from FIV^- cats exhibited little or no mTGF-β expression. $CD8^+$ lymphocytes from FIV^+ cats also displayed greater surface TGF-β RII expression when compared to FIV^- cats. Treatment of $CD8^+$ cells with anti-TGF-β RII antibody and Treg cells with anti-TGF-β antibody prior to the IFN-γ ELISpot assay inhibited Treg mediated immune suppression. Furthermore, feline $CD8^+$ lymphocytes exhibited up regulation of Smad 2/3 phosphorylation and down regulation of IFN-γ message when treated with soluble TGF-β, suggesting that $CD4^+CD25^+$mTGF-β^+ Treg cells mediated $CD8^+$ suppression via the TGF-β / TGF-βRII signaling pathway.

Originally presented at the Keystone Symposia—TGF beta Family in Homeostasis and Disease, Santa Fe, NM.

ABSTRACT #348

SERUM TOTAL GLOBULIN AND IgA, IgE, IgG AND IgM CONCENTRATIONS IN ALASKAN SLED DOGS PARTICIPATING IN THE IDITAROD TRAIL SLED DOG RACE.

Erica McKenzie[1]; Christopher Lupfer[2]; Manoj Pastey[2]; Heidi Banse[3]; Kenneth Hinchcliff[4]; Stuart Nelson, Jr.[5]; Michael Davis[6]; Mark Payton[7]. [1]Department of Clinical Sciences, Oregon State University, Corvallis, OR. [2]Department of Biomedical Sciences, Oregon State University, Corvallis, OR. [3]Department of Large Animal Medicine, University of Georgia, Athens, GA. [4]Faculty of Veterinary Science, University of Melbourne, Werribee, Victoria, Australia. [5]Iditarod Trail Committee, Wasilla, AK. [6]Department of Physiological Sciences, Oklahoma State University, Stillwater, OK. [7]Department of Statistics, College of Arts and Sciences, Oklahoma State University, Stillwater, OK.

This study characterized hypoglobulinemia in racing sled dogs. Serum was collected from all dogs participating in the 2007 Iditarod within one month of race start. In addition, serum was collected from 118 dogs that finished, and retrospectively matched with each dog's pre-race sample. Blood was also obtained 3 months later from 51 dogs that finished the race. Serum globulin was determined by subtraction from chemically determined serum total protein (TP) and albumin. Serum [IgA], [IgE], [IgG] and [IgM] were determined by ELISA.

TP was lower immediately after (5.8 ± 0.5 mg/dl) and three months after racing (6.0 ± 0.4) than before racing (6.4 ± 0.5). Immediately after racing, 28.6% of dogs were hypoproteinemic compared to ~2% of dogs before and three months after racing. Mean serum globulin was below reference range for the general canine population (<2.7 mg/dl) in all three groups. Serum [IgG] was significantly lower before and immediately after racing, than three months after racing (8.21 ± 4.95, 7.97 ± 5.62 and 18.88 ± 5.76 mg/ml respectively). Serum [IgM] and [IgE] displayed a similar pattern ([IgM]: 0.97 ± 0.46, 0.88 ± 0.44, 1.21 ± 0.39 mg/ml; [IgE]: 0.07 ± 0.07, 0.05 ± 0.06, 0.12 ± 0.10 mg/ml). Serum [IgA] was higher immediately after (1.50 ± 1.07 mg/ml) and three months after racing (1.43 ± 0.82) than before racing (1.23 ± 1.00).

Hypoglobulinemia is prominent in racing sled dogs although a previous study demonstrated increases in specific antibody titers during racing. Hypoglobulinemia may reflect exercise effects or need for more appropriate references ranges for exercising dogs.

ABSTRACT #349

EVALUATION OF DIETS FOR THEIR ABILITY TO GENERATE "SATIETY" IN CATS.

E Servet, Y Soulard, C Venet, V Biourge. Royal Canin Research Center, Aimargues, France.

Today, around 30% of cats are overweight or obese in the Western world. To limit this serious condition, various dietary strategies designed to make cat lose weight already exist. However, in the field, the success of these feline weight loss programs is not as good as expected from laboratory studies. This is probably due to the fact that food restriction leads to increased begging, mewing and/or agressivity. One solution would thus be to design a diet inducing a "satiety effect" that could limit begging and help respect more easily recommended daily feeding. The goal of this study was thus to assess the ability of various dietary strategies to generate "satiety" (spontaneous food and/or energy intake reduction) in cats.

Sixteen adult non-obese colony cats were included in the study. Four different experimental dry-expanded diets were evaluated: Diet 1 (protein: 41%, fat: 10%, total dietary fiber (TDF): 16% as fed, ME: 3200 kcal/kg), Diet 2, the same diet but containing a high-water-binding-capacity fiber (ME: 3115 kcal/kg), Diet 3 (prot: 46%, fat: 10%, TDF: 10%, ME: 3365 kcal/kg) and Diet 4 (prot: 36%, fat: 10%, TDF: 21%, ME: 3090 kcal/kg). Four groups of 4 cats were randomly fed all diets according to a 4-week-Latin-Square design, with a 2-day-transition and a 5-day-measurement period for each diet. Diets were given *ad libitum* from 2-pm till 8-am daily and water was freely available. Food consumption was monitored by constant electronic weighing. Satiety criteria were: total energy intake (kcal/kgBW/day), meal size (intra-meal satiety or satiation in g/meal) and time interval (inter-meal satiety: time between 2 meals generated after consumption of 1 kcal of food during previous meal in min:sec/1 kcal). Data are expressed as mean ± SD.

Cats consumed their diets adequately. Results are shown below:

Criteria	Diet 1	Diet 2	Diet 3	Diet 4
Energy intake (kcal/kgBW/d)	43.8 ± 5.9^{ab}	41.9 ± 5.4^{a}	39.6 ± 6.3^{a}	48.9 ± 6.3^{b}
Meal size (g/meal)	6.5 ± 1.5^{ab}	7.3 ± 1.8^{bc}	6.1 ± 1.3^{a}	7.7 ± 2.1^{c}
Time interval (min:sec/1 kcal)	$07'11''^{ab}$	$10'08''^{c}$	$09'32''^{bc}$	$05'43''^{a}$

Data with different subscripts significantly differ at $p<0.05$.

Our results show that it is possible to discriminate the diets according to their "satiety effect" based on feeding pattern and that macronutrient composition will affect total energy intake, intra-meal satiety and inter-meal satiety. In cats, it appears that limiting the amount of protein (with a fiber substitution) is a strategy to limit spontaneous food/energy intake. Also, the nature of the fiber can increase further the efficacy, especially fibers with a high water-binding-capacity which potentially generate a bulking effect in the stomach (fullness). These observations might be useful in the design of diet to better manage feline obesity.

ABSTRACT #350

OXIDATIVE STRESS AND NEUTROPHIL FUNCTION FOLLOWING ORAL SUPPLEMENTATION OF A SILIBININ-PHOSPHATIDYLCHOLINE COMPLEX IN CATS.

CB Webb, KW McCord & DC Twedt. Colorado State University, Fort Collins, CO.

The purpose of this study was to determine the effect of oral supplementation of a silibinin-phosphatidylcholine complex SPC on parameters of oxidative stress and neutrophil function in healthy cats.

Blood samples were collected from 10 purpose-bred cats prior to and immediately after five days of oral supplementation of SPC (10 mg/kg; supplied by Nutramax Laboratories). Leukocytes were isolated from heparinized peripheral blood and incubated with monochlorobimane (mBCl). Monochlorobimane conjugates with reduced glutathione (GSH) resulting in a fluorescent signal that can be detected with flow cytometry. Leukocytes were also incubated with dihydrorhodamine 123 (DHR) and then mixed with *E. coli* conjugated to the fluorescent marker Alexa 488. DHR fluoresces in the presence of free radicals such that this combination of markers allows the measurement of *E. coli* phagocytosis and the subsequent oxidative burst by flow cytometry. The antioxidant enzymes superoxide dismutase and glutathione peroxidase, along with the reduced-to-oxidized glutathione ratio (GSH:GSSG) and a measure of lipid peroxidation (malondialdehyde) were measured using spectrophotometry in erythrocytes and whole blood.

A significant increase in intracellular GSH content was found in neutrophils of cats supplemented with SPC, with fluorescence intensity increasing from 515.3 ± 191.5 to 592.7 ± 178.1 ($P = .026$). A significant increase in phagocytic function was also noted, with the percent of neutrophils exhibiting maximal phagocytosis and oxidative burst increasing from 37% ± 11.8 to 45% ± 17.5 ($P = .049$) following 5 days of SPC supplementation. Other measures of oxidative stress did not change significantly.

Silibinin is a flavonoid with antioxidant properties used in treating a variety of human diseases, including liver disease, diabetes mellitus, and cancer. One of silibinin's proposed mechanisms of action is to increase intracellular glutathione content. Oral supplementation of SPC in cats appears to increase neutrophil GSH content and phagocytic function, both of which would be potentially beneficial in cats suffering from diseases associated with oxidative stress.

ABSTRACT #351
INFLUENCE OF AGE ON SERUM COBALAMIN AND FOLATE CONCENTRATIONS IN HEALTHY CATS. NK Parnell[1], GE Moore[1], JS Suchodolski,[2] JM Steiner[2]. [1]Purdue University, West Lafayette, IN, [2]Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

The prevalence of both cobalamin and folate deficiency increases with increasing age in humans, and these deficiencies have been associated with vascular disease and neurocognitive disorders. In the United States, the average age of the pet cat population has increased over the past decade. In the cat, alterations in intestinal tract physiology develop with increasing age. These changes result in decreased digestive efficiency, which could culminate in deficiencies of either cobalamin and/or folate. Therefore, the purpose of this study was to evaluate a possible effect of age on serum cobalamin and folate concentrations in the healthy cats. It was hypothesized that serum cobalamin and folate concentrations would decrease with increasing age.

Eighty-eight healthy cats were recruited and placed into one of four groups based on age-life stage: Group 1: less than one year, Group 2: 1 to <8 years, Group 3: 8 to <13 years, and Group 4: ≥ 13 years. Serum cobalamin (reference range: 290–1,500 ng/L) and folate concentrations (reference range: 9.7–21.6 μg/L) samples were measured in samples which were obtained after a 12 hour fast.

Overall, two cats (2.3%) had subnormal cobalamin concentrations and 8 cats (9.1%) had subnormal folate concentrations. For Group 1 (n = 21), the median serum cobalamin concentration was 1,113 ng/L (range: 267–4,143 ng/L) and the median folate concentration was 19.9 μg/L (range: 9.6–39.8 μg/L). Cats of Group 2 (n = 21) had a median cobalamin concentration of 1,524 ng/L (range: 197–4,282 ng/L) and a median folate concentration of 15.3 μg/L (range: 6.7–26 μg/L). The median cobalamin concentration for Group 3 (n = 23) was 953 ng/L (range: 494–2,333 ng/L) and median folate concentration was 12.8 μg/L (range: 7.2–23.5 μg/L). For Group 4 (n = 23), the median cobalamin concentration was 811 ng/L (range: 293–3,136 ng/L) and the median folate concentration was 14.4 μg/L (range: 6.7–35.9). Both the median serum cobalamin and folate concentrations were significantly different between age groups ($p = 0.008$ and $p = 0.025$, respectively). For cobalamin concentrations, the greatest difference was between Groups 2 and 4 ($p = 0.004$). Serum cobalamin concentration was inversely correlated with age (Spearman $r = -0.353$; $p < 0.001$). Serum folate concentration was more weakly inversely correlated with age (Spearman $r = -0.243$; $p = 0.023$). The median serum cobalamin concentration in cats 8 years and older (870.5 ng/L) was significantly lower than that of cats under the age of 8 (1,274 ng/L) ($p = 0.002$).

While some cats in this healthy population were identified as having subnormal cobalamin or folate concentrations, the majority were not. However, this study suggests that, as cats age, their serum cobalamin and folate concentrations decrease significantly. Clinically this could be important as cats that are greater than 8 years of age may be more likely to become deficient when affected by conditions that can alter cobalamin and folate homeostasis.

ABSTRACT #352
GHRELIN SECRETION IS UNRELATED TO DIET COMPOSITION IN CATS. L. Martin[1], H Dumon[1], V,B Siliart[1], T Lutz[2], V Biourge[3] and P Nguyen[1]. [1]Ecole Nationale Vétérinaire de Nantes, France, [2]Vetsuisse Faculty University of Zurich, CH, Switzerland, [3]Royal Canin, Aimargues, France

The understanding of the relationship between diet composition and obesity is a current topic. Although satiety is controlled by many factors, hormones implicated in the control of food intake and glucose metabolism should be considered. The aim of this study was to compare the effect of two meals with different protein-to-fat ratios on fasting and postprandial plasma glucose, ghrelin, insulin, and amylin concentrations. Fourteen neutered cats were fed a high protein low fat (HPLF—48% energy from protein, 28% from fat) diet and a low protein high fat (LPHF—28% energy from protein, 46% from fat) diet in a randomized cross-over design. Cats were categorized according to their body fat mass (BFM) ($p < 0.001$): group NO: BFM < 30%, normal cats (n = 5); group OB: BFM > 31% obese cats (n = 9). For each diet there was a 6-week adaptation period before the test meal. They were offered 70 g/d. Blood samples for hormone assays were collected at the end of the 6-wk period before the meal (fasting) then immediately (T0), 30, 60 and 100 minutes after the test meal (35 g food/10 minutes) (post-prandial—pp). Commercially available kits previously validated for use in cats were used. Since data were non-normally distributed, non-parametric tests were used to examine the effects of diet and BFM. Values of $p < 0.05$ were considered significant.

Mean body weight did not differ between groups and during the study. Spontaneous food intake (g/d) did not differ between diets but total energy intake (kcal ME/d), energy (kcal ME) per kg BW or per kg fat-free mass were higher for LPHF diet (respectively $p = 0.014$, $p = 0.013$ and $p = 0.013$). There was no effect of BFM on these parameters. Fasting glucose and hormone concentrations were affected by neither the type of diet or BFM. The HPLF diet induced greater pp variations of insulin ($p = 0.033$) and amylin ($p = 0.035$) than the LPHF diet and, inversely, changes in pp glucose were higher with the LPHF diet ($p = 0.001$). Post-prandial variations of ghrelin were not affected by the composition of the meal. Unexpectedly, BFM had no effect on post-prandial insulin and amylin. However, time course of pp glucose was higher in obese cats than in lean cats ($p < 0.001$). Moreover, change in pp ghrelin was significantly affected by BFM ($p = 0.019$): pp ghrelin decreased in lean cats while it increased in obese cats.

The study showed an effect of the diet composition on post-prandial changes in glucose, insulin and amylin plasma concentration. The HPLF diet induced a higher post-prandial secretion of insulin and amylin than the LPHF diet, suggesting a direct effect of amino acids on insulin secretion. Ghrelin was not affected by the type of diet, suggesting a limited effect of the composition of the diet on satiety. Unexpectedly, the most marked effect on ghrelin secretion was related to body composition, suggesting a dysregulation of food intake in obese cats unrelated with food composition.

ABSTRACT #353
DIETARY ERYTHORBIC ACID DOES NOT AFFECT URINE OXALATE CONCENTRATION IN HEALTHY ADULT CATS. S Yu and KL Gross. Hill's Pet Nutrition, Inc., Topeka, KS.

Erythorbic acid (isoascorbic acid or D-araboascorbic acid) is a stereoisomer of ascorbic acid (vitamin C) and is used as a processing aid in some pet foods. Similar to vitamin C, erythorbic acid can be absorbed, metabolized, and excreted in the urine of animals when ingested. Therefore, erythorbic acid could potentially be metabolized in the body to oxalate that is excreted in the urine. Increased urinary oxalate concentration is known to increase the risk of urinary calcium oxalate stones in cats and other animals. The objective of this study was to investigate whether dietary erythorbic acid affects urine oxalate concentration in cats.

Twenty-four healthy adult cats were used in an unbalanced cross-over study. A nutritionally complete and balanced dry cat food was used as a control food, which contained an undetectable amount of erythorbic acid (< 0.5 ppm). Three test foods were made by adding various amounts of erythorbic acid to the control food at the expense of starch. The test foods contained 90, 203, or 348 ppm erythorbic acid. All cats were fed the control food for 2 weeks (washout period) and then fed one of the test foods or the control food for another 4 weeks (test period). Blood samples and 24-hour urine samples were collected at the end of the washout period and the test period. Food intake was measured daily and body weight weekly.

Intake of erythorbic acid did not affect urine oxalate concentration ($r = 0.26$, $p = 0.083$) nor urine oxalate excretion corrected by urine creatinine ($r = 0.18$, $p = 0.235$). Similarly, intake of erythorbic acid had no effect on urine vitamin C concentration ($r = 0.11$, $p = 0.451$), urine volume ($r = -0.04$, $p = 0.817$), or urine pH ($r = 0.24$, p

= 0.102). Plasma erythorbic acid was undetectable (< 0.5 ppm) in cats fed any of the test foods. Plasma vitamin C concentration was not affected by dietary erythorbic acid. Urine erythorbic acid was undetectable (< 0.5 ppm) in cats fed the test foods containing less than 348 ppm of erythorbic acid. Urine erythorbic acid concentration was 8.24 ± 7.61 ppm in cats fed the food containing 348 ppm erythorbic acid. Food intake and body weight were similar among dietary groups during the study.

This study demonstrates that dietary erythorbic acid up to 348 ppm does not affect urine oxalate concentration, urine pH, and urine volume in healthy adult cats.

ABSTRACT #354

LIPID METABOLIC RESPONSE OF DOMESTIC FELINES TO DIETARY OMEGA-6 AND OMEGA-3 POLYUNSATURATED FATTY ACIDS. M. McClure, R. Angell[1], K. Bigley[1], K. Fennell[1], J.E. Bauer[1,3]. [1]Companion Animal Nutrition Lab., [3]Faculty of Nutrition, Texas A&M University, College Station, TX.

Despite the unique aspects of feline fatty acid metabolism, few studies have systematically characterized this species' response to dietary polyunsaturated fatty acids. Consequently, this study was designed to investigate effects of diets varying only in fatty acid (FA) composition on triacylglycerol (TAG), total cholesterol (TC), non-esterified fatty acid (NEFA), lipoprotein-cholesterol distributions, and plasma phospholipids (PL). Twenty nine clinically normal, sexually intact, young adult female cats were randomized into three groups (n = 9,10,10). Each group was fed a complete and balanced, commercial, dry extruded type basal diet, supplemented with 8g oil/100g diet. The oils used and the resulting diets fed were: high-oleic sunflower oil (H diet) with 82% oleic acid, Menhaden fish oil (M diet) with high amounts of long chain n3 fatty acids (LCn3FA), and safflower oil (S diet) with 75% linoleic acid. Arachidonic acid (20:4n6) content of the diets was: 0.03 for H, 0.09 for M, and 0.03 for S (g FA/kg diet). Diets were fed for 28 days with blood collections on days 0, 14, and 28. Using PROC MIXED analysis in SAS with $p < 0.05$ as significant, the M diet showed a significant TAG lowering effect despite the already low normal feline TAG levels. No main time or diet effects were found with TC or NEFA. Lipoprotein electrophoresis revealed a significant lowering of the pre-beta band (i.e. TAG-rich VLDL) in the M group consistent with plasma TAG lowering. Plasma PL FA profile revealed statistically significant diet and time effects in addition to time x diet interactions. Many of these changes were significantly different at day 14, but by day 28 were less pronounced indicating a transitional metabolic response at day 14 versus day 28. Anticipated diet effects included: statistically significant accumulation of 18:1n9 in the H group, 18:2n6 in the S group, and LCn3FA in the M group. Despite high dietary 18:2n6 in the S group PL-20:4n6 was not increased over the other groups. In comparison to other species, only modest amounts of PL-20:4n6 were found in these cats. Furthermore, a significant increase in the in 20:2n6 in the S group demonstrated that chain elongation of excess 18:2n6 occurred in deference to its Δ6 desaturation. These findings further substantiate the known low Δ6 desaturase activity of cats. Similarly, increased relative amounts of 20:1n9 in the M diet resulted in its chain elongation and accumulation to 24:1n9 in the M group. It is noteworthy that the M diet did not blunt incorporation of 20:4n6 into PLs. This effect was minimized because fish oil provided 3 times more 20:4n6 than the vegetable oils, and cats have low Δ6 conversion. Additionally, blunting of 20:4n6 into PLs was not seen due to tissue 20:4n6 content being diet dependent. In conclusion, fish oil results in PL-LCn3FA enrichment without negative effects on 20:4n6 and lowers plasma TAG levels. Whether TAG lowering in normal cats is beneficial is unknown at this time, although n3 FAs may help treat hepatic lipidosis in this species.

ABSTRACT #355

THE EFFECT OF SOCIAL INTERACTION ON PHYSIOLOGIC RESPONSE IN CATS FED TWO LEVELS OF DIETARY SODIUM AS COMPARED TO POTASSIUM. SC Zicker[1], SR Lowry[1], CA Kirk[2]. [1]Pet Nutrition Center, Hill's Pet Nutrition, Inc. Topeka, KS. [2]University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

The purpose of this study was to examine the interaction between a change of social interactions and different concentrations of dietary sodium (n = 2) compared to potassium (n = 1) on physiologic response in cats. Thirty, healthy adult DSH cats (15 M, 15 F) were randomly assigned to one of three groups for a three phase feeding trial. Average age for each group was 4.7, 4.9 and 4.7 years, respectively. During the two weeks of Phase I, all cats were housed individually and received a dry food containing 1.1% sodium (Higher Sodium = HS) dry matter basis (DMB) as an initial adaptation period. During Phase II all cats remained individually housed but were randomly assigned into 3 dietary groups. Ten cats remained on HS, ten cats were fed a food with 0.35% sodium (Lower Sodium = LS) DMB and ten cats were fed a food with 2.0% potassium (P) DMB. In Phase III, cats remained on the same foods as in Phase II but were housed in a group by dietary group instead of housed as individuals. Individual housing units allowed for cats to view other cats through windows but not interact; however, some time each day was allotted for daily physical interactions with people. During group housing, cats were grouped together, by diet, in separate large rooms, and allowed to jump to resting boards or cubbies, daily physical interaction with people as before, as well as unlimited interaction with cohorts. Urine and blood samples were collected on the last day of Phase I and II and on days 1, 5 and 12 of Phase III. Urine was analyzed for cortisol:creatinine ratio (UCC), and specific gravity. Blood samples were analyzed for aldosterone, cortisol, and osmolality. Data were analyzed using SAS, PROC MIXED methods. UCC showed no difference among groups in response to food fed. However, UCC and serum cortisol were significantly increased in Phase III for all groups on days 1 and 5 compared to Phase II. Urine specific gravity was significantly higher on day 1 of Phase III compared to Phase II. In contrast, serum osmolality displayed a significant group by period interaction between Phase II and Phase III. This was probably attributable to the HS and P dietary groups having a decrease in serum osmolality at day 5 of Phase III. Curiously, there was no significant increase in serum osmolality during day 1 of Phase III to reflect the increased urine specific gravity response. Finally, aldosterone displayed a highly significant group by period interaction. During the individual housing period the response was as anticipated with cats transitioning from HS to LS foods having an increased aldosterone secretion. However, during Phase III, the HS food group had a significant increase in aldosterone secretion in spite of no change in serum osmolality on day 1 and a lower serum osmolality on day 5. The LS and P did not display increased aldosterone during Phase III. Aldosterone response to social adaptation appeared to be dependent on sodium content of the food with higher sodium foods having a paradoxical increase in the face of normal to decreased serum osmolality.

ABSTRACT #356

GLOBAL METHYLATION OF CYTOSINE IS NOT INFLUENCED BY AGE OR DIET IN BEAGLE DOGS. SC Zicker[1], F Domann[2]. [1]Pet Nutrition Center, Hill's Pet Nutrition, Inc., Topeka, KS. [2]University of Iowa, Iowa City, IA.

Mammalian genomes contain about 5-fold fewer CpG dinucleotides than expected based on random probability. Regions of the genome that have retained "near expected" numbers of CpG dinucleotides are called CpG islands and these CpG islands constitute the 5′ ends of approximately half of all genes. This becomes important when one considers that CpG dinucleotides are also the substrate for enzymatic modification of cytosine to 5-methylcytosine. Importantly, age associated changes in gene expressions have been attributed, at least in part, to changes in DNA methylation within the transcriptional control regions of such genes. The purpose of this study was to determine the endogenous DNA methylation in canine lymphocytes and to further determine the role of aging and an antioxidant rich diet on the level of DNA methylation. Twenty-four Beagle dogs were used in this study. Ten young dogs (aged 2–4 years), 14 dogs greater than 12 years of age on either a control (n = 7) or antioxidant enriched food (n = 7) for a period of 3 years prior to samples. Lymphocytes were utilized to

harvest RNA free DNA and 5 methylcytosine was determined by reversed-phase HPLC. Results for each group were as follows: young dogs 4.34% ± 0.77; old control dogs 4.37% ± 0.72; old antioxidant dogs 4.38% ± 0.68 as a percent of cytosine methylated (± SD). There were no significant differences between any of the groups. The data is consistent with levels recorded for other mammalian species. Although there were no differences detected in global methyation it remains possible that regional methylation differences may exist. A redistribution of methylated cytosines within the genome could lead to a changing pattern of gene expression in aging canines without changing the overall level of 5-methylcytosine.

ABSTRACT #357
DOGS OF DIFFERENT BREEDS ARE APOLIPOPROTEIN E4 HOMOZYGOUS BY PCR-RFLP. SC Zicker[1], P Ward[2], E Head[3]. [1]Pet Nutrition Center, Hill's Pet Nutrition, Topeka, KS. [2]Department of Pathology, University of California, Irvine, CA. [3]Department of Neurology, University of California, Irvine, CA.

In dogs, high density lipoproteins (HDL) are the predominant plasma lipoprotein fraction (75–85%). This differs from humans where low-density lipoproteins (LDL) predominate. Because of the high HDL level of lipoproteins in dogs they have been considered to be atherosclerosis-resistant. Although dogs are considered to be atherosclerosis resistant, reports have shown that atherosclerosis does exist in older dogs and in higher prevalence in some specific disease states. Recently in humans it has been shown that apolipoprotein E has several distinct allotypes which may be associated with distinct disease physiologic processes. Apolipoprotein E4 in humans associates with very low density lipoproteins (VLDL) while apolipoprotein E3 associates with HDL. In transgenic mice Apo E3 and Apo E4 associate equally with VLDL and intermediate density lipoproteins IDL. The Apo E3 and Apo E4 alleles both bind the LDL family of receptors with equal affinity. Of recent interest is the finding that ApoE4/ApoE4 associated with VLDL inhibits the anti-apoptosis effects of HDL and thus may yield some insight on possible mechanisms of the homozygous increased risk factor for atherosclerosis. The current study utilized 25 dogs representing 9 different pure breeds, and 3 mixed breeds, to survey the prevalence of apolipoprotein allele distribution in canids. Screening for the three common APOE alleles (E2, E3 and E4) was achieved using PCR-RFLP (polymerase chain reaction - restricted fragment length polymorphism) analysis exploiting the restriction enzymes *Afl* III and *Hae* II. These enzymes recognize the allele specific nucleotide substitutions at codons 112 and 158, respectively. Results showed that all dogs assessed by this methodology were homozygous for apolipoprotein E4 allele. It is possible that the finding of dogs being apolipoprotein E4 homozygous may be important in the pathopysiologic development of atherosclerosis in at risk disease populations or in furthering the understanding of cholesterol metabolism in dogs.

ABSTRACT #358
LOW GLYCEMIC INDEX STARCH PLUS DIACYLGLYCEROL BENEFICIALLY MODIFIES OBESITY HORMONES DURING CANINE WEIGHT LOSS. D. Nagaoka[1], Y. Mitsuhashi[1], K. Bigley[1], T. Umeda[2], K. Otsuji[2], J.E. Bauer[1,3]. [1]Companion Animal Nutrition Lab, College of Veterinary Medicine, and [3]Faculty of Nutrition, Texas A&M University, College Station, TX, USA. [2]Kao Corp., Tokyo, Japan.

Obesity is the most common nutritional disorder in small animal medicine and is associated with several metabolic diseases. Decreasing its incidence is thus important in animal health. We previously reported that a low glycemic index starch/diacylglycerol oil dietary combination (LGIS/DAG) offered several benefits during canine weight loss. Here we report additional data investigating LGIS/DAG effects on obesity hormones. Twelve obese Beagles with body condition scores of 8.4 ± 0.1 (SEM) out of 9, and 48.9 ± 3.3% body fat were randomly divided into four groups. Four diets were studied: all included chicken by-product meal and either low- (LGIS, high amylose corn) or high- (HGIS, waxy corn) glycemic index starch; either diacylglycerol (DAG) or triacylglycerol (TAG) dietary oil; and vitamin/mineral pre-mixes for canine maintenance (25% protein, 39% fat and 36% nitrogen-free extract, respectively (energy basis) and ca. 4200 kcal/kg DM). After a 10 week (n = 3 per group) weight loss regimen, obesity was re-induced in all dogs to pre-diet body weights. After obesity re-induction, the beagles were then assigned into exactly opposite diet groups and weight loss again induced (10 weeks). All dogs were offered the equivalent calories each day to maintain their initial obese body weights. They were weighed weekly with food consumption recorded daily. During week 3, fecal samples were collected for diet digestibility trials. On week 1 and 8, post-prandial blood samples were collected via jugular catheter for glucose and gastric inhibitory polypeptide (GIP) determination using cooked chicken breast meat in place of chicken by-product meal in the diet mixture. On weeks 1, 4, 8 and 9, fasting blood samples were collected for adiponectin and leptin analyses. Data were analyzed by repeated measures ANOVA and Tukey post-hoc tests. The LGIS diet groups lost more weight than the HGIS diet group (2% vs 1% per week) due to lower digestibilities of the LGIS diets. Significantly lower plasma leptin concentrations were found, consistent with weight loss in all groups and especially the LGIS/DAG group. Plasma adiponectin concentrations were significantly higher and plasma insulin significantly lower in the LGIS/DAG group compared with all other diet groups while glucose concentrations were similar. Although there were no statistically significant differences among the plasma GIP concentrations, the LGIS diet groups tended to be lower over time compared with the HGIS groups. These findings indicate that the LGIS/DAG dietary combination beneficially alters obesity hormones and, together with our earlier findings, appears to be preferred for healthy canine weight loss.

ABSTRACT #359
EFFECT OF A SHORT-TERM INFUSION WITH SOYBEAN OIL-BASED LIPID EMULSION ON PHAGOCYTOSIS OF CANINE PERIPHERAL BLOOD POLYMORPHONUCLEAR NEUTROPHILIC LEUKOCYTES. JH Kang, MP Yang. Chungbuk National University College of Veterinary Medicine, Cheongju, Chungbuk, Republic of Korea.

The objective of this study was to examine the effect of a short-term infusion with soybean oil (SO)-based lipid emulsion (LE) on functions of canine peripheral blood polymorphonuclear neutrophilic leukocytes (PMNs). Twenty-four healthy Beagles were randomly assigned to receive 4 treatments (6 dogs per treatment). Treatment A consisted of IV infusion of 0.9% NaCl solution (control treatment). Treatments B, C, and D consisted of IV infusion of SO-based LE (Intralipose 20%) that supplied 40, 100, and 200% of daily energy requirements, respectively. All treatments were infused for 2 hours. To evaluate PMNs function, blood samples were collected before IV infusion of SO-based LE, immediately after infusion, and 24 hours after completion of the infusion. The phagocytic capacity and oxidative burst activity (OBA) were analyzed simultaneously by use of flow cytometry, and the actin polymerization was assayed by use of flow cytometry and confocal microscopy. The Cdc42 activation levels in phorbol myristate acetate-treated PMNs were determined by affinity precipitation using Cdc42 activation assay kit.

All treatments had no significant effect on the phagocytic capacity of PMNs. However, the values for OBA and actin polymerization were reduced in only treatment D and restored to normal values 24 hours after infusion. The activation of Cdc42 was also inhibited by treatment D. In conclusion, these results suggest that the short-term infusion with a supraphysiologic dose of SO-based LE can decrease the immune functions of canine PMNs, but infusions with clinically relevant doses of SO-based LE may not trigger any significant inhibitory effects.

ABSTRACT #360
FREQUENCY OF THE MUTANT MDR1 ALLELE IN A SAMPLE OF SHEPHERD DOGS IN FRANCE CORRELATION WITH FRENCH PHARMACOVIGILANCE DATA. C. Hugnet[1], X. Pineau[2], F. Buronfosse[2], S. Queffélec[2], G. Queney[2]. [1]Clinique vétérinaire des Lavandes, La Bégude de Mazenc, France, [2]Veterinary Pharmacovigilance Center of Lyon (CPVL), Ecole

Nationale Vétérinaire de Lyon, Marcy L'Etoile, France, [3]Antagène, Limonest, France.

The multidrug resistance gene (MDR1) produces P-glycoprotein (P-gp) that belongs to the ATP Binding Cassette protein superfamily. P-gp is involved in the active cellular efflux of a large number of drugs and transports many structurally and pharmacologically unrelated hydrophobic compounds including macrocyclic lactones, anticancer agents, immunosuppressive agents, steroid hormones, calcium channel blockers, β-blockers, cardiac glycosides, antibiotics . . . In the central nervous system (CNS), P-gp is found in the capillary endothelial cells that form the blood brain barrier. So, the role of P-gp is particulary important for the protection against accumulation in the CNS of macrocyclic lactones (or other P-gp substrate drugs) and its subsequent neurotoxicity. P-gp is also disposed on the biliary canicular membrane of hepatocytes and on the apical side of enterocytes, and is a key player in both biliary and intestinal secretion of P-gp substrate drugs.

A 4-bp deletion mutation of MDR1 gene was described in ivermectin-sensitive collies. This mutation generates a premature stop codon, preventing synthesis of the complete and active protein product.

Between june 2003 and december 2007, 264 dogs (97 Australian Shepherds, 81 Collies and 86 white German Shepherds) living in France were tested in Antagene laboratory or in Washington State University Laboratory by PCR for genotyping MDR1. Only 13.6% of the collies studied were homozygous for the normal allele (normal), 40.7% were heterozygous (carrier), and 33.3% were homozygous for the mutant allele (affected). For Australian Shepherds and white German Shepherds, respectively, 9.3% and 3.5% of tested dogs were affected; in addition 43.3% and 17.4% were carrier. Some of the tested dogs were clinically sensitive to macrocyclic lactones or loperamide; all of the dogs with signs of toxicosis were homozygous for the mutant allele or heterozygous. Macrocyclic lactones and loperamide were prescribed at dosages that cause no adverse effects in normal dogs. In purebred or mixed dogs of these breeds, veterinarians should perform a test before prescribing any P-substrate drug to avoid toxicosis in affected or carrier dogs.

ABSTRACT #361
BIOAVAILABILITY FOLLOWING ORAL ADMINISTRATION OF A SILIBININ-PHOSPHATIDYLCHOLINE COMPLEX IN CATS. CB Webb, BJ Samber, D Gustafson, & DC Twedt. Colorado State University Clinical Sciences Department, Fort Collins, CO.

The milk thistle extract silimarin or its active product (silibinin) is shown to have antioxidant effects, one of which is increasing hepatic glutathione concentration. A number of human clinical trials have demonstrated the therapeautic effects of this compound for treating liver disease. Complexing silibinin with phosphatidylcholine (SPC) increases its oral uptake and bioavailability. The bioavailability of SPC was recently demonstrated in dogs but because of the unique characteristics of feline hepatic metabolism the bioavailability may be different. The purpose of this study is to determine the oral bioavailability of a silibinin-phosphatidylcholine complex (SPC) in healthy cats.

Five cats were administered 10 mg/kg of SPC (provided by Nutramax Laboratories) as a powder combined with cornstarch (31% silibinin) in a capsule. Blood samples were drawn prior to SPC administration, and then at the 0.5, 1, 2, 4, 6, 8, and 12 hour time points following SPC administration. Blood samples drawn for silibinin analysis were collected in lithium-heparin tubes, centrifuged, and the plasma stored at −70 °C until analyzed. Following a 14 day washout period a single IV dose of silibinin (90.5% pure; 55 mg/cat dissolved in absolute ethanol, resulting in a 50mg silibinin dose per cat) was administered to the same 5 cats. Post-treatment samples were drawn at 5 minutes, 15 minutes, and 0.5, 1, 2, 4, 6, 8, and 12 hour time points. A liquid chromatography-mass spectrometry-mass spectrometry method was used to quantify plasma levels of silibinin. A pharmacokinetic (PK) comparison of IV silibinin versus oral dosing of SPC in cats showed the following: C_{max} of 87.1 μM ± 44.2 IV and 5.8 μM ± 3.1 oral; AUC_{0inf} of 86.6 μM•hr ± 81.4 IV and 6.1 μM•hr ± 3.5 oral; $t_{1/2}\lambda$ of 2.51 hr ± 1.63 IV and 3.16 hr ± 1.74 oral; V_z of 5.65 ± 3.66L IV and 309L ± 165 oral; CL of 1.88 L/hr ± 0.99 IV to 74.7 L/hr ± 47.1 oral. Comparing these PK parameters for a 10/mg/kg dose of SPC in cats with those of dogs (45 mg/kg) and humans (62 mg/kg) following oral silibinin administration showed the following: C_{max} of 5.8 μM ± 3.1 in cats, 0.33 μM ± 0.25 in dogs, 96.1 μM ± 37.3 in humans; $AUC_{04\,hr}$ of 5.80 μM•hr ± 3.46 in cats, 0.69 μM•hr ± 0.52 in dogs, 165.1 μM•hr ± 66.6 in humans; CL/F of 22.89 L/hr ± 14.33 in cats, 11,850 L/hr ± 12,187 in dogs, 63.24 L/hr ± 27.50 in humans. Oral SPC has a bioavailability of 6–7% in cats. The distinct species differences are best explained by the feline's limited capacity for hepatic glucuronidation of xenobiotics. Oral administration of SPC effectively raised plasma levels of silibinin in these healthy cats. Confirmation of the bioavailability of this complex suggests that oral administration of SPC could be an effective treatment option in with liver disease involving oxidative stress or depletion of hepatic glutathione concentrations.

ABSTRACT #362
ASSESSING PERFORMANCE OF A GENETIC TEST FOR DETERMINING THE BREED COMPOSITION OF MIXED BREED DOGS USING F1 HYBRIDS. AL Watson, PG Jones, AJ Martin, RJ Stark, PJ Markwell. Waltham Centre for Pet Nutrition, Melton Mowbray, UK.

Mixed breed dogs comprise approximately 50% of the dog population in the USA. A single nucleotide polymorphism (SNP) based test designed to determine the breed composition of mixed breed dogs has recently been made commercially available[1]. The aim of this study was to assess the performance of this test in known mixed breed dogs. First cross (F1) hybrids containing breeds from six of the seven American Kennel Club breed groups were included in the study.

Blood samples were collected under veterinary supervision from 85 F1 hybrids bred from parents registered to a recognised Kennel Club. DNA was extracted and typed at more than 300 different SNPs across the genome using selective hybridization and PCR amplification, followed by a discriminatory single base-pair primer extension reaction. The SNP variants were detected by mass spectrometry. A Bayesian generative model was then used to infer the family tree of a dog from comparison of detected genotypes with 134 breed signatures developed previously from more than 8000 pure bred dogs. Inference was performed on eleven different family tree models, and the best-fit model selected using the deviance information criterion.

Fifteen different breeds, the most numerous of which were Labrador Retriever, Golden Retriever and Poodle were represented amongst the 85 dogs. Sensitivity of breed detection, calculated as true positive calls (TP) / (true positive calls + false negative calls), was 95%. Positive predictive value (TP / TP + false positive calls) of breed detection was 84%.

These data indicate that this type of test can be used as a first step in understanding the genetics of mixed breed dogs and may have application in both clinical practice and research [1]WISDOM Panel TM MX, Mars Veterinary, Rockville, MD.

ABSTRACT #363
PRELIMINARY OBSERVATIONS OF THE BREED COMPOSITION OF MIXED BREED DOGS IN THE USA. PG Jones, AJ Martin, RJ Stark, SMD Davison, PJ Markwell. Waltham Centre for Pet Nutrition, Melton Mowbray, Nottingham, UK.

Mixed breed dogs comprise approximately 50% of the dog population in the USA. A SNP based test designed to determine the breed composition of mixed breed dogs has recently been made available commercially[1]. The aim of this study was to collect preliminary data on the breed composition of mixed breed dogs in the USA.

Blood samples were collected in veterinary hospitals from 891 dogs. DNA was extracted and typed at more than 300 different SNPs across the genome using selective hybridization and PCR amplification, followed by a discriminatory single base-pair primer extension reaction. The SNP variants were detected by mass spectrometry. A Bayesian generative model was then used to infer the family tree of a dog from comparison of detected genotypes with 134 breed signatures developed previously from more than 8000 pure bred dogs. Inference was performed on eleven different family

tree models, and the best-fit model selected using the deviance information criterion.

The number of breeds detected per dog averaged 3.3, with German Shepherd Dog the most commonly detected breed (Table). Twenty-seven of the dogs were considered to be pure bred.

Breed	Percentage of total breeds detected	Breed	Percentage of total breeds detected
German Shepherd Dog	7.3	Golden Retriever	2.5
Labrador Retriever	5.1	Cocker Spaniel	2.5
Chow Chow	4.8	Beagle	2.3
Rottweiler	3.4	Siberian Husky	2.3
Boxer	3.3	Poodle (miniature)	2.1

This study provides a first assessment of the mixed breed dog population of the USA. The data suggest that this test could be of value in both veterinary practice and research to determine the breeds present within mixed breed dogs.

[1]WISDOM Panel™ MX, Mars Veterinary, Rockville, MD

ABSTRACT #364

EVALUATION OF LAPAROSCOPIC ASSISTED PANCREATIC BIOPSIES IN 11 HEALTHY CATS. K Cosford[1], A Carr[1], C Schmon[1], S Myers[1], S Taylor[1], J Steiner[2], and J Suchodolski[2]. [1]Western College of Veterinary Medicine, Saskatoon, Sask., Canada. [2]Texas A&M University College of Veterinary Medicine, College Station, TX.

Definitive diagnosis of pancreatitis continues to be a challenge in cats. In many instances, a definitive diagnosis is only possible with histopathologic examination of tissue samples. Biopsies can be obtained via exploratory surgery; however, this is considered relatively invasive. Laparoscopy is a less invasive procedure used to obtain biopsies from various organs. The purpose of this study was to determine the clinical effects of laparoscopic pancreatic biopsy in cats. Additional goals were to ascertain whether any significant clinicopathological abnormalities arose, to establish that the punch type biopsy instrument could indeed provide adequate pancreatic tissue for histopathology, and to reassess the pancreatic biopsy site grossly and histopathologically one month later.

The study population consisted of 11 cats (6 biopsy, 5 sham operated control cats). Body weight, temperature, pulse, respiratory rate, and caloric intake were monitored for one week prior and one week after the surgical procedure. The cats were also monitored for any indications of gastrointestinal tract disturbances. At 0, 6, 24 and 72 hours post-procedure, a complete blood cell count, serum biochemistry profile, urine specific gravity and feline pancreatic lipase immunoreactivity, were evaluated.

A Wilk-Shapiro test was performed to determine if the data was normally distributed. If normally distributed, a 2 sample t-test was run on the differences between pre and post procedure parameters for experimental and control groups. If the data was not normally distributed, a Wilcoxon rank sum test was run on the differences between pre and post procedure parameters for experimental and control groups.

None of the clinical parameters deviated significantly from controls. Clinicopathological changes included statistically significant changes within the following parameters as compared to time zero elevated blood urea nitrogen at 72 hours, decreased bicarbonate at 6 hours, and decreased total serum calcium at 24 hours in comparison to controls. The pancreatic tissue collected was deemed to provide excellent samples for histopathological analysis.

One month after the original procedure, the cats underwent a second procedure to reevaluate the effects of manipulation and biopsy on the pancreas. There were no gross pancreatic abnormalities in the control cats after one month. Of the 6 cats biopsied, gross examination revealed adhesions of omental fat (2/6), and neovascularization (3/6) at the previous biopsy sites. Moreover, when the original pancreatic biopsy sites were re-sampled one month later, during a second procedure, a subclinical mild to moderate chronic pancreatitis could be identified histopathologically in 5/6 cats.

These findings document the safety and feasibility of laparoscopic assisted pancreatic biopsy in healthy cats.

ABSTRACT #365

INDUCTION OF OXIDATIVE STRESS AND INFLAMMATION IN A CANINE CHONDROCYTE CULTURE MODEL. DL Dycus[1], AY Au[2,3,4], MW Grzanna[2], CG Frondoza[1,2,3]. [1]Mississippi State University, Mississippi State, MS. [2]Nutramax Laboratories, Inc., Edgewood, MD. [3]Johns Hopkins University, Baltimore, MD. [4]Syracuse University, Syracuse, NY.

Osteoarthritis (OA) is a chronic progressive disease affecting diarthrodial joints. It is characterized by cartilage breakdown resulting in pain and joint dysfunction. The etiology of OA is still unclear but evidence points to the critical role of pro-inflammatory mediators, particularly cytokines and prostaglandins. Cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are suspected to induce reactive oxygen species (ROS). Overproduction of these molecules may be a cause of oxidative stress resulting in chondrocyte apoptosis. ROS generation may thus contribute to the pathogenesis of OA. However, little is known about the effect of oxidative stress on canine chondrocytes and its role in the development of OA. To address this problem, we determined whether oxidative stress can be induced by cytokines in a canine chondrocyte culture model. We evaluated whether the chondrocytes continue to proliferate and maintain the cartilage phenotype. In addition, we subjected chondrocytes to oxidative stress by activation with cytokines. Oxidative stress was assessed by measurement of reduced glutathione (GSH) levels and superoxide dismutase (SOD) enzyme activity. As positive control for inhibiting oxidative stress, we used silybin phosphatidylcholine complex (SPC). This agent has anti-oxidant, as well as anti-inflammatory properties, and is used for hepatoprotection (Marin®).

The phenotype expression was verified by immunostaining and Western blot analyses of aggrecan, as well as collagen types I and II. Chondrocytes (5×10^5 cells/well) were incubated with control media alone or SPC (298 ng/ml) for 24 hrs. The cultures were re-incubated with control media alone or the combination of IL-1β (10 ng/ml) and TNF-α (1 ng/ml) for 24 hrs or 48 hrs. The spent culture supernatant was measured for PGE-2 by ELISA. Cell lysates were assayed for GSH and SOD. Statistical significance was set at $p < 0.05$ using one-way ANOVA, Tukey post-hoc test. Cultured canine chondrocytes continued to produce type II collagen and aggrecan. There was no detectable production of type I collagen. Activation significantly increased GSH levels by 37% and decreased SOD levels by 50%. Activation also significantly increased production of PGE-2, a pain producing substance. Pretreatment with SPC significantly reduced GSH levels, increased SOD activity, and decreased PGE-2 production. The present study demonstrates that the canine chondrocyte model maintains the cartilage phenotype and respond to cytokines. Here we demonstrate that oxidative stress and inflammation can be induced by cytokines and suppressed by SPC. The chondrocyte culture model helps clarify cellular mechanisms involved in oxidative stress and inflammation. Moreover, this model may be used to screen potential agents that minimize induction of oxidative stress and inflammation in cartilage.

ABSTRACT #366

ASSOCIATION BETWEEN BIOCHEMICAL VALUES FOR SAMPLES COLLECTED USING VACUTAINER™ AND PEDIATRIC SAMPLING TUBES. SR Shadwick, JC Whittemore. Department of Small Animal Clinical Sciences, University of Tennessee, College of Veterinary Medicine, Knoxville TN.

Anemia caused by diagnostic sampling is the main indication for blood transfusion in human neonates. Diagnostic sampling may pose the same risk to dogs and cats given their size. Pediatric sampling tubes may be used to decrease sampling volumes, but their accuracy under clinical conditions has not been reported.

Samples were collected from 15 clinically healthy dogs and 15 clinically healthy cats. Three mL of blood were collected from each animal and transferred into lithium heparin Vacutainers™ (2.5 mL) and pediatric tubes (0.5 mL) by one individual blinded to the study purpose. Complete biochemical profiles were performed within 12 hours on all samples. Results were compared by paired t-test using SPSS 15.0 for Windows. A p-value of <0.01 was considered significant.

There were no clinical or statistical differences for feline samples. For canines, statistically significant differences were present for eight parameters. Results were the same or higher for pediatric tubes for ALP activity, calcium, total protein, albumin, globulin, and cholesterol. None of these differences was clinically significant. Glucose values were the same or lower on pediatric tubes with a mean difference of 7.1 mg/dl.

These results suggest that pediatric sampling tubes provide clinically equivalent biochemical data to Vacutainers™ while minimizing total diagnostic sampling volumes. Excluding glucose results in dogs, results for both collection tubes may be evaluated using the same reference intervals. For glucose monitoring, use of the same collection method may be warranted. Comparison of complete blood count parameters is ongoing.

ABSTRACT #367
QUANTITATIVE MYOCARDIAL IMAGING (X-STRAIN™) AND RIGHT VENTRICULAR FUNCTION IN ADULT DAIRY CATTLE. GD Hallowell[1] and TJ. Potter[2]. [1]Shepshed, Leicestershire, UK. [2]Royal Veterinary College, London, UK.

X-strain™ is a novel technique that allows determination of myocardial strain (S) and strain rate (SR) for assessment of regional myocardial function that is angle independent. This makes it an ideal tool for the evaluation of the right ventricle (RV) as it rotates on its axis in a non-uniform manner when it contracts. The technique has not previously been applied to bovine echocardiography and may enable better evaluation of RV cardiovascular function in clinical patients with cor pulmonale, myocarditis or pericarditis. The aims of this study were to report normal values and assess repeatability for right ventricular myocardial tissue velocities (TV), S and SR in healthy adult dairy cows. Eight healthy adult Holstein Friesian cattle (656 ± 11 Kg) were recruited and examined on three consecutive days. Standard echocardiographic images were obtained and offline analysis of RV function was performed using three cardiac cycles of the right parasternal long axis projections optimised to show either the left ventricular outflow tract (LVOT) or left ventricle (LV) and using a short axis projection at the level of the mitral valve (MV). Basal, mid and apical regions of the right ventricular free wall and right, mid and left regions of the interventricular septum (IVS) were compared both on the same view and on comparable views (LVOT and LV view). Data, shown as mean (±SEM), were normally distributed and analysed using a repeated measures ANOVA and Student's T-test where appropriate. Where no significant differences were found between regions/projections, data were combined. Repeatability was determined using intra-class correlation coefficients (ICC). Measures of free wall function were not affected by region and were consistent between the two different long axis projections (TV 4.5 ± 0.73 cm/s, S $-16 \pm 4.1\%$ and SR $-1.29 \pm 0.23\ s^{-1}$). IVS S and SR were not affected by region (S $-12 \pm 2.6\%$ SR $-1.25 \pm 0.61\ s^{-1}$) although IVS TV differed between the regions assessed (basal 6.8 ± 0.59 cm/s, mid 3.81 ± 0.88 cm/s and apical 2.53 ± 0.59 cm/s). All measures of IVS function were consistent between the two different long axis projections. With the exception of SR of the basal free wall all X-strain measures demonstrated good repeatability (ICC > 0.85). This technique is repeatable and values are comparable for those reported for the human right ventricle. X-strain may provide a superior method for assessing RV function in cattle with different forms of cardiac disease compared to 2D echocardiography. Evaluation in the clinical setting is warranted.

ABSTRACT #368
PLASMA INSULIN-LIKE GROWTH FACTOR-1 CONCENTRATION IN HORSES WITH EQUINE PITUITARY PARS INTERMEDIA DYSFUNCTION. Dianne McFarlane. Department of Physiological Sciences, Oklahoma State University Center Veterinary Health Sciences, Stillwater, OK.

Insulin-like growth factor (IGF-1) is a pluripotent hormone with a role in growth, metabolism, and cell survival. IGF-1 has been shown to protect neurons from toxic and inflammatory-induced damage and IGF-1 deficiency has been reported to occur with neurodegenerative disease. Equine pituitary pars intermedia dysfunction (PPID) is a neuroendocrine disease that results from dopaminergic neurodegeneration. We hypothesized deficiency in IGF-1 contributes to development of PPID. The aim of this study was to determine the plasma IGF-1 concentration in equids with PPID.

Plasma was collected from animals with or without clinical evidence of PPID. Disease status was confirmed by plasma α-MSH concentration or postmortem examination. IGF-1 was determined by immunoradioassay, insulin-like growth factor binding protein-3 (IGFBP-3) by ELISA. Relative IGF-1 was calculated [IGF-1 (nmol/L)/IGFBP-3 (nmol/L)]. Mean results from animals with PPID were compared to controls by t-test.

Plasma IGF-1 and IGFBP-3 concentration was greater in normal ponies compared to normal horses (IGF-1: 437 ± 140 ng/ml, n = 25 versus 249 ± 105 ng/ml, n = 30, $P < 0.001$; IGFBP-3: 794 ± 292 ng/ml, n = 25 versus 419 ± 317 ng/ml, n = 30, $P < 0.0001$). Plasma IGF-1 concentration in horses with PPID was significantly greater than in healthy horses (442 ± 210 ng/ml, n = 25 versus 249 ± 105, n = 30 ng/ml, $P < 0.01$). While relative IGF-1 was greater in horses with PPID, it failed to reach significance ($P = 0.08$). Plasma IGFBP-3 concentration was similar in healthy and PPID horses. There was no difference in any IGF-related measurement in ponies with PPID compared to healthy ponies. These unexpected findings warrant further investigation to determine the role of IGF-1 in horses with PPID.

ABSTRACT #369
^{13}C PHENYLALANINE FOR ASSESSMENT OF HEPATIC FUNCTION IN HORSES. N. Sasaki, N. Tsuzuki, H. Yamada. Department of Veterinary Clinical Science, Obihiro University of Agriculture and Veterinary Medicine, Japan.

It is difficult to establish a definitive diagnostic method for hepatitis in horses as this condition is similar to other colicky diseases. Based on its metabolic pathway, ^{13}C Phenylalanine (L-[1-^{13}C] Phenylalanine) is expected to be a substratum for diagnose of liver diseases. However, basic study on ^{13}C Phenylalanine breath test in horses has not been undertaken. In this study, we examined the validity and experimental conditions for use of ^{13}C Phenylalanine breath test in horses.

Six healthy thoroughbreds (1 stallion, 3 mares and 2 geldings) were dosed with 1 ml/kg of distilled water and 2.5 mg/kg, 5 mg/kg, or 10 mg/kg ^{13}C Phenylalanine (Tokyo Gas Chemical Corporation, Japan) through a nasogastric tube. Breath samples were collected at 5 minute intervals for 20 minutes, at 10 minute intervals for 60 minutes, at 15 minute intervals for 180 minutes, and at 30 minutes intervals for 240 minutes after dosing. Analysis of breath samples was performed using a spectrophotometer (POCone, Otsuka Electronics Corporation, Japan). The time corresponding to maximum $\Delta^{13}CO_2$ in $\Delta^{13}CO_2$ graph was marked as t_{max} and maximum $\Delta^{13}CO_2$ was marked as C_{max}.

Two horses, suspected to have hepatic disease by serum biochemical analysis, were considered as cases (case 1 and case 2) and were dosed with ^{13}C Phenylalanine at 10 mg/kg. Breath test in these two cases was carried out under the same conditions as for healthy horses, except for the dosage.

In both the 5 mg/kg and 10 mg/kg groups, clear maximum $\Delta^{13}CO_2$ was observed earlier than the respective healthy horses under the test. However, in the 2.5 mg/kg group t_{max} points were scattered over test time horizon, and no distinctive maximum $\Delta^{13}CO_2$ was observed. Standard deviation of C_{max} in the 5 mg/kg group was lower than that in the 10 mg/kg group.

In case 1, t_{max} was not distinct from that obtained for healthy horses. However, t_{max} in case 2 appeared little lower than that observed for healthy horses. C_{max} in case 1 was remarkably lower than that of healthy horses. In addition, $\Delta^{13}CO_2$ values and cumulative $^{13}CO_2$ output for both case 1 and case 2 were lower than those of healthy horses over the test time.

In the 5 mg/kg and 10 mg/kg groups, clear maximum $\Delta^{13}CO_2$ was observed, and C_{max} in the 5 mg/kg group had a standard deviation lower than that in the 10 mg/kg group. These findings indicate that 5 mg/kg is the appropriate dose of ^{13}C Phenylalanine for breath test in horses. During the test, distinctive t_{max} and decreased $\Delta^{13}CO_2$ (after a peak) were observed. Thus, the time framework set for the test was judged to be sufficient. Reference values of t_{max} and C_{max}

for healthy horses under this test were 32.5 ± 14.6 min and 32.1 ± 11.0‰, respectively.

Case 1 had lower C_{max} than those of the healthy horses, and both case 1 and case 2 showed cumulative $^{13}CO_2$ output lower than those of the healthy horses. This implies that C_{max} and cumulative $^{13}CO_2$ output could be considered as symptomatic indicators for liver disease.

ABSTRACT #370
PERITONITIS SECONDARY TO UTERINE TEARS IN POSTPARTUM MARES: RETROSPECTIVE COMPARISON OF SURGICAL VERSUS MEDICAL TREATMENT. LH Javsicas,[1] NM Slovis,[2] DE Freeman[1], and S Giguère[1]. [1]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL; [2]McGee Medicine Center, Hagyard Equine Medical Institute, Lexington, KY.

Peritonitis secondary to uterine tears is a common problem in mares during the first week postpartum. Surgery has been suggested to be the best option for preservation of the mare's life and breeding soundness. Due to the risks associated with anesthesia and the potential additional costs incurred, a critical comparison of medical and surgical treatment of postpartum peritonitis mares is warranted. The objectives of this retrospective study were to determine the factors associated with survival in mares with peritonitis secondary to uterine tears and to compare the value of medical versus surgical treatment. Forty-nine postpartum mares diagnosed with peritonitis based on abdominocentesis or with a uterine laceration confirmed at surgery were included in the study. Mares with rupture of a gastrointestinal viscus or vaginal lacerations were excluded. Information on age, breed, physical examination findings, laboratory testing, abdominocentesis, treatment, breeding performance following discharge, length of hospital stay, and hospital bill was obtained from each medical record. The overall survival rate was 75.5%. In the univariable analysis, nonsurvivors were significantly more likely to have gastric reflux, a higher heart rate and anion gap, and a lower total CO_2 and leukocyte count, compared to survivors. Fourteen mares were treated medically and thirty-four were treated surgically. Admission variables, survival rate, hospital bill, duration of hospital stay, and likelihood to foal following discharge were not significantly different between mares treated medically or surgically. Based on these results, medical treatment of postpartum mares with peritonitis should be considered a reasonable alternative to exploratory laparotomy.

ABSTRACT #371
IN VITRO EFFECTS OF LACTIC ACID ON BIOELECTRIC PROPERTIES OF EQUINE NONGLANDULAR SQUAMOUS MUCOSA. B. Buchanan[1], F. Andrews[1], S. Elliott[1], R.Al. Jassim[2], C. McGowan[2], A. Saxton[1]. [1]University of Tennessee, College of Veterinary Medicine, Knoxville, TN. [2]University of Queensland, School of Animal Studies, Gatton Campus, QLD, Australia.

Gastric ulcers of the nonglandular (NG) squamous mucosa are common in horses. Volatile fatty acids (VFAs), byproducts of carbohydrate fermentation by resident bacteria, have been implicated in causing NG gastric ulcers. Lactic acid (LA), also produced by bacteria, may cause ulcers when exposed to the equine NG mucosa.

The purpose of this study was to investigate the *in vitro* effects of LA on tissue NG mucosal bioelectric properties, sodium transport and tissue resistance. Gastric tissues obtained from 13 horses were studied in Ussing chambers. Short-circuit current (Isc) and potential difference (PD) were measured and electrical resistance (R) and conductance (G) calculated for tissues after addition of HCl and various concentrations of LA (5, 10, 20, or 40 mM) in normal Ringer's Solution (NRS). Permeability to [^{14}C] Mannitol was measured in 2 additional horses. Tissues were examined for histopathologic changes after routine staining with H&E.

Mucosa exposed to HCl in NRS at pH 1.5 and 4.0 had a significant decrease in Isc and PD, whereas or tissues exposed to LA (5, 10, and 20 mM) in NRS did not show a significant decrease in Isc and PD, more than what was seen with HCl exposure alone. However, mucosa exposed to 40 mM lactic acid in NRS, at pH 1.5, tended to show an increased G, decreased R and increased permeability to [^{14}C] Mannitol. Histologic changes were consistent with HCl-induced (pH < 4.0) acid damage.

HCl induced alteration in bioelectric properties of equine NG mucosa, whereas addition of LA (5, 10, 20 mM) did not induce alter tissue bioelectric properties NG mucosa, other than that seen with HCl exposure alone. However, a high concentration of LA (40 mM) exposure at pH 1.5, increased NG tissue permeability, without significantly altering sodium transport in tissues. Lactic acid, produced by resident bacteria in the stomach of horses fed a high-grain diet, may increase equine NG mucosal permeability by a different mechanism than VFAs, which may be important in the pathogenesis of Equine Gastric Ulcer Syndrome (EGUS).

ABSTRACT #372
THE EFFECT OF SEABUCKTHORN EXTRACT IN THE TREATMENT AND PREVENTION OF GASTRIC ULCERS IN HORSES. R. Reese[1], F. Andrews[1], S. Elliott[1], A. Saxton[1], R.B. McMullin[2]. [1]University of Tennessee, Knoxville, TN; [2]Seabuck LLC, Midvale, UT.

Non-glandular (NG) gastric ulcers are common in horses. Current pharmaceutical treatments are expensive and alter the acidic environment of the stomach. Seabuckthorn (*Hippophae* rhamnoides) has been shown to be useful for the treatment of gastric ulcers in humans. The purpose of this study was to evaluate the efficacy of a commercially sold extract of seabuckthorn berry (SBC: SeaBuck™ Complete Liquid) in the treatment and prevention of gastric ulcers in horses.

This study was a blinded 2-period cross-over design using 8 adult female horses. Each treatment period consisted of a control group, that received feed only, and a treated group that received SBC (3oz) mixed with feed twice a day. Horses were treated for 60 days and then subjected to an alternating feed-deprivation period to induce or worsen ulcers. Gastroscopy was performed on each horse before each treatment period (day 0), on day 30, day 60, and following the alternating feed-deprivation period (day 67). Gastric juice was aspirated and pH was recorded along with overall NG gastric ulcer score, NG ulcer severity score, and NG ulcer number score. Between each treatment period the horses had a 4-week washout period. All horses received the two treatments. The data was analyzed using a cross over ANOVA model with significant differences considered, $P < 0.05$.

Mean overall NG gastric ulcer score decreased on Day 30 in both groups compared to ulcer scores on Day 0, whereas ulcer scores increased on Day 60 in the SBC-treated group, compared to the control group. However, on Day 67 after the alternating feed-deprivation period, overall NG gastric ulcer score increased in the control group from 1.1 to 2.0, whereas the mean gastric ulcer score in the SBC-treated group remained the same. Mean NG ulcer number and severity decreased in both groups on Day 30, compared to Day 0 and increased on Day 60 in both groups. However, after the feed-deprivation period, mean NG ulcer number and severity stayed the same for the SBC-treated group, while the mean NG number and severity scores increased in the control group. The SBC did not effect the acidic environment of the stomach, as mean gastric juice pH was 2.32(±.24) in the SBC-treated group compared to 2.25(± .26) in the control group. During, the feed-deprivation period, ulcer scores improved or stayed the same in 7/8 (88%) SBC-treated horses, compared to 2/8 (25%) of the control horses.

While trends were seen, SBC did not significantly decrease NG gastric ulcer score, number, or severity when compared to untreated controls. SBC was not effective in the treatment of NG gastric ulcers in horses in this study, but trends showed it aided in preventing worsening of gastric ulcers during the feed-deprivation period. Thus, SeaBuck™ Complete Liquid has been found to be efficacous in the prevention of gastric ulcers during stress without altering normal stomach pH.

ABSTRACT #373
USE OF A WIRELESS CAPSULE, SMARTPILL™, TO MEASURE GASTROINTESTINAL PH, PRESSURE AND TRANSIT TIME IN A HORSE. S. Elliott[1], R. Reese[1], R. Denovo[1], D. Barthel[2], M. Lyman[2], G. Daniel[1], F. Andrews[1]. [1]University of Tennessee, Knoxville, TN; [2]SmartPill Corporation, Buffalo, NY.

Currently methods such as nuclear scintigraphy, gastric cannulation, and indwelling pH probes, for evaluating gastric emptying

(GE), gastric and intestinal pH, and luminal pressure in horses are invasive and costly. Recently, a wireless ambulatory capsule technology, the SmartPill™ GI Monitoring System (SP), was introduced to measure gastrointestinal (GI) pH, pressure and temperature, and provide GE and transit time in humans. The purpose of this study was to evaluate the SmartPill for measurement of intraluminal GI pH, pressure, and temperature, GE time, small and large bowel transit time (SLBTT) and total transit time (TTT). Also, SP GE study was compared to gastric emptying scintigraphy (GES).

A 12-year-old female mixed breed horse weighing 454 kg was used to evaluate the SP technology. The evening before the SP study at 8:00 PM, a muzzle was placed and feed withheld. The following morning, the SP was preloaded in the end of a nasogastric tube and SP administered into the esophagus. An enhanced wireless data receiver was attached to a girth strap and gastrointestinal pH was collected every 5 seconds for 24 hours, along with luminal pressure and temperature until the SP was passed in the feces. Once the SP was expelled from the horse, feed was withheld and the horse was again muzzled overnight. The following morning the horse was fed a radioactive meal (20mCi, 99mTcDisofenin) mixed with 1 kg of a complete pelleted feed (Purina® Horse Chow). Once the meal was consumed GES was performed in a standard manner for 4 hours.

Sixty-eight percent of the data packets were received from the SP during the study period and the SP was recovered intact from the horse's feces. GE time measured by the SP and GES ($T_{7\%}$) were 10.3 h and 4.1 h, respectively. The TTT of the SP was 46.8 h and SLBTT was 36.5 h. Gastric pH ranged from less than 1 to 7.5. The pH increased to 8 in the small intestine and abruptly decreased to pH 7.0, 23.3 h after administration. pH remained 7.0 until the SP was excreted from the horse. GI temperature ranged from 37.5 °C in the stomach to 38.5 °C in the large intestine. Rectal temperature taken daily during the study averaged 37.2 °C

The GE measured by the SP was substantially longer than GE measured by the GES, which was probably due to the larger size and weight of the SP compared to the pelleted feed. The TTT (46.8 h) measured by the SP was similar to previous reports in horses, where TTT was 40 h. Gastric juice pH and pressure profiles were similar to previous reports. Rectal temperature was lower than GI temperature measured by the SP, which was expected. The SmartPill was easy to administer and shows promise as an ambulatory method for measuring GI pH, temperature, pressure, gastric emptying time and bowel transit time in health and disease.

ABSTRACT #374
USE OF ULTRASOUND TO EVALUATE OUTCOME FOLLOWING COLIC SURGERY FOR EQUINE LARGE COLON VOLVULUS. MK Sheats, VL Cook, SL Jones, AT Blikslager, AP Pease. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Large colon volvulus (LCV) is one of the most fatal forms of colic. In horses with LCV, diagnosis can be made pre-operatively using ultrasound to identify edema in the wall of the ventral colon; however, the post-operative response of the equine large colon wall after surgically corrected LCV has not been investigated. This study was designed to test the hypothesis that a longer time to colon wall involution following surgical correction of large colon volvulus would correlate with an increased rate of post-operative morbidity and mortality. Transcutaneous abdominal ultrasound was used to determine colon wall involution. Outcome was evaluated by comparing time to colon wall involution between 1) survivors and non-survivors and 2) horses that developed multiple organ dysfunction syndrome (MODS) during the post-operative period to those that recovered without evidence of MODS. Multiple organ dysfunction was identified in any horse having evidence of compromise in 2 or more organ systems.

Horses that were admitted to the North Carolina State University Veterinary Teaching Hospital between September 1, 2006 and September 5, 2007, had surgical correction of LCV and were recovered from anesthesia were included in this study. Ultrasonographic examination of the ventral large colon was performed at the time of anesthetic recovery and then every 6 to 8 hours until the colon wall returned to normal thickness (<5 mm). Images were obtained approximately 2 cm caudal to the xiphoid using a Megas ES ultrasound machine and an 8–5 MHz curvilinear probe. Physical examination was recorded every 6 hours for the first 72 hours, and then every 8 hours for the duration of hospitalization. Complete blood count and serum biochemistry were collected every 48 to 72 hours. Owner permission was obtained for participation in the study and post-operative management was at the discretion of the attending clinician. A 1-way ANOVA was used to compare colon wall involution time between the designated groups of horses.

Fifteen horses met the initial inclusion criteria. Two horses were excluded due to euthanasia prior to colon wall involution. Of the 13 remaining horses, 5 developed MODS during hospitalization and 8 did not. Three of the 5 horses diagnosed with MODS were either euthanized or died postoperatively, while 2 survived to discharge. The 8 horses that did not develop MODS all survived to discharge. Horses that recovered without evidence of MODS had a significantly shorter time to colon wall involution ($<$ 5 mm) compared to horses diagnosed with MODS (Mean $\pm$ SEM: 19.2 hours $\pm$2.8 vs. 40.8 hours $\pm$ 6.7 respectively, $P = 0.006$). There was no significant difference in mean time to colon wall involution between survivors and non-survivors.

A shorter time to colon wall involution was positively correlated with decreased postoperative morbidity in horses presented for surgical correction of large colon volvulus without resection.

ABSTRACT #375
THROMBOELASTOGRAPHY (TEG) FOR EARLY DETECTION OF HEMOSTATIC ABNORMALITIES IN HORSES WITH COLIC. J Mendez, P Vilar, M Mudge, G Couto. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Hemostatic changes are common in horses with colic, and frequently result in secondary complications such as disseminated intravascular coagulation (DIC), thrombophlebitis, or laminitis. Recently, horses with severe gastrointestinal disorders had widespread intravascular fibrin deposits, with evidence of hypercoagulation and reduced fibrinolysis (Cotovio et al. 2007). Thrombelastography provides data about the entire hemostatic system, from the beginning of coagulation through clot formation, and fibrinolysis. The purpose of this study was to evaluate and compare the hemostatic parameters using TEG in healthy horses to those in horses diagnosed with colic.

Blood samples were collected from horses with colic ($n = 25$) upon admission to the emergency service. Samples were collected into 2.7 ml Vacutainer tubes containing 3.2% buffered sodium citrate; they were placed in the thrombelastograph[a] (TEG)® cup and recalcified by adding $CaCl_2$ 30–120 minutes after sampling. A group of healthy horses ($n = 25$) based on normal physical examination and clinical pathology were used as controls in this study. CBC, plasma fibrinogen concentration, prothrombin time (PT), and activated partial thromboplastin time (aPTT) were performed. Specific TEG parameters including R-time, K-time, angle, MA, "G" value and LY60 value were compared between groups.

There were significant differences ($p<0.05$) in TEG parameters between the horses with colic and the control group; horses with colic had higher platelet count, higher fibrinogen concentration, shorter "K-time", wider angle "α", and increased maximum amplitude (MA) and "G" value, changes consistent with hypercoagulability. However, there were no significant differences in fibrinolysis, as evaluated by the "LY60" value.

In conclusion, thromboelastography can be a useful test to detect early hemostatic changes such as tendency to hypercoagulability in horses with colic.

ABSTRACT #376
NORMAL TEG® PARAMETERS IN THE HORSE: EX VIVO EFFECTS OF TISSUE FACTOR. BM Brainard, MAF Lopes, SR Hutsell, KL Epstein, MH Barton, JN Moore. University of Georgia, Athens, GA.

Hypocoagulability is associated with a poor prognosis in horses presenting with colic. Thrombelastography (TEG®) is a stall-side test that can be used to rapidly evaluate citrated blood samples for evidence of coagulopathy. Tissue factor (TF) may be added to speed the initiation of clot formation for TEG analysis. TEG was evaluated in citrated blood from 16 normal horses 30 minutes after collection using TF-activated (1:100 in 4% bovine albumin) and non-TF activated protocols. The median (range) values for TF-TEG were R =

7.2 (3–10) min, K = 2.8 (1.5–5) min, Ang = 55 (40–67) deg, and MA = 62 (53–68) mm. The median (range) values acquired for non-TF TEG were R = 14 (9–25) min, K = 3.6 (2–10) min, Ang = 48.1 (28–64) deg, and MA = 59 (51.6–69) mm. R values were significantly different between TF and non-TF TEG protocols ($p < 0.01$), illustrating the effect of TF on initiation of coagulation. The remaining TEG variables were not significantly different between the two protocols, indicating that either technique is suitable for evaluating these characteristics. R values in both groups were significantly different between two operators (and cohorts of horses; $p = 0.01$). Non-TF K and Ang values were also different between operators ($p < 0.01$), a distinction that was not true for the TF-activated parameters. It is prudent to perform both TF- and non-TF-activated TEG when evaluating horses for coagulopathies. Use of TF as activator may minimize inter-operator variability.

ABSTRACT #377
PROINFLAMMATORY CYTOKINE GENE EXPRESSION BY EQUINE EPIDERMAL EPITHELIAL CELLS UPON EXPOSURE TO BACTERIAL COMPONENTS IN VITRO. B Leise, C Yin, JK Belknap. The Ohio State University College of Veterinary Medicine, Columbus, OH.

The host response to bacterial components involves the innate immune system through recognition of, pathogen-associated molecular patterns (PAMPs). These PAMPs are recognized by different pattern recognition receptors (PRRs), such as the toll-like (TLR) and NOD receptors present on the surface (and cytoplasm) of the host cells; subsequent activation of these receptors can result not only in a protective response, but commonly in inflammatory injury to the host cells and tissue. The response to various bacterial ligands has been well documented in human epidermal and visceral (i.e. renal, intestinal) epithelial cells, where inflammatory signaling due to PRR binding plays an important role in both superficial and systemic sepsis. However, little information exists regarding equine epithelial cells and their response to bacterial ligands and toxins. The epidermal epithelial cell response is not only important clinically from the perspective of skin infection or wound healing in the horse, but is also important in laminitis where the laminar epidermal epithelial cells are at the point of failure in laminitis. Therefore, we investigated the response of equine epidermal epithelial cells to bacterial components likely to be present in the septic equine patient at risk of sequelae such as laminitis. Equine epidermal epithelial cells were cultured from skin of horses euthanized for reasons other than systemic illness; cells (passage 3-4) from two horses were exposed to lipopolysaccharide (LPS, TLR4 ligand; 100 ng/ml, 500 ng/ml, and 5 μg/ml), gram positive peptidoglycan (PGN, NOD2 receptor ligand;100 ng/ml, 500 ng/ml, 5 μg/ml, and 10 μg/ml), lipoteichoic acid (LTA, TLR2 ligand; 1 μg/ml and 10 μg/ml), bacterial DNA (CpG, TLR9 ligand; 1 uM), gram negative flagellin (100 ng/ml, TLR5 ligand; 500 ng/ml, and 5 μg/ml) or maintained in media (controls) for 4 and 24 hours. Conditions were performed in triplicate. After incubation, epithelial cells were harvested and RNA isolated to quantify interleukin-1β, interleukin-6 and interleukin-8 mRNA expression via real-time PCR. Significant ($P < 0.05$) increases in IL-1β, IL-6, and IL-8 gene expression were present after stimulation with LPS when compared to controls. Increased expression of both IL-6 and IL-8 were also noted in response to flagellin stimulation when compared to control culture. Overall responses were greatest at 4 hours. No significant increases in cytokine expression in epidermal epithelial cells were present after stimulation with PGN, LTA, or CpG. The cytokine expression present after stimulation of equine epithelial cells by LPS is similar to reports in the human literature. However, the negative response to gram positive ligands is not consistent with responses of human keratinocytes in culture. These findings are consistent with the observations that, in regards to sepsis-related sequelae such as laminitis, horses are much more sensitive to gram negative sepsis and bacterial toxin systemically compared to gram positive sepsis.

ABSTRACT #378
MATRIX METALLOPROTEINASES AND STRUCTURAL TISSUE DAMAGE IN EQUINE LAMINITIS. JP Loftus[1], P Johnson[2], C Yin[3], JK Belknap[3] SJ. Black[1]. [1]University of Massachusetts, Amherst, MA; [2]University of Missouri, Columbia, MO; [3]Ohio State University, Columbus, OH.

Equine Laminitis affects approximately 1% of horses in the United States, causing both acute and chronic lameness. The acute case is characterized initially by pain, which is associated with inflammation but little to no derangement of the laminae. As the disease progresses to chronic laminitis, the integrity of the lamellae becomes impaired, resulting in distal displacement of the third phalanx. A prominent hypothesis for the pathogenesis of the disease is that activation of matrix metalloproteinases (MMPs) leads to destruction of the extracellular matrix components that lend structural integrity to the lamellae. The purpose of the study was to use zymography and histologic techniques to investigate the concentrations and localization of MMP-2 and MMP-9 in laminar samples from both experimental and clinical cases of laminitis. Samples from three categories of laminitis were examined: i) laminitis induced with a gastric bolus of starch-grain gruel (CHO; Obel Grade 3, n = 7), ii) clinical chronic (CHR, n = 4) and iii) clinical chronic aggravated (CHR-AG, n = 5) laminitis and were compared to controls (n = 4). Protein extracts and thin sections of lamellar tissue were analyzed by gelatin zymography and (immuno) histochemistry, respectively. Pro-MMP-9 was elevated in most samples from the CHO, acute and CHR-AG groups. Pro-MMP-2 was elevated most consistently in the CHO and CHR-AG groups and was accompanied by increased processing to MMP-2. When zymography results were compared with H&E stained sections from the same individual, there was no overall association between elevated MMP and the extent of tissue pathology. Interestingly, in CHO-induced laminitis, MMP-2 levels were generally positively associated with tissue pathology. In contrast, in horses suffering from chronic forms of the disease MMP-2 levels appeared to have an inverse association with tissue pathology. Thin sections of laminae from 3 CHO treated horses and 3 controls were stained with an anti-equine MMP-9 mAb, which predominantly stained leukocytes (primarily neutrophils). There was no obvious association between the presence of MMP-9 positive cells and areas of overt tissue damage. These results suggest that i) MMP-2 may be involved in mechanisms of tissue repair as well as mechanisms of pathology, ii) MMP-9 is unlikely to play a direct role in gross tissue damage and iii) careful consideration of MMP inhibitors is warranted, as their therapeutic use may have deleterious effects with respect to tissue remodeling and wound healing.

ABSTRACT #379
CLONING OF EQUINE CD40 LIGAND: AN IMPORTANT MOLECULE IN THE REGULATION OF CELLULAR & HUMORAL IMMUNE RESPONSES. BA Sponseller, SK Clark, JM Hostetter, DE Jones. College of Veterinary Medicine, Iowa State University, Ames, IA.

CD40 Ligand, or CD154, is a 33 kD, type II transmembrane protein with sequence homology to the tumor necrosis factor (TNF) superfamily of cell surface signaling molecules. It is expressed on activated B and T lymphocytes, natural killer cells, mast cells, and dendritic cells. CD154 binds to CD40, a 50 kD glycoprotein, also a member of the TNF receptor superfamily. The CD40 receptor is expressed on the surface of immune cells including monocytes, thymic epithelial cells, B lymphocytes, and dendritic cells. Binding of CD154 with CD40 contributes to the activation of antigen presenting cells. Without CD154, antigen presentation by both dendritic cells and macrophages is markedly impaired, as is macrophage-mediated killing of both intracellular and extracellular pathogens. Dendritic cell production of IL-12, an important cytokine in driving a type I response, is induced by the CD154/ CD40 interaction. Type II responses are elicited when CD154 binds cell surface CD40 on mature B cells, resulting in B cell activation. Recent studies comparing expression of CD154 between human adults and neonates have revealed that there is a decrease in expression of CD154 by neonatal CD4+ T lymphocytes, suggesting that decreased expression of CD154 may play a role in acquired diseases of the neonate. Given the importance of the CD40/CD154 interaction, we initiated studies to characterize equine CD154 in order to determine its role in maturation of immune responses in the horse. Here, we report the cloning, sequence analysis, and expression of the equine CD154 in Chinese hamster ovary (CHO) cells and characterization of the recombinant protein by Western blot analysis. Sequence alignments of the translated equine sequence revealed distinct regions of homology with those of ovine, bovine, porcine, feline, canine, murine and human species. Similar to other species, equine CD154

also has a hydrophobic signal anchor region typical of type II membrane proteins. In contrast to human CD154, which contains 2 potential N-linked glycosylation sites, equine CD154 contains but one site, with a similar location is described in other domestic species. In the horse it is unknown as to whether or not the putative N-glycan sites are glycosylated. Nonetheless, the observed cross-reactivity of a murine monoclonal antibody to human CD154 in Western blot analysis suggests that the extracellular protein domain shares considerable homology with human, porcine, and murine CD154. The cloning and sequence analyses of equine CD154 provide the basis for future studies of equine neonatal immunology with regard to intracellular and extracellular infections.

ABSTRACT #380
MOLECULAR BASIS FOR ESCAPE OF EQUINE INFECTIOUS ANEMIA VIRUS FROM TYPE-SPECIFIC NEUTRALIZING ANTIBODY. BA Sponseller, RA Friedrich, SK Clark. College of Veterinary Medicine, Iowa State University, Ames, IA.

Equine infectious anemia virus (EIAV) is a lentivirus that causes a lifelong infection of equids. While surveillance efforts have reduced the frequency of disease in the United States, absence of an eradication program requiring testing of all horses results in maintenance of viral reservoirs. It has been estimated that the annual cost of surveillance to horse owners approaches US $50 million. Infection with EIAV typically results in recurrent episodes of fever, thrombocytopenia, inappetance and depression during the acute and chronic stages of infection. While EIA is fatal in some animals, most eventually control viremia and become inapparent carriers. A hypervariable region (V3) of the viral envelope surface glycoprotein (SU) contains epitopes recognized by neutralizing antibodies and is termed the Principal Neutralizing Domain (PND). From a pony experimentally infected with the Wyoming wild-type virus, we previously characterized genetic variation within the PND. In addition, we characterized the neutralizing antibody response throughout infection. Using chimeric infectious clones, it was demonstrated that a type-specific antibody response occurred with transition from the acute to the chronic stage of infection. Also, viral escape from the type-specific response was associated with 3 amino acid changes within the PND. In this study, we used site-directed mutagenesis to develop a panel of infectious molecular clones in order to elucidate the molecular basis for escape from the type-specific neutralizing antibody response. We determined that a single, putative asparagine (N)-glycan in the upstream region of the PND conferred resistance to the autologous neutralizing antibody response observed, but that presence of a charged histidine did not contribute to viral escape. N-linked glycans are sugar moieties that are attached to the surface of the viral envelope and aid in cloaking viral epitopes recognized by serum antibodies. Our studies indicated that changes confined to the PND can be sufficient for escape from a type-specific neutralizing antibody response. In addition, viral genotypes derived from other EIAV infected horses that were resistant to neutralization became susceptible with elimination of a single putative N-glycan in the PND. These results suggest that the putative N-glycan located in the upstream region of the EIAV PND is, in fact, glycosylated, and that this site is key to viral resistance to neutralizing antibody. In addition, these results indicate that neutralizing antibody rapidly selects for escape mutants which enhance viral persistence. Ongoing studies within the hypervariable PND are aimed at identifying the epitope(s) shielded by the upstream, putative N-glycan.

ABSTRACT #381
DETECTION AND CHARACTERIZATION OF EQUINE HERPESVIRUS-1 STRAINS IN SUBMANDIBULAR LYMPH NODES AND TRIGEMINAL GANGLIA FROM HORSES USING REAL-TIME PCR. N. Pusterla, S. Mapes. School of Veterinary Medicine, University of California, Davis, CA.

Equine herpesvirus-1 (EHV-1) is an important, ubiquitous equine viral pathogen that causes significant economic losses to the equine industry and produces well-documented syndromes of respiratory disease, abortion, neonatal foal death and myeloencephalopathy. Latency and reactivation are key features of the biology and epidemiology of EHV-1. Functional latency of EHV-1 is routinely established in circulating and lymph node T lymphocytes and in neurons within the trigeminal ganglia. The aim of this study was to detect and to characterize the strains of EHV-1 from submandibular lymph nodes and trigeminal ganglia collected from horses using real-time PCR.

One hundred and eleven horses, two mules and one donkey euthanized for reasons other than acute respiratory or neurologic diseases were included in this study. There were 42 mares, 54 geldings, 7 stallions and 11 foals with ages ranging from 2 months to 39 years (mean 14.3 years). Tissue samples including a piece of submandibular lymph node, and both trigeminal ganglia were collected during post-mortem examination in all animals, while uncoagulated blood samples and nasal swabs were collected from 43 animals prior to euthanasia. All samples were processed for nucleic acid purification within 24 hours of collection and tested for EHV-1 using real-time PCR assays targeting the glycoprotein B (gB) gene and the polymerase (ORF 30) gene. The latter assay allowed the characterization of EHV-1 into neuropathogenic and non-neuropathogenic strains. In order to detect as few as one target gene (gB and ORF 30), each purified DNA sample underwent a precipitation and preamplification step.

None of the 43 uncoagulated blood samples and nasal swabs and none of the 114 submandibular lymph nodes tested PCR positive for the gB gene and the ORF 30 gene of EHV-1. A total of 15 trigeminal ganglia collected from 9 horses tested PCR positive for the gB gene of EHV-1. Six and 3 horses tested PCR positive for the gB gene of EHV-1 in both and only one trigeminal ganglia, respectively. Eight trigeminal ganglia harbored non-neuropathogenic EHV-1 strains, while two trigeminal ganglia tested PCR positive for the neuropathogenic ORF 30 gene. Five trigeminal ganglia tested PCR positive for both the neuropathogenic and the non-neuropathogenic ORF 30 gene of EHV-1.

In conclusion, 7.9% of study horses had molecular evidence of EHV-1 in their trigeminal ganglia. The results provide evidence that trigeminal ganglia serve as a latency site of EHV-1. This was in sharp contrast to the submandibular lymph nodes, since none of them tested PCR positive for EHV-1. Non-neuropathogenic strains of EHV-1 were more commonly detected than neuropathogenic strains in the trigeminal ganglia of the study horses.

ABSTRACT #382
EVALUATION OF AN AIR TESTER FOR THE SAMPLING OF AEROSOLIZED EQUINE HERPESVIRUS-1. N. Pusterla, S. Mapes. School of Veterinary Medicine, University of California, Davis, CA.

Air sampling devices have been developed and are routinely used in pharmaceutical, medical and industrial settings to document the presence of air-borne pathogens. The aim of this study was to evaluate the capability of a commercially available air tester to sample aerosolized equine herpesvirus-1 (EHV-1) for detection by real-time PCR.

A commercial EHV-1 whole virus vaccine (Rhinomune, Pfizer Animal Health, New York, NY, USA) was nebulized at different doses, followed by the collection of air samples and environmental swabs at different distances from the nebulizer (shedding source). Air samples (500 l) were collected using a portable air monitoring system (M Air T, Millipore, Billerica, MA, USA) at 14.5, 9.6, 4.8, 1.5 and 0.5 m from the nebulizer. The principle of the portable air tester used in this study is based on the sampling of a pre-determined air volume through a sieve directly onto a retrievable agar plate. During the entire study event (from the beginning of nebulization to the collection of the last sample) 40 cm rayon swabs (Fox Converting Swabs, Green Bay, WI, USA) previously soaked in PBS were left in the room in a vertical position at 14.5, 9.6, 4.8, 1.5 and 0.5 m from the nebulizer to document environmental contamination with EHV-1. Following the last sample collection, PBS soaked rayon swabs were collected from the gloves, surgical mask, coat and hair from the person performing the experiment. The collected samples (agar plates and swabs) were processed for nucleic acid purification within 1 hour of collection using an automated nucleic acid extrac-

tion system (CAS-1820 X-tractor Gene, Corbett Life Science, Sydney, Australia). All purified DNA samples were assayed for the presence of the glycoprotein B (gB) gene of EHV-1 by real-time TaqMan PCR and the results were expressed as EHV-1 gB gene copies per liter of sampled air or whole swab.

The detection of EHV-1 in air samples and swabs correlated directly with the nebulized dose as well as the distance at which the samples were collected. Swabs collected from gloves, mask and coat of the person performing the experiment tested PCR positive for EHV-1 when high doses of EHV-1 were nebulized.

In conclusion, the study results have shown that aerosolized EHV-1 can be sampled from the air via a commercial air tester for real-time PCR detection. The air sampling protocol used for the study will require more validation and optimization for detection limits using naturally or experimentally infected horses in the future. The study has also shown that environmental swabs may capture aerosolized EHV-1 virus and that the virus may be detected from gloves, coat and mask of attending personnel. The risk of contaminated environment and personnel needs to be further investigated as possible transmission source.

ABSTRACT #383
CLINICAL OBSERVATIONS OF EQUINE INFLUENZA IN A NAÏVE FOAL POPULATION. JE Axon, CM Russell, GA Tyner, ME Burbury, JB Carrick. Scone Veterinary Hospital (SVH), Liverpool St, Scone, NSW, Australia.

Equine Influenza (EI) was detected on the first Thoroughbred stud in Scone on September 2, 2007. Patients in the SVH intensive care unit (ICU) became infected and showed clinical signs 2 weeks later. During the ensuing 6 weeks 22 foals were admitted to the ICU with respiratory signs attributed to EI infection. There were estimated to be 4,000 foals and 1000 late pregnant mares in the region exposed to EI. Three syndromes were associated with EIV infection of foals which resulted in hospitalization and treatment. All dams of the foals had clinical signs of EI.

Acute respiratory distress: Seven foals (2–12 days of age) presented with severe dyspnea. Auscultation of the thorax revealed minimal breath sounds or wheezes and crackles over the entire thorax. Thoracic ultrasound revealed numerous comet-tail artifacts which coalesced to form sheets and long thin superficial areas of consolidation. Thoracic radiographs revealed changes typically associated with interstitial pneumonia. Foals had normal or slightly elevated WBC and fibrinogen concentration, and severe hypoxemia with hypo-, normo- or hypercapnia. Treatment included broad-spectrum antimicrobial and anti-inflammatory therapy, oseltamivir phosphate (Tamiflu), nebulization and INO_2 insufflation. All foals that presented with ARDS died. Post-mortem examination confirmed bronchointerstitial pneumonia due to EIV.

Tachypnea without secondary bacterial infection: Fifteen foals (2–12 days) presented with an elevated respiratory rate. The foals were bright, nursing intermittently and had normal or slightly purple discolored mucous membranes. Auscultation of the chest revealed wheezes and crackles over the entire thorax. Thoracic ultrasound and radiographs, clinicopathologic results and treatment regimes were similar to the ARDS foals. All foals responded to treatment and survived.

Bronchopneumonia with secondary bacterial infection: Older foals (>10 days of age) presented with an increased respiratory rate, fever and elevated WBC count and fibrinogen concentration. Wheezes and crackles were audible on auscultation over the entire thorax. Thoracic ultrasound revealed widespread comet-tail artifacts, large areas of consolidation and fluid bronchograms. Radiographs revealed mixed bronchointerstitial pattern and alveolar pattern. Transtracheal aspirates indicated septic suppurative inflammation and culture grew *Streptococcus equi* sp. *zooepidemicus*. Treatment included broad-spectrum antimicrobial therapy, oseltamivir phosphate and INO_2 insufflation. All foals responded well to treatment and survived.

Infection of in-hospital patients: Critically-ill neonates hospitalized during the outbreak developed signs of EIV infection. The majority remained bright and continued to nurse, however the disease was more severe in 6 foals. Three foals died due to complications associated with EI.

Foals born subsequent to the outbreak did not show clinical signs of EI. This may be due to partial transfer of colostral immunity or the lack of in-contact infected horses. No foals discharged from hospital have returned with respiratory problems.

Originally presented at the Rossdale & Partners Foal Care Course (www.lifelearn.co.uk), Newmarket, January 2008.

ABSTRACT #384
SURVIVAL OF *STREPTOCOCCUS EQUI* IN AN OUTDOOR ENVIRONMENT. JS Weese[1], C Jarlot[1], P Morley[2]. [1]University of Guelph, Guelph, Ontario, [2]Colorado State University, Fort Collins, CO.

Streptococcus equi is an important and highly infectious pathogen in horses. Strict infection control measures are required to prevent or control *S. equi*. One aspect that is frequently discussed is survival of *S. equi* on environmental surfaces. Studies performed in laboratories on sterile surfaces have reported prolonged environmental survival of *S. equi*; however, it is unclear whether these results are applicable to the field situation where factors such as sunlight, temperature extremes, variations in temperature and humidity and competing microflora may affect survival. The objective of this study was to evaluate *S. equi* persistence in an outdoor environment on surfaces found on equine farms.

A field strain of *S. equi* was grown in pure culture and suspended in either an equal volume of phosphate buffered saline or mucus collected from the upper respiratory tract of euthanized horses that was determined to be *S. equi*-free. One ml of inoculum was spread onto multiple 15 cm sections of bare wood, painted wood, metal and rubber. Moistened swabs were used to sample the inoculated sites immediately after inoculation (day 0), on day 1, then daily until 2 consecutive negative samples were obtained. Swabs were inoculated onto blood agar for anaerobic and aerobic incubation. Enrichment culture using Todd-Hewitt broth was also performed. Meteorological data were collected daily. Kruskall-Wallis test was used to compare duration of persistence between saline and mucous inoculum groups. Chi-square test was used for categorical comparisons.

Eight replicates were performed between July 5 and September 21, 2007. The mean concentration of the *S. equi* inoculum was 2.1×10^8 colony forming units (CFU)/ml (SD 1.5×10^8, range 1.3×10^7–4.8×10^8). Survival was short term with *S. equi* identified from only 53/64 (83%) samples after 1 day. The longest duration of persistence was 3 days, which occurred in only 4 (6.3%) samples. There was no difference in persistence on different materials ($P = 0.95$), with greater than 1 day survival in only 2/16 (13%) of wood surfaces and 3/16 (19%) metal, rubber or painted wood surfaces. Persistence was longer in *S. equi* inoculated in respiratory mucus compared to saline ($P = 0.02$). When surface materials were combined, there was significantly shorter survival on samples inoculated during sunny periods for both mucus and saline groups (both $P \leq 0.01$).

This study indicates that *S. equi* persists for only a short time in the outdoor environment, at least under these conditions. It is possible that survival could be longer given different weather conditions, and further study is warranted. The longer survival of *S. equi* inoculated in mucus is not surprising as mucus could be both physically protective and provide a nutritional source, and indicates that persistence studies should be performed using organic materials such as mucus that would expected to be deposited with *S. equi* in the natural situation.

ABSTRACT #385
INVESTIGATION OF NEUROLOGIC EQUINE HERPES VIRUS 1 EPIDEMIOLOGY FROM 1984–2007. GA Perkins, LB Goodman, K Tsujimura, GR Van de Walle, SG Kim, E Dubovi, N Osterrieder. Cornell University, College of Veterinary Medicine, Ithaca, NY.

A single nucleotide polymorphism in the equine herpesvirus 1 (EHV-1) DNA polymerase gene (ORF30 A_{2254} to G) is causally associated with clinical signs of equine herpes myeloencephalopathy (EHM) (PLoS Pathog 2007;3:e160). The purpose of our study was to investigate the association of this genetic marker with EHV-1 disease prevalence in field isolates from North America over the past twenty-four years.

EHV-1 isolates cultured at the Cornell University Animal Health Diagnostic Laboratory from 1984–2007 were retrieved along with

their clinical histories. The DNA was prepared and allelic discrimination performed using real-time PCR as described by G. Allen (J Vet Diagn Invest 2007;19:69–72) and confirmed by partially sequencing the ORF30 region. The odds ratio was computed by logistic regression.

There were a total of 179 EHV-1 isolates. About 90% of isolates were from New York (117), Pennsylvania (27) and Virginia (13) and the rest were from 9 other states. The majority of samples were from fetal tissue, placenta and blood. Three isolates were from fatal disease in other species (alpaca, zebra, and fallow deer) and had the mutant ORF30 G_{2254} detected by PCR (3/3) and by sequencing (2/3). These isolates were not included in further analysis. PCR and sequencing were in 100% agreement. There were 11% (19/176) isolates that were defined as the mutant ORF30 G_{2254} by PCR.

Number of EHV-1 Isolates categorized by the ORF30 genotype and clinical sign

	EHM	Respiratory Abortion	
ORF30 G_{2254}	16	3	19
ORF30 A_{2254}	5	152	157
	21	155	176

The odds of having neurologic disease with the ORF30 G_{2254} genotype versus the ORF30 A_{2254} are 162 times greater (95% confidence interval 35–742). Combining our data with the results reported by Nugent et al. (J Virol 2006;80:4047–4060) gives an odds ratio of 490. Despite this strong statistical significance, 24% (5/21) of horses with neurologic disease in our study population had the "non-neurologic" form (ORF30 A_{2254}), suggesting that other factors may also contribute to the onset of EHM.

ABSTRACT #386
SEROLOGIC DIAGNOSIS OF EQUINE BORRELIOSIS AND ANAPLASMOSIS: EVALUATION OF AN IN-CLINIC ELISA (SNAP®4Dx®). R. Chandrashekar, D. Daniluk, C. Esty. IDEXX Laboratories, Westbrook, ME.

Borrelia burgdorferi, the causative agent for Lyme disease, and *Anaplasma phagocytophilum* (Aph) (formerly *Ehrlichia equi*), the causative agent for granulocytic anaplasmosis, infect a wide range of mammalian hosts. Serological studies in horses indicate that incidence of equine *Borrelia* and *Anaplasma* infection is increasing in the northeastern United States, the Midwest, Texas and California. Clinical disease in horses has been associated with lameness, stiffness, joint swelling, lethargy, fever, weight loss, uveitis, and potentially with neurologic disease and foal mortality.

The SNAP®4Dx® assay (IDEXX Laboratories, Westbrook, ME) is a commercially available in-office test kit for the simultaneous detection of antibodies to *B. burgdorferi, A. phagocytophilum, Ehrlichia canis* and *Dirofilaria immitis* antigen in blood, plasma or serum of dogs. The test kit is an ELISA that uses synthetic peptides, C6 derived from the IR6 region within the *Borrelia* membrane protein VlsE, and a peptide derived from the immuno-dominant p44 protein of *A. phagocytophilum*. Studies with canine samples suggested that SNAP 4Dx was useful in endemic areas because it can be conveniently and reliably used in the clinic to determine the infection status of a dog (ACVIM, 2006). We evaluated the performance of SNAP 4Dx for the detection of antibodies to Lyme and Aph in equine serum samples from northeastern United States and Alaska. A total of 164 equine samples obtained from the University of Connecticut were tested by SNAP 4Dx and QualiCode™ *B. burgdorferi* IgG/IgM Western Blot Kits (Immunetics, Cambridge, MA) and Aph immunofluorescence assay (IFA). Of the serum samples tested, 109 were positive for Lyme by SNAP 4Dx and 54 were positive for Aph by SNAP 4Dx. Twenty four samples were co-infected with Lyme and Aph. Of the 109 samples that tested positive for Lyme by SNAP 4Dx, only 106 were positive by Western Blot Assay. The three discordant samples were positive by IFA and had a low reciprocal antibody titer of 64. All the 54 samples that were positive for Aph by SNAP 4Dx tested positive by IFA. Thus, relative to Lyme Western Blot Assay, SNAP 4Dx had a sensitivity and specificity of 100 and 95%, respectively, for the detection of antibodies to Lyme. Relative to IFA, SNAP 4Dx had a sensitivity and specificity of 100% for the detection of antibodies to Aph.

These results indicate that SNAP 4Dx can be successfully used to detect antibodies to *B. burgdorferi* and *A. phagocytophilum* in infected horses.

ABSTRACT #388
EFFECT OF SELENIUM SOURCE ON SELENIUM STATUS AND IMMUNE FUNCTION IN HORSES. J Montgomery, M Wichtel, J Wichtel, M McNiven, F Markham, J Sheppard, J McClure. Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada.

The specific effects of Se source (organic vs. inorganic) on Se status and immune function have not been adequately examined in the horse. We compared measures of Se status and innate and adaptive immunity in horses receiving inorganic, organic or no supplementary Se.

Fifteen Standardbred horses, previously unvaccinated for rabies, were assigned randomly to 3 groups: the Control group received no supplementary Se, while the Inorganic and Organic groups received sodium selenate or a commercial Se yeast product, respectively, at a rate calculated to deliver 0.3 ppm of diet dry matter. Supplementary Se was mixed with barley and fed once daily with the maintenance diet of hay and oats. The basal diet contained $<$ 0.05 ppm Se throughout the study. Blood samples were obtained at the beginning of the study and monthly thereafter for the experimental period of four months. Serum, and blood cell Se concentrations, blood glutathione peroxidase activity, neutrophil phagocytosis, lymphocyte proliferation in response to mitogen ConA, and antibody production in response to rabies vaccination were measured.

Measures of Se status were increased in all horses receiving supplementary Se when compared to horses in the Control group. Further, horses in the Organic group had higher serum and blood cell Se concentrations when compared to horses in the Inorganic group, with the greatest difference in blood cell Se. Mean Se concentrations at the end of the study for the Control, Inorganic and Organic groups were 0.030 ± 0.003, 0.144 ± 0.009 and 0.166 ± 0.017 ppm Se in serum, and 0.085 ± 0.024, 0.299 ± 0.018 and 0.442 ± 0.044 ppm Se in blood cells, respectively (all group comparisons differ $P<0.05$). Glutathione peroxidase activity was higher in the Inorganic and Organic groups when compared to the Control group ($P<0.001$), but there was no significant difference between the Inorganic and Organic groups. Phagocytosis activity increased in all groups during the course of the study but tended to increase more in the horses in the Organic group. Mean increase in phagocytosis index for the Control, Inorganic and Organic groups were 15 ± 9, 16 ± 10 and 26 ± 21 percent, respectively ($P<0.10$ for the AVOVA contrast Organic vs. Inorganic and Control). Other measures of immune function did not differ significantly between groups.

To conclude, supplementation of adult horses with organic Se resulted in significantly higher serum and blood cell Se concentrations when compared to supplementation with inorganic Se, but this difference was not reflected to the same degree in blood glutathione peroxidase activity. This is the first study looking at the effect of Se supplementation and Se source on phagocytosis activity in adult horses. When compared to unsupplemented controls, phagocytosis activity tended to be enhanced in horses supplemented with organic Se, but not in horses supplemented with inorganic Se. This latter finding is the first to suggest an effect of Se source on phagocytosis activity in horses and is the subject of further investigations in mares and their foals.

ABSTRACT #389
EFFECT OF DIET ON SERUM ELECTROLYTES, BLOOD PH AND MARKERS OF BONE REMODELING IN HORSES. J.M. Newquist[1], R.M. Enns[2], A.E. Hill[1], J.M. MacLeay[1]. [1]Dept. of Clinical Sciences, Colorado State University, Fort Collins, CO; [2]Dept. of Animal Sciences, Colorado State University, Fort Collins, CO.

High dietary acid load (DAL) has been associated with metabolic acidosis in many species. Racehorses are typically fed grain-rich di-

ets, which represent high DAL that may decrease blood pH to result in calcium mobilization from bone as a buffer. In young racehorses under the pressures of growth and bone remodeling due to high impact exercise, high DAL may hinder normal bone development and predispose these athletes to injury. This paper describes two studies investigating a possible association between grain consumption and acidosis, alterations in serum electrolytes, and bone remodeling in horses.

Study 1: Thirty-nine racehorses and 11 pastured horses were used in a pilot study. Data was collected at a single time point and included grain intake (kg), work status, age, gender, breed, blood pH, iCa, HCO_3, TCO_2, BE, BEecf, K, Cl, PTH and Osteocalcin (OC). Grain intake was not significantly associated with lower pH, but lower mean pH and increased HCO_3 and TCO_2 were observed with grain consumption. Significant differences between working and sedentary horses were seen in iCa, BE, BEecf, TCO_2 and HCO_3. Together with the observed increase in blood pH after exercise, this suggested grain-induced acidosis may be offset by exercise-induced alkalosis. A tendency for higher grain intake to result in increased OC was also noted. This suggested increased bone turnover may be associated with grain consumption.

Study 2: Ten sedentary horses were used in this 24 hour study, 5 fed grass hay only and 5 fed grain and grass hay. Data collected included hourly measurements of blood pH, iCa, HCO_3, TCO_2, BE, SID, K, Cl, Na, glucose and OC. Mean pH in the grain group relative to the control group was significantly lower after eating, but was significantly higher at later collections. Grain consumption was significantly associated with lower HCO_3, TCO_2, and BE. SID was significantly lower in the grain group near the time of decreased buffer and pH, supporting DAL as causal.

These data support that high DAL significantly affects blood pH and acid-base balance; however, no evidence of increased bone turnover was seen, as demonstrated by lack of significant increase in iCa and OC. Further research using a more sensitive indicator of bone turnover and/or a longer time frame and/or exercising horses may help determine if high DAL plays a role in bone turnover in the horse.

ABSTRACT #390
EXERCISE TRAINING IN OBESE HORSES DECREASES FAT MASS INDEPENDENT OF SUBCUTANEOUS FAT THICKNESS. R Carter, J McCutcheon, E Valle, R Geor. Virginia Tech, Blacksburg, VA.

Increasing physical activity is a common recommendation to reduce adiposity and improve insulin sensitivity in obese horses. The present study evaluates the influence of exercise training without dietary restriction on adiposity and basal insulin and glucose concentrations in obese horses. Twelve Arabian geldings (median body condition 8, scale 1–9) were fed hay at 2.4% bodyweight. Four horses remained sedentary (CON) and 8 horses (EX) were exercised 4 times per week for 4 wk at low intensity (30 min trot) followed by 4 wk at higher intensity (10 min trot, 20 min canter, 3° incline) exercise. Prior to and after each training period, basal blood samples, body condition scores, subcutaneous fat thickness (ultrasonic assessment), and total body water (deuterium oxide dilution) were measured. Data were analyzed by the Kruskal-Wallis test and are reported as median (interquartile range). All variables did not change ($P > 0.10$) in CON throughout the study. In EX, body condition score and subcutaneous fat thickness did not change ($P > 0.10$). Fat mass calculated from total body water decreased ($P < 0.05$) from 96 (88–101) kg to 73 (71–78) kg after low intensity exercise and to 61 (57–68) kg after higher intensity exercise. Bodyweight, calculated fat-free mass, and basal glucose and insulin concentrations did not change ($P > 0.05$) with exercise training. The results of this study demonstrate a decrease in fat mass with exercise training independent of changes in subcutaneous fat thickness, visual appearance of adiposity, or basal glucose and insulin concentrations.

ABSTRACT #391
ANALYSIS OF PERFORMANCE FOLLOWING JUGULAR THROMBOPHLEBITIS IN HORSES. P. Moreau, G. Beauchamp, J.P. Lavoie. Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.

Little is currently known on the effects of jugular thrombophlebitis on future athletic performance of horses. The aim of the study was to evaluate the impact of jugular thrombophlebitis on performance.

Records from 91 horses with thrombophlebitis were studied. Signalment, history, clinical signs, diagnosis, and treatment were reviewed retrospectively from medical files. Performance was evaluated in two ways: a questionnaire was sent to the owners to obtain their subjective assessment of their horse's performance compared to the pre-thrombophlebitis level. In Standardbreds, the records of pre and post thrombophlebitis races were reviewed, when available.

Thrombophlebitis was diagnosed in 37 horses upon admission (Group 1) while 54 horses developed thrombophlebitis when hospitalized for an unrelated condition (Group 2). 37 owners (33%) answered the questionnaire and racing records were available for 31 horses. Twelve horses had died, no performance information was available for an additional 25 horses, 6 horses had never performed, so only performances of 48 horses were assessed. Owners reported that all but one non-racing horse had performances equivalent or improved following discharge. According to the owners' evaluation or race time evaluations, 64% of horses presented with thrombophlebitis (Group 1) and 85% of the horses who developed thrombophlebitis during hospitalization (Group 2) returned to their previous performance level. 84% of Standardbred racehorses returned to racing. In these horses, there were no significant differences between racing time prior to, and following thrombophlebitis. No significant difference in performance was also noted regardless of the primary diseases, the presence of unilateral or bilateral thrombophlebitis, or the treatment administered.

Results of the present study suggest that athletic performance of pleasure and performance horses is not affected by thrombophlebitis even if bilateral. However, thrombophlebitis in Standardbred racehorses is associated with a decreased return to racing, but when they do, their performances are not impaired.

ABSTRACT #392
EFFECTS OF OXIDANT STRESS ON EQUINE SKIN EPITHELIAL PROINFLAMMATORY CYTOKINE AND MMP GENE EXPRESSION. TL Westerman, C Yin, JK Belknap. The Ohio State University College of Veterinary Medicine, Columbus, OH.

The epithelial cell is not only a target of inflammation, but has also been found to respond to inflammatory stimuli via expression of signaling molecules important in inflammatory injury including proinflammatory cytokines, chemokines and matrix metalloproteases (MMPs). We have recently found evidence of laminar oxidant stress in the early stages of laminitis. Cellular oxidant stress plays a central role in epithelial cell dysfunction in human sepsis-related inflammatory injury of organs; reactive oxygen and nitrogen species (ROS and RNS) responsible for cellular oxidant stress are produced by infiltrating leukocytes, and also reportedly by the host cells themselves during inflammatory signaling induced by Toll-like receptor-4 (TLR4) ligands. Due to these findings, we compared the inflammatory response of the equine epidermal epithelial cell (a critical cell type at the site of laminar failure) exposed directly to ROS to the epithelial response elicited by exposure to the TLR4 ligand lipopolysaccharide (LPS). Skin epithelial cells were obtained for primary culture from two horses. Epithelial cell cultures (passage 3'4) were exposed for 1H and 4H to ROS or LPS (5 μg/ml). ROS included H_2O_2 (50 uM, 200 uM, 1 mM) and xanthine/xanthine oxidase (X/XO, 100 uM/3 mU, 200 uM/30 mU; superoxide radical generation confirmed with nitro blue tetrazolium chloride). All experiments were performed in triplicate. Real time-quantitative PCR was used to assess mRNA concentrations of heme oxygenase-1 (HO-1, indicator of redox-stimulated gene expression), proinflammatory cytokines (IL-1β, IL-6 and IL-8) and MMPs (MMP-2 and MMP-9). Although variability in gene expression existed between cultures from the two horses, several patterns were evident. HO-1 gene expression was induced by oxidant stress (both H_2O_2 and X/XO) at 4H ($P < 0.05$) but, in contrast to most reports, was not induced by exposure to LPS. IL-6, which was markedly induced by LPS at 1H and 4H ($P < 0.05$), underwent minimal induction with oxidant stress. The potent neutrophil chemokine IL-8 was induced to a similar extent by both LPS ($P < 0.05$) and oxidant stress ($P < 0.05$). MMP-9 expression was significantly induced at 4H by oxidant stress but was unaffected by LPS. In conclusion, oxidant

stress appears to produce a specific inflammatory response that differs markedly from the response elicited by LPS. Both the absence of HO-1 induction by LPS and the distinct differences in induction of cytokine expression between ROS and LPS exposure indicate that LPS may not cause significant changes in the redox state of the epidermal cells, and the TLR signaling is working through different signaling mechanisms than ROS. Coinciding HO-1 and MMP-9 upregulation indicates oxidant stress may be a contributing factor to MMP dysregulation reported to occur in laminitis. Future comparison of skin and laminar epithelium cell culture response will be helpful in understanding the heightened susceptibility of the laminar epithelium to injury during sepsis.

ABSTRACT #393

PHARMACOKINETICS AND URINE DETECTION OF TRAMADOL IN ADULT HORSES. AJ Stewart, DM Boothe, C Cruz-Espindola, E Baird, J Springfield. Auburn University College of Veterinary Medicine, Auburn, AL.

The purpose was to determine the pharmacokinetic profile of tramadol and metabolites ODT and N-desmethyltramadol (NDT) in serum and urine of adult horses.

Six adult horses (482–564 kg) received intravenous (4.4 mg/kg over 3 minutes) and oral (10 mg/kg) tramadol separated by a six-day washout period in a randomized crossover design. Urine and blood samples were collected over 48 hours. Tramadol, ODT, and NDT concentrations were measured using high performance liquid chromatography in serum and urine. Non-compartmental pharmacokinetic analysis was implemented.

Maximum (mean ± sd) serum concentration after intravenous tramadol administration for T, ODT and NDT was $Cmax[T_{IV}]$ = 1330 ± 519 ng/ml, $Cmax[ODT_{IV}]$ = 0 ng/ml and $Cmax[NDT_{IV}]$ = 66.7 ± 20.2 ng/ml. The calculated parameters for half-life (t1/2), volume of distribution (Vd), area under the curve (AUC) and total body clearance (Cl) after intravenous tramadol administration were: $t1/2[T_{IV}]$ = 193 ± 260 min; $Vd(T_{IV})$ = 14.3 ± 11.5 L/kg, $AUC(T_{IV})$ = 65,234 ± 41,017 min*ng/ml and $Cl[T_{IV}]$ = 123 ± 143 ml/min/kg.

Maximal serum concentration after oral tramadol administration for T, ODT and NDT was $Cmax[T_{oral}]$ = 115.1 ± 51.5 ng/ml, $Cmax[ODT_{oral}]$ = 61.1 ± 28.1 ng/ml and $Cmax[NDT_{oral}]$ = 168 ± 78.7 ng/ml. After oral tramadol administration $t1/2[T_{oral}]$ = 74.8 ± 52.8 min, $t1/2[ODT_{oral}]$ = 52.3 ± 23.4 min, $t1/2[NDT_{oral}]$ = 135 ± 45.4 min. The oral bioavailability of tramadol was 14.1 ± 10.7%. Concentrations of ODT remained above the minimum reported therapeutic range (for humans > 40 ng/ml) for 60–120 minutes in 4 of 6 horses after oral tramadol administration. The relative bioavailability for NDT was 130 ± 123%. Duration of urine detection of T and NDT after oral tramadol administration was 20.3 ± 4.8 and 22 ± 7.8 hrs after oral administration, respectively.

Tramadol has poor oral absorption, but relatively slow clearance in horses compared to other species. Tramadol and NDT can be detected in the urine for approximately 1 day after single dose oral administration.

ABSTRACT #394

PHARMACOKINETICS OF THE GASTROKINETIC AGENT MOSAPRIDE CITRATE AFTER SINGLE ORAL ADMINISTRATION IN HORSES. K. Okamura[1], N. Sasaki[2], M. Fukunaka[2], H. Yamada[2], H. Inokuma[2]. [1]Department of Animal Health Products, Dainippon Sumitomo Pharma. Co., Ltd., Osaka, Japan. [2]Department of Veterinary Clinical Science, Obihiro University of Agriculture and Veterinary Medicine, Japan.

Mosapride citrate, a selective 5-HT_4 receptor agonist, is a gastrokinetic agent used clinically in the treatment of patients with gastrointestinal motility dysfunctions in Japan. In horses, it has been reported that oral administration of mosapride citrate can promote motility in the small intestine and cecum. However, the pharmacokinetics of mosapride citrate has not been cleared. In this study, we report mosapride citrate pharmakokinetic data obtained following after single oral doses.

Seven healthy Thoroughbreds (1 mare and 6 geldings) were dosed with 1,000 ml of distilled water and 0.5 mg/kg, 1.0 mg/kg, or 1.5 mg/kg mosapride citrate (Dainippon Sumitomo Pharma, Japan) through a nasogastric tube. The study was conducted as a Latin-square design; thus, each horse received each dosage. There was a 1-week washout period between subsequent treatments. Jugular venous blood was collected just before mosapride administration and at 15, 30, 60, 120, 180, 360, 480 minutes after dosing. Serum mosapride concentrations were measured by a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method.

Mean serum levels of mosapride reach the maximum 60–120 minutes after administration, with levels of 0.029 ± 0.004, 0.047 ± 0.007, 0.101 ± 0.014 μg/ml at doses of 0.5, 1.0, 1.5 mg/kg, respectively. Thereafter, serum levels of mosapride decreased with $t_{1/2}$s of 185–233 minutes. Mosapride showed the C_{max}s of 0.031 ± 0.004, 0.060 ± 0.009, 0.104 ± 0.012 μg/ml and $AUC_{0\text{-}480}$s of 7.6 ± 1.0, 15.1 ± 2.1, 25.7 ± 3.1 μg/ml.min at doses of 0.5, 1.0, 1.5 mg/kg, respectively. The C_{max} and $AUC_{0\text{-}480}$ increased in proportion to the dose, indicating linear pharmacokinetics of mosapride up to 1.5 mg/kg.

The pharmacokinetic profiles of mosapride in horses are quite different from that in humans. The average $t_{1/2}$ in horses in this study was almost 2-fold longer than the 2.0 hours reported in healthy adult humans. Therefore, it is thought that it is suitable to reduce the number of doses a day in horses to half. In humans, the therapeutic dosage regimen of mosapride citrate is 5 mg, three times a day. Mean while, it has been reported that the optimal orally administered dosage of mosapride citrate in horses is 1.5 to 2 mg/kg. Therefore, we arrived at the following conclusion: the predicted therapeutic dosage regimen of mosapride citrate in horses is 1.5 to 2 mg/kg, a time or two times a day.

ABSTRACT #395

MEASUREMENT OF BIOMARKERS OF OXIDATIVE STRESS IN PLASMA, RESPIRATORY FLUIDS AND TISSUES COLLECTED FROM RECURRENT AIRWAY OBSTRUCTION AFFECTED HORSES AND THEIR CONTROLS. R Tan, C Thatcher, V Buechner-Maxwell, U Christmann, M Crisman, S Werre. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Multiple biomarkers of oxidative stress have been measured and used in human medicine to diagnose and monitor airway disease. The purpose of the study was to determine if relationships existed between clinical status of RAO-affected horses or controls and; concentrations of isoprostanes and isofurans in plasma, EBC and BALF; mRNA expression of interleukin 4 (IL4) and gamma interferon (INFγ) in airway inflammatory cells of BALF; and mRNA expression of inducible nitric oxide synthase (iNOS), extracellular glutathione peroxidase (GPx-3), and cytosolic superoxide dismutase (SOD-1) from bronchial mucosal biopsies. In addition, EBC was evaluated as a non-invasive method to diagnose and monitor RAO-affected horses.

Eight pairs of non-RAO-affected (controls) and RAO-affected horses were used in this study. Horses were maintained on pasture for a minimum of 4 weeks to minimize exposure to respirable debris and sample 1 (remission) was taken after horses were determined to be in remission based on clinical parameters. Environmental challenge was performed one week later and continued until RAO-affected horses reached a clinical score of at least 5 (out of 8) or they stayed in the barn greater than 72 hours, at which time sample 2 (challenge) was collected. Sample 3 (recovery) was post-environmental challenge collected after paired horses were returned onto pasture with reduction in clinical score by 2 points, or after 1 week. Plasma, serum, EBC, and BALF were collected at each sample time. Bronchial mucosal biopsies were collected at challenge. A stable isotope dilution gas chromatography negative ion chemical ionization mass spectrometry (GC-MS) method was used to measure isoprostanes and isofurans. Measurement of IL4 and INFγ in BALF cells was by real time PCR, and iNOS, GPx-3, and SOD-1 from bronchial mucosal biopsies by quantitative PCR.

Isofurans could only be detected in EBC in three samples from RAO-affected and five samples from control horses. Isoprostanes were not detected in any EBC sample. In BALF and plasma no significant difference in isoprostanes or isofuran concentrations were found between either group or sample time. mRNA expression of IL4 and INFγ from cells harvested from BALF and iNOS, GPx-3, and SOD-1 from bronchial mucosal biopsies were also not significantly different.

None of the biomarkers measured could differentiate disease status of RAO-affected horses from their controls. This may indicate lack of test sensitivity, study power or a minimal role of the selected biomarkers in RAO. The use of EBC to measure isoprostanes or isofurans did not yield additional diagnostic information.

ABSTRACT #396
PRIMARY BRONCHIAL EPITHELIAL CELL CULTURES FROM HEAVEY HORSES EXPOSED TO HAY DUST EXHIBIT EARLY UP-REGULATION OF CXCL2. DM Ainsworth[a], HN Erb[b], MB Matychak[a], CL Reyner[a], JC Young[a]. [a]Department of Clinical Sciences and [b]Department of Population Medicine & Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY USA.

In heavey horses, IL-8 gene and protein expression in bronchial biopsies are up-regulated (Ainsworth et al, 2006) but the inciting stimuli are unknown. The objective of this study was to determine if, in response to hay dust exposure, chemokine and cell surface receptor (CSR) expression increases in primary bronchial cell cultures (BEC) from heavey horses.

BEC were established from 6 diseased and 6 control horses stabled and fed hay for 2 weeks. Cultures were incubated for 6 or 24 hours with solutions of PBS, hay dust, LPS and β-glucan. IL-8, CXCL2, IL-1β, TLR2, TLR4 and IL-1R1 gene expressions were measured by kinetic PCR. Group differences were detected using a Wilcoxon rank sum test with a Bonferroni correction.

Following PBS treatment, there were no differences between the 2 groups in chemokine or CSR expression. For each group, HDS and LPS but not β-glucan treatment up-regulated IL-8, CXCL2 and IL-1β expression. No treatment up-regulated TLR2, TLR-4 or IL-1R1 expression relative to PBS. The single group difference was an early CXCL2 up-regulation in hay dust-treated BEC from heavey horses. These data suggest that epithelial IL-8 or IL-1β but not CXCL2 expression requires signals from luminal or interstitial lung cells not present in BEC. (Funded USDA 2004-01235.)

ABSTRACT #397
BRANCHIAL REMNANT CYSTS IN HORSES: 7 CASES. RD Nolen-Walston[1], EJ Parente[1], KA Kalck[2], FM Andrews[2], JE Madigan[3], JB Engiles[1]. [1]New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA. [2]College of Veterinary Medicine, University of Tennessee, Knoxville, TN. [3]School of Veterinary Medicine, University of California, Davis, CA.

The branchial arches are the embryologic origin of multiple structures of the mammalian head and neck. In horses, there are several studies of 4th branchial arch anomalies which present as laryngeal cartilage malformations, but only isolated cases of branchial cysts have been described. This retrospective multicenter study aims to identify and characterize the presentation, clinical characteristics, treatment, outcome, and histology of suspected branchial cysts in horses.

An email survey of diplomats of the ACVIM and ACVP identified 7 cases of branchial cysts. There was no sex or breed predilection, and age at presentation was bimodal, with 3/7 cases presenting at <6 months of age, and the remaining 4 at >8 years of age (range 9–21 years). The younger group presented for respiratory distress or stridor, whereas the older horses typically were evaluated for esophageal obstruction. A firm, non-painful, spherical mass was palpable in the throatlatch region in all patients (right-sided in 6/7). Sonographic examination typically revealed an encapsulated mass 3–10 cm in diameter, containing minimally echoic fluid and frequently small spherical, hyperechoic bodies, with aspirates consistent with chronic hemorrhage. Six cases were treated via surgical excision. Of these, one was euthanized intraoperatively due to severe laryngeal anomalies, and the remainder survived to discharge. Post-operative complications were common, and included seroma (3/5), pneumonia, and laminitis. Preliminary histology and immunostaining for pancytokeratin markers confirmed squamous to ciliated pseudostratified columnar epithelial cyst lining in multiple cases, consistent with branchial cysts described in humans. At follow-up right recurrent laryngeal neuropathy was identified in 4 cases.

ABSTRACT #398
TEMPORAL AGREEMENT OF HISTAMINE BRONCHOPROVOCATION TESTING IN HORSES USING OPEN PLETHYSMOGRAPHY. RD Nolen-Walston, RC Boston, PA Wilkins. New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.

Open plethysmography with histamine challenge is a simple, noninvasive field test for measuring airway reactivity in horses with suspected Inflammatory Airway Disease (IAD). Horses (n = 9, adult mares) were tested with a commercial system (Open Pleth™, Ambulatory Monitoring Inc.), and underwent bronchoprovocation via nebulization with exponentially increasing concentrations of histamine from 2 to 32 mg/ml. Baseline Δflow (a measure of gas trapping due to airway obstruction), and the concentration of histamine needed to obtain a 35% increase in Δflow (PC35), were measured. A PC35 of <6 mg/ml histamine is associated with airway hyperreactivity. Measurements were obtained at days 1 and 3, and again after 90 days with agreement being reflected in the association of repeated measures of PC35, determined by Fischer's Exact Test and concordance analysis (rho 'ρ'). Animals were categorized into 1 of 5 groups of responsiveness at each time point based on their individual test results. Each animal maintained the same degree of airway reactivity, excepting 2 with increased reactivity at 90 days. Short-term measurements (days 1 and 3) had strong association ($P = 0.002$) and concordance ($\rho = 0.97$). Long-term association (90 day interval) was also significant, ($P = 0.038$), while concordance was low ($\rho = 0.63$). By observation, differences in ambient temperature may have impacted longer time frame results, perhaps due to cold air-provoked bronchospasm in some animals. Airway reactivity measured by histamine bronchoprovocation and open plethysmography is highly reproducible over a short time period, but may be unacceptable over a period of 90 days with large ambient temperature variation.

ABSTRACT #399
EFFECT OF AMINOCAPROIC ACID ON THE SEVERITY OF EXERCISE-INDUCED PULMONARY HEMORRHAGE. MM Durando, EK Birks. University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA.

Exercise-induced pulmonary hemorrhage (EIPH) is considered to be an important cause of reduced performance in racehorses. Numerous treatments are used to attempt to minimize EIPH and/or the consequences of bleeding. However few controlled scientific studies have evaluated the efficacy of most of the available treatments. The diuretic furosemide is commonly administered to limit EIPH. Because of perceived ineffectiveness of furosemide to prevent EIPH, the anti-fibrinolytic drug aminocaproic acid (Amicar®) has also been administered along with furosemide, in an attempt to decrease EIPH severity. This study was conducted to determine if the administration of Amicar along with furosemide would have any effect on the incidence/severity of EIPH in TB racehorses.

Horses were voluntarily enrolled by their trainers and were required to participate in 2 races as part of the study. Participating horses received one of two treatments prior to each race in a blinded, randomized cross-over design. Treatments consisted of either furosemide + Amicar (F+A; 250 mg furosemide i.v. 4 hours prior to racing and 5 gm Amicar)i.v. 2 hr prior to racing) or furosemide only (F). Of 43 horses completing both treatments, 22 received F+A and 21 received F as the initial treatment. All horses had videoendoscopy of their airways and bilateral bronchoalveolar lavage (BAL) 12–15 hours following each race. Lungs were lavaged with 200 ml sterile saline per side. Recovered BAL fluid was evaluated by clinical pathologists blinded to treatment, for RBC and nucleated cell counts, and WBC differential analysis. Data were analyzed by repeated measures ANOVA to examine possible effects of treatment and/or race order on RBC number, WBC number and differential cell count. Significance was $P < 0.05$.

No significant effect of treatment or race order was observed for total RBC numbers (mean ± SEM, F 1271 ± 530 vs F+A 1550 ± 442 cells/μl, $P = 0.62$). However, the right side had significantly greater RBC numbers than the left side after both treatments (mean ± SEM right vs left; F 1833 ± 999 vs 410 ± 124 cells/μl and F+A 2330 ± 703 vs 415 ± 119 cells/μl, $P < 0.01$). No significant effect on

WBC numbers was observed with regard to either treatment/race order (right $P = 0.77$, left $P = 0.47$), or lung side ($P = 0.29$).

This study demonstrated that pre-race administration of Amicar in addition to the diuretic furosemide had no effect on the severity of EIPH, compared with furosemide alone. WBC enumeration and distributions were also not affected by treatment. Interestingly, significantly more RBC were recovered from the right lung. Whether this is related to the direction of the races or the predominant lead the horse races on is unknown. Additional studies with larger numbers of horses racing at various racetracks are warranted. Variations in race direction/configuration and surfaces would also be important to study.