

## ABSTRACT #1

CLINICAL AND CLINICOPATHOLOGIC FINDINGS IN DOGS SEROREACTIVE TO *BARTONELLA HENSELAE* ANTIGENS. Robert A. Goodman and Edward B. Breitschwerdt. North Carolina State University Veterinary Teaching Hospital, Department of Clinical Sciences, Raleigh, NC.

Bacteria of the genus *Bartonella* are increasingly reported as pathogens that induce chronic infections in humans and dogs. In humans, infection with *Bartonella henselae* is most commonly associated with an acute, febrile lymphadenopathy referred to as cat scratch disease. In a minority of patients infection results in atypical manifestations such as endocarditis, encephalitis, granulomatous hepatitis and splenitis, peliosis hepatis, neuroretinitis, and bacillary angiomatosis.

*Bartonella vinsonii* subsp. *berkoffii* causes persistent bacteremia, immunosuppression, granulomatous inflammation, endocarditis, and hemoglobinuria in dogs. Infrequently, *B. henselae* DNA has been detected in dogs with disease manifestations including, peliosis hepatis, granulomatous hepatitis, fever, thrombocytopenia, and neurologic dysfunction. To date, *B. henselae* has not been isolated from the blood or tissues of a dog. A recent serosurvey of dogs from the Southeastern United States identified a significantly increased seroprevalence in sick dogs (10.8% in healthy dogs v. 28.5% in sick dogs).

This study was designed to assess the clinical relevance of seroreactivity (reciprocal titer of 64 or greater by IFA) to *B. henselae* antigens through a cross-sectional epidemiologic study. Clinical, hematological, biochemical and cytological findings were extracted from the medical records of 40 *B. henselae* seroreactive and 45 non-seroreactive dogs also submitted for testing for exposure to vector borne pathogens. Statistical analysis was conducted utilizing odds ratios, descriptive statistics, the Student's t-test, Chi-square test, and Wilcoxon rank sum test.

There was no statistical difference between *B. henselae* seroreactive and non-seroreactive dogs when analyzed by disease category, hematological, biochemical, or cytological findings. However, 2 of the 4 cases of granulomatous meningoencephalitis, 3 of the 4 cases of IMHA, 2 of the 3 cases of infective endocarditis, 2 of the 3 cases of lymphoid neoplasia, and 5 of the 10 cases of polyarthritis were seroreactive to *B. henselae* antigens. Additionally, 18 of the 34 thrombocytopenic and 14 of the 27 neutrophilic dogs were *B. henselae* seroreactive. Failure to detect statistical differences among these groups may be due to a lack of correlation, due to small sample size, or due to multifactorial influences on disease expression. Prospective clinical examination of specific disease syndromes will be required to determine the significance of *B. henselae* antibodies in dogs.

## ABSTRACT #2

GENOTYPIC ANALYSIS OF *GIARDIA DUODENALIS* IN DOMESTIC CATS. R. Vasilopoulos, L.G. Rickard, A. Mackin, C. Huston, and G.T. Pharr. College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

*Giardia duodenalis* is an intestinal flagellated protozoan parasite that affects many mammalian species, including cats, dogs, and humans. Recent studies, using molecular biological techniques, indicate *G. duodenalis* is a species complex, with several different genotypes identified (Assemblages A to G). Previous studies on isolates recovered from domestic cats in different geographic locations indicate that most *G. duodenalis* isolates can be assigned either to Assemblage A (a potentially zoonotic form of the parasite found in many different animal species) or Assemblage F, which is a cat-specific group. The objectives of our study were to determine the prevalence of *G. duodenalis* in the northeastern Mississippi and northwestern Alabama region, and to use molecular genotyping

techniques to determine the distribution of the various subtypes in our geographic region.

Fecal samples collected from 250 domestic cats of various breeds, gender, ages, and clinical signs from northeastern Mississippi were examined for the presence of *G. duodenalis* cysts using a direct immunofluorescent assay (DFA). Thirty-five fecal samples were *Giardia* DFA positive (35/250; prevalence of 14%; 95% CI = 9.9-18.9%). Genomic DNA was prepared from *Giardia* cysts and partial glutamate dehydrogenase (gdh) gene fragments were amplified by polymerase chain reaction using specific *G. duodenalis* primers. Amplicons were cloned into plasmid vectors and sequenced using an automated DNA sequencer. The resultant sequences were compared to the existing sequences stored in Genebank.

Preliminary results revealed that 66% (4/6) of *G. duodenalis* isolates analyzed thus far could be assigned to Assemblage A, an assemblage shared between humans, cats, and other vertebrates. Only 33% (2/6) of these isolates could be assigned to the cat-specific Assemblage F. To the best of our knowledge, the prevalence rates for the different assemblages of feline *Giardia* have not been reported.

Studies in people have demonstrated that different genotypes of *G. duodenalis* are associated with significant differences in clinical signs at presentation, disease severity, and zoonotic potential. The Assemblage A group of organisms are believed to have the greatest zoonotic potential. Our somewhat surprising results suggest that the majority of *G. duodenalis* isolates in our geographic area are potentially zoonotic. It is possible that in cats, as has been demonstrated in humans, different *G. duodenalis* genotypes may have the potential to cause different forms of disease. Knowledge of the distribution of genotypes in a particular geographic area may lead to a greater ability to predict the types of clinical signs, and the human health risk, associated with *Giardia* infection in local cats.

## ABSTRACT #3

INCIDENCE OF INFECTIOUS COMPLICATIONS IN CANINE AND FELINE RENAL TRANSPLANT RECIPIENTS. E. Kadar, J. Sykes, L. Bernstein, A. Kyles, C. Gregory. School of Veterinary Medicine, University of California, Davis, CA.

Renal transplantation has been available for treatment of end-stage renal failure in cats for fifteen years and has more recently become available for dogs. The role of infection in morbidity and mortality of human renal transplant recipients is well documented. Studies on the incidence of infectious complications in feline and canine transplant recipients are lacking.

The medical records of 192 renal transplant recipients operated between 1987 and 2002 were reviewed. Seven animals were lost to follow up, 169 cats and 16 dogs were included in the study. Standard screening tests for pre-existing infectious disease were conducted in all renal transplant recipients. The standard immunosuppressive regimen in cats included prednisone, cyclosporine and rarely azathioprine. Some variation existed in canine immunosuppressive regimens.

Bacterial and fungal infections were documented via microbial culture; FIV and FeLV infections were diagnosed by positive serology. Upper respiratory tract infections (URI) were diagnosed based on clinical signs and microbial culture when available. Hemoplasmosis was diagnosed on whole blood PCR; protozoal infections were diagnosed by demonstration of the organisms.

The median age of feline renal transplant recipients was 8y (range 0.4-17), 107/169 were male castrated, 58 were female spayed, 2 male and 2 female. Forty-seven infectious complications were documented in 43/169 (25%) feline renal transplant recipients, the median time from transplantation to infection was 2.5 months [25<sup>th</sup>-75<sup>th</sup> percentile 1-11.9]. Of the 47 infectious complications in feline renal transplant recipients, bacterial infections were most common (n=25, 53%), followed by viral (n=13, 28%), fungal (n=6, 13%) and

protozoal (n=3, 6%) infections. The median time to bacterial infection was 2.8[1-17.4], URI 0.2[0.1-7.1], protozoal infection 1[0.7-1.8m], fungal infection 6[2.3-34.1]. 17/43 (40%) of cats died or were euthanized as a result of infection. Overall, infection (13%) was secondary only to rejection (20%) as a cause of death amongst feline renal transplant recipients.

Median age of canine renal transplant recipients was 3y (range 0.3-11). 8 infectious episodes were documented in 7/16 canine renal transplant recipients (43%), the median time to infection was 0.6 months [25<sup>th</sup>-75<sup>th</sup> percentile 0.4-3.4]. Bacterial infections were most common in dogs and 3/8 infections were fatal.

Infection is an important cause of morbidity and mortality in feline and canine renal transplant recipients. 50% of infectious episodes occurred within 2.5 months of transplantation in cats and within 18 days in dogs.

#### ABSTRACT #4

CHARACTERIZATION OF THE SYSTEMIC INFLAMMATORY RESPONSE IN DOGS NATURALLY INFECTED WITH *ANGIOSTRONGYLUS VASORUM*. M. Kjelgaard-Hansen, A.L. Jensen, J. Koch, A.T. Kristensen. Department of Clinical Studies, The Royal Veterinary and Agricultural University, Denmark.

Several experimental studies have been conducted on dogs infected with *Angiostrongylus vasorum*, where changes in a wide range of clinical, hematological, pathological (including several inflammatory) parameters have been reported as the dog passes through the various phases after a point-inoculation with infective L3-larvae. In dogs residing in endemic areas, continued inoculation with varying numbers of larvae occurs thus an inseparable continuum of infection phases likely takes place. There are no previous reports on the levels of acute phase proteins (APP) such as alpha<sub>1</sub>-acid glycoprotein (AGP), haptoglobin (Hp), C-reactive protein (CRP), fibrinogen (Fib) or albumin (Alb) in dogs naturally infected with *A. vasorum*. The objective of the present study was to evaluate the level of these APP's and a selection of other inflammatory parameters (segmented and band neutrophils, eosinophils and rectal temperature) to characterize the inflammatory response at the time of diagnosis in clinically symptomatic and naturally infected dogs residing in endemic areas.

Data and measurement of inflammatory parameters were obtained at the time of diagnosis of angiostrongylosis (positive Baermann test) from eleven client-owned dogs presented at our University Small Animal Veterinary Teaching Hospital. Measurements of APP and leukocyte counts were performed using routine methods at our laboratory.

Abnormal levels of the inflammatory parameters were distributed as follows: CRP was increased (>10 mg/L) in 73 % (8/11) and >30 mg/L in 18% (2/11), Hp (>2 g/L) in 18 % (2/11), Fib (>4 g/L) in 9 % (1/11) and Alb was decreased (<26 g/L) in 18 % (2/11), whereas AGP was within normal (<1 g/l) for all 11 dogs. Eighteen percent (2/11) were febrile (>39.3 °C), neutrophilia was present in 73 % (8/11) as either an elevated number of segmented or band neutrophils (>12.1 10<sup>9</sup>/L or >0.3 10<sup>9</sup>/L, respectively) and eosinophilia (>1.2 10<sup>9</sup>/L) was detected in 27 % (3/11) of the dogs.

An APP pattern of increased major APP (CRP [response time from stimulus ≈ 24h]) and normal intermediate APP (Hp and AGP [response time ≈ 6 days]) usually indicates an acute systemic inflammation. However, all dogs were passing larvae and have thus passed at least one prepatent period, hence the infection must be chronic in time at the time of diagnosis. The APP pattern detected in this study is more likely a result of differences in the APP response to a moderate inflammatory stimulus, which is supported by the finding that only 18% (2/11) dogs have more than only moderately increased CRP levels (>30 mg/L). Further, eosinophilia is a more consistent finding than neutrophilia in experimental studies. Hence, dogs naturally infected with *A. vasorum* develop a systemic inflammatory

response which at the time of diagnosis seem to be a moderate response and the results indicate that the inflammatory response seen after experimental point-inoculations may not fully resemble that seen after naturally-occurring continuous inoculations.

#### ABSTRACT #5

USE OF A PRIMARY CARE VETERINARY MEDICAL DATABASE FOR SURVEILLANCE OF SYNDROMES AND DISEASES IN DOGS AND CATS. G.E. Moore<sup>1</sup>, M.P. Ward<sup>1</sup>, J. Dhariwal<sup>1</sup>, C.C. Wu<sup>1</sup>, N.W. Glickman<sup>1</sup>, H.B. Lewis<sup>2</sup>, L.T. Glickman<sup>1</sup>. <sup>1</sup> School of Veterinary Medicine, Purdue University, West Lafayette, IN; <sup>2</sup> Banfield, The Pet Hospital, Portland, OR.

The reported prevalence of syndromes or diseases in veterinary medicine is often based on small numbers and highly selected animal populations, e.g. referral hospitals or humane shelters. The predictive value of diagnostic tests used in primary care settings however, is greatly influenced by the prevalence of those diseases they are intended to detect. In addition, determining the prevalence of selected zoonotic diseases among primary care patients is important for public health. Most medical record keeping systems used today in primary care practices are inadequate for disease surveillance. The growth of large corporate primary care veterinary practices with multiple geographic locations and a standardized computerized medical record system provides the data needed to initiate companion animal surveillance programs on a national level, with applicability to both animal and human health.

The purpose of this study was to use a subset of medical records from Banfield, The Pet Hospital, with approximately 360 clinics and hospitals in 44 states, to estimate the prevalence of selected canine and feline infectious diseases. These fully computerized medical records contain information on patient demographics, physical examination, laboratory tests, diagnoses, and treatments, all linked by a unique pet ID number and hospital visit code. Patient records of 21,201 dogs and 6,704 cats from 6 hospitals in a southeastern urban center for an 18-month period were studied. . Of tests routinely recommended to screen healthy pets, 177 (2.8%) of 6203 heartworm adult antigen tests in dogs were positive. In cats, 23 (1.3%) of 1763 FeLV ELISA tests and 15 (0.9%) of 1757 FIV ELISA tests were positive, and were lower than published seroprevalence rates of 5.3% for FELV and 2.3% for FIV in unowned free-roaming cats in the same geographic area. For diagnostic tests usually administered to sick pets, 85 (35.6%) of 239 canine parvovirus fecal antigen tests were positive while 0 (0%) of 6 heartworm adult antigen tests were positive in cats. The concordance between a positive parvovirus antigen test in dogs and a diagnosis of parvovirus in the medical record was 82.8% (kappa = 0.63). Patients' addresses were converted into map coordinates for geospatial and temporal cluster analysis using GIS software. The results will be presented using data from all Banfield hospitals and >1,000,000 patients.

The computerized medical records of Banfield hospitals proved useful for estimating disease prevalence in companion animals. Other uses include identifying disease clusters possibly related to environmental risk factors or transmission of infectious agents, alerting veterinarians to the occurrence of epidemics of disease in their area, evaluating the effectiveness of disease prevention strategies or the efficacy of therapies, and determining the rate of adverse events associated with specific veterinary biologicals or pharmaceuticals.

## ABSTRACT #6

EVIDENCE OF NATURAL *ANAPLASMA PHAGOCYTOPHILA* INFECTION IN DOGS FROM WESTERN WASHINGTON STATE. JK Shinozaki<sup>1</sup>, FM Poutout<sup>1</sup> and PJ Stockwell<sup>2</sup>. <sup>1</sup>Phoenix Central Laboratory for Veterinarians, Everett, WA and <sup>2</sup>Clinical Research Center, Marshfield Clinic Research Foundation, Marshfield, WI.

*Anaplasma phagocytophila* is a recently designated species unifying the former taxa *Ehrlichia equi*, *Ehrlichia phagocytophila*, and the human granulocytic ehrlichiosis (HGE) agent. Infection of dogs in the Pacific Northwest region of the United States with *A. phagocytophila* has not been previously reported. The purpose of this report is to describe the clinicopathologic, serologic, and molecular findings in dogs residing in western Washington State that were naturally infected with *A. phagocytophila*.

Medical records of 6 client-owned dogs from western Washington State with ehrlichial-like morulae observed in circulating neutrophils were studied retrospectively for determination of the etiologic agent. The mean age of infected dogs was 8.3 years. All cases were diagnosed between April and October. There was no history of travel in 4 of the 6 dogs during the 6 months prior to presentation. One dog had traveled to Oregon, Idaho, and Montana. One dog had traveled through Idaho, Montana, and Canada (Alberta and Saskatchewan). None were known to have exposure to ticks. Clinical signs were nonspecific and most commonly included fever (5/6 dogs), lethargy (5/6 dogs), and anorexia (5/6 dogs). The most common hematologic and biochemical abnormalities were lymphopenia (6/6 dogs), thrombocytopenia (5/6 dogs), and high serum alkaline phosphatase activity (5/6 dogs). Antibody titers to *Ehrlichia canis* and *Ehrlichia equi* were obtained from 4 and 5 dogs, respectively, for which stored sera were available for indirect fluorescent antibody testing. No dog seroreacted to *E. canis* antigens. All dogs were seropositive for *E. equi*. Portions of the 16S rRNA gene amplified by the polymerase chain reaction from EDTA anticoagulated whole blood of 5 dogs were directly sequenced. Comparison of these sequences to all bacterial sequences in the GenBank database identified homologies with *A. phagocytophila*. Treatment with doxycycline or tetracycline resulted in rapid resolution of signs in all dogs.

Based on serological and molecular evidence, we conclude that these dogs were infected with *A. phagocytophila*. *A. phagocytophila* should be considered as a differential for dogs residing in western Washington State that present with lethargy, anorexia, or fever, particularly in conjunction with lymphopenia, thrombocytopenia, and/or increased serum alkaline phosphatase.

## ABSTRACT #7

IMPORTANCE OF HEMOTROPHIC *MYCOPLASMA* SPECIES IN SWISS CATS AND DETECTION OF A NEW *MYCOPLASMA*-LIKE AGENT IN A CLINICALLY ILL CAT. B. Willi<sup>1</sup>, F.S. Boretti<sup>2</sup>, H. Lutz<sup>1</sup>, C.E. Reusch<sup>2</sup>, R. Hofmann-Lehmann<sup>1</sup>. <sup>1</sup>Clinical Laboratory and <sup>2</sup>Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

*Haemobartonella felis*, the causative agent of Feline Infectious Anemia, has recently been reclassified within the group hemotrophic *Mycoplasma* and two different species have been identified: *Mycoplasma haemofelis* and *Candidatus M. haemominutum*. Newly developed molecular methods have facilitated sensitive, specific identification and quantification of these infectious agents. The goal of the present study was to investigate the prevalence of hemotrophic *Mycoplasma* in the Swiss cat population, to characterize the *Mycoplasma* species in positive cats and to identify potential risk factors for infection and disease.

For these purposes, EDTA blood and plasma samples from 750 cats have been collected between March and December 2003. DNA was extracted from 200µl of blood and samples have been analyzed by conventional and quantitative TaqMan real-time PCR assays. The

16S rRNA gene of some positive samples were sequenced. Each cat was tested for FeLV and FIV infection by ELISA and case histories were compiled and evaluated.

Preliminary results from conventional and TaqMan PCR indicate that *Cand. M. haemominutum* infection can be detected in 9.3% of Swiss cats, whereas *M. haemofelis* was found in only 1.0% of the tested population. In addition, a so far uncharacterized *Mycoplasma*-like agent was identified in a clinically ill cat by means of conventional PCR and sequencing of the PCR product. The sequence was not detectable by TaqMan PCR. The agent was readily transmittable via inoculation of infectious blood into two SPF cats. One of them was immune-compromised by administering corticosteroids. Both cats became PCR positive one week after infection. The immunosuppressed cat developed a severe anemia with a drop of PCV from 33% to 17%, whereas the untreated animal showed a moderate decrease of PCV from 34% to 26%. An increase in osmotic fragility of the erythrocytes was observed. An additional TaqMan PCR assay was established to detect also this newly found agent.

Our results show that several hemotrophic *Mycoplasma* species are common in the Swiss cat population. As demonstrated for other countries, *Cand. M. haemominutum* was more prevalent than *M. haemofelis*. In addition, a new *Mycoplasma*-like agent was detected, which seems to be pathogenic and was undetectable by so far described TaqMan PCR assays.

## ABSTRACT #8

ANTIBODY RESPONSES TO FIV VACCINATION. Levy JK<sup>1</sup>, Crawford PC<sup>1</sup>, Slater MR<sup>2</sup>. <sup>1</sup>University of Florida, Gainesville FL, <sup>2</sup>Texas A&M University, College Station FL.

Vaccination of cats with Fel-O-Vax FIV induces antibodies that interfere with diagnosis of FIV infection using the currently licensed antibody-based ELISA kits. The purpose of this study was to determine the magnitude and duration of FIV test interference following vaccination. The time from vaccination to seroconversion as detected by the 2 ELISA tests (IDEXX SNAP and PetChek) (n=26) and the percent of cats remaining positive 1 year after vaccination (n=16) was determined. Presence of FIV antibodies in serum and milk of vaccinated queens (n=12) and passive transfer of these antibodies to their kittens (n=55) was also determined. The performance of available licensed and unlicensed antibody-based diagnostic tests for FIV (SNAP, PetChek, Western blot, and IFA) was evaluated with plasma from unvaccinated, uninfected cats (n=42), cats vaccinated with Fel-O-Vax FIV (n=41), and FIV-infected cats (n=41).

FIV antibodies were detected by SNAP and PetChek and persisted through 1 year in all cats tested. The SNAP test became positive earlier (2.3±0.5 weeks; range 2-3) than the PetChek (6.9±2.5 weeks; range 4-14). Antibodies were detected in serum of vaccinated queens and in milk throughout lactation. All kittens were positive for antibodies after nursing. At 8 weeks, 55% of kittens tested by SNAP and 63% of kittens tested by PetChek remained positive. All kittens were negative for FIV antibodies at 12 weeks of age. Test performance for the 4 antibody tests is reported in the table.

	SNAP	PetChek	Western blot	IFA
Sensitivity	100%	100%	98%	100%
Specificity-Unvaccinated Cats	100%	100%	98%	90%
Specificity-Vaccinated Cats	0%	0%	54%	22%

Currently available antibody-based FIV diagnostic tests are highly sensitive for FIV infection. Specificity is also high for the 2 ELISA tests and for the Western blot and lower for the IFA. All cats vaccinated with Fel-O-Vax FIV developed antibodies that were detected by all 4 tests. These antibodies appear in the first few weeks following vaccination and persist for at least a year. Kittens readily absorb colostral antibodies from FIV-vaccinated queens, and these antibodies interfere with FIV diagnosis past the age of weaning in a

majority of kittens. Positive FIV antibody test results may indicate that a cat is infected with FIV, is vaccinated against FIV, and/or has nursed from a vaccinated or infected cat. Currently available antibody-based FIV diagnostic tests cannot distinguish between antibodies induced by infection or vaccination and should be interpreted with caution.

#### ABSTRACT #9

PCR FOR DIAGNOSIS OF FIV INFECTION. Crawford PC<sup>1</sup>, Slater MR<sup>2</sup>, Leutenegger CM<sup>3</sup>, Levy JK<sup>1</sup>. <sup>1</sup>University of Florida, Gainesville FL, <sup>2</sup>Texas A&M University, College Station FL, <sup>3</sup>University of California-Davis, Davis CA.

Cats vaccinated with Fel-O-Vax FIV test “false-positive” for FIV infection when tested with the antibody-based kits currently licensed for diagnosis of FIV. The polymerase chain reaction (PCR) utilizes the detection of specific nucleic acid sequences for diagnosis of infectious diseases. PCR has been proposed as an alternative to antibody testing to avoid the interference of vaccination with FIV diagnostic testing. The purpose of this study was to determine diagnostic performance of several commercially available FIV PCR tests.

Blood was collected from SPF cats that were neither infected with nor vaccinated against FIV (n=42), SPF cats that were vaccinated with Fel-O-Vax FIV (n=41), and FIV-infected cats (n=41). FIV-infected cats included those with both experimental (n=19) and natural infections (n=22) representing FIV strains A, B, and C. FIV infection status in all cats was confirmed by virus isolation. Blinded blood samples were submitted to 3 laboratories in the United States and Canada offering FIV PCR to veterinary practitioners. All laboratories tested fresh blood samples (PCR 1-3), and 1 laboratory also tested samples submitted as dried blood smears (PCR 4). Sensitivity, specificity, and correct result were calculated for all PCR tests. Results of PCR testing are summarized in the table.

	PCR 1	PCR 2	PCR 3	PCR 4
Sensitivity	76%	93%	51%	41%
Specificity (unvaccinated)	100%	81%	81%	81%
Specificity (vaccinated)	95%	66%	44%	51%
Correct Result	90%	80%	59%	58%

All tests misidentified both uninfected and infected cats. False-positive results by all laboratories were higher in FIV-vaccinated cats than in unvaccinated SPF cats, suggesting that vaccination interferes with the performance or interpretation of FIV PCR tests. Performance of FIV PCR diagnostic tests currently marketed to veterinary practitioners in North America varies significantly in diagnostic accuracy. FIV PCR tests are an alternative to serological tests, but results should be interpreted with caution.

#### ABSTRACT #10

ANTIBIOTIC SENSITIVITY PROFILES UNDERESTIMATE THE PROPORTION OF RELAPSING INFECTIONS IN CATS WITH CHRONIC RENAL FAILURE AND URINARY TRACT INFECTION. Freitag<sup>1</sup>, R.A. Squires<sup>1</sup>, J. Schmid<sup>2</sup>, J. Elliott<sup>3</sup>. <sup>1</sup>Institute for Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, NZ. <sup>2</sup>Institute for Molecular Biosciences, Massey University, Palmerston North, NZ. <sup>3</sup>Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK.

Urinary tract infections (UTI) caused by *Escherichia coli* are common in cats with stable chronic renal failure (CRF), particularly in females. These infections may be clinically inapparent and recur frequently after treatment. Recurrent UTI may contribute to the progression of feline CRF, or cause other health problems, so these infections merit study. Clinicians commonly use antibiotic sensitivity profiles to distinguish between relapses and reinfections. However,

the precision with which antibiograms distinguish feline uropathogenic *E. coli* clones has not been thoroughly investigated.

We studied 17 cystocentesis-derived *E. coli* isolates from five cats with CRF. Three cats were found to be infected on two occasions, one cat on three occasions, and one cat on eight occasions over a period of nearly two years. The interval between successive diagnoses in individual cats ranged from 6 weeks to 2.5 years. Cats received antibiotic therapy each time UTI was diagnosed. A repeat urine culture 4-7 days after antibiotic therapy confirmed urine sterility in the majority of cases. Sensitivities to 8 antibiotics were determined by Kirby-Bauer disc diffusion testing. Of these 8 antibiotics, only three were useful in discriminating isolates; non-identical profiles were interpreted as evidence of reinfection rather than relapse. All *E. coli* isolates were later genetically characterized by pulsed-field gel electrophoresis (PFGE) and virulence factor genotyping (VFG) using multiplex PCR to determine the presence or absence of 25 putative urovirulence genes.

PFGE and VFG findings were 100% in accord and suggested that 8/12 recurrent *E. coli* infections were relapses and 4 were reinfections. One cat was found to be infected consecutively 6 times with one *E. coli* clone over a period of 427 days. Using PFGE/VFG as our gold standard, antibiograms incorrectly categorized 5/8 relapses and 1/4 reinfections in 3/5 cats. Sensitivity and specificity of antibiograms for detection of relapse were 37.5% and 75%, respectively.

These data indicate that individual *E. coli* clones can cause recurrent UTI in cats with CRF over a prolonged period (at least 427 days) despite intermittent and apparently successful antibiotic therapy. Whether the clones that cause such relapses persist in the external environment, the cat’s gastrointestinal tract, or, as has recently been shown in mice, inside epithelial cells within the cat’s urinary tract remains to be determined and has practical implications for monitoring and therapy. Kirby-Bauer disc diffusion test results cannot be relied upon to distinguish precisely between relapses and reinfections in cats with uropathogenic *E. coli* infections, in this study tending to underdiagnose relapses.

#### ABSTRACT #11

VIRULENCE GENOTYPES OF FELINE URINARY *ESCHERICHIA COLI* ISOLATES FROM NEW ZEALAND AND GREAT BRITAIN DIFFER. Freitag<sup>1</sup>, R.A. Squires<sup>1</sup>, J. Elliott<sup>2</sup>. <sup>1</sup>Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, NZ. <sup>2</sup>Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK.

Some of the strains of *E. coli* that cause urinary tract infection (UTI) in dogs and cats are reportedly indistinguishable from strains that cause serious extraintestinal infections in humans, despite the use of highly discriminating molecular tests including macrorestriction analysis and extended virulence factor genotyping. There is little incriminating evidence concerning feline urinary *E. coli*, but the potential for zoonotic transmission from pet cats to humans has nevertheless been suggested. One of several factors complicating this field of study is that human urinary *E. coli* isolates from different geographic regions have been shown to differ genetically. It is to be expected that feline urinary *E. coli* isolates will also differ according to geographic origin, but this has not been studied. Such information would assist future epidemiological investigations of this potential, unproven, zoonotic association.

We retrospectively studied 36 cystocentesis-derived *E. coli* isolates from cats with UTI, 15 from New Zealand, and 21 from UK, by extended virulence factor (VF) genotyping using multiplex PCR to determine the presence or absence of 25 genes encoding fimbrial structures, toxins, siderophores, and protectins. We also carried out macrorestriction analysis of *Xba*I-digested bacterial DNA from each isolate using pulsed-field gel electrophoresis (PFGE).

PFGE revealed 36 distinct band patterns, the closest DICE similarity between any two clones being 69%. According to UPGAMA cluster analysis of VF profiles, the 36 isolates segregated into 3 groups at a similarity level of approximately 60%, two of which contained exclusively UK isolates, the third being mixed. Cross-validated discriminant analysis based on the VF genotypes correctly categorized 15/15 NZ isolates and 17/21 UK isolates. Four P fimbrial-encoding genes *papA*, *papEF*, *papC*, and *papG III* were present in all of the New Zealand isolates, but in a significantly smaller proportion, 43%, of UK isolates (Fisher's exact test,  $p < 0.0005$ ). Conversely, the protectins colicin (*cvaC*), serum survival factor (*iss*), and serum resistance factor (*traT*) were absent from all of the NZ isolates but present in 42%, 48%, and 67% of UK isolates, respectively (Fisher's exact test,  $p \leq 0.005$ ).

This study has revealed substantial VF genotypic differences between feline urinary *E. coli* isolates from UK and New Zealand and calls into question the use of VF genotypes to study the zoonotic potential of feline urinary *E. coli* unless the source of isolates is carefully considered. In this retrospective study, clinical information concerning the NZ cats was incomplete, whereas all of the UK cats had been diagnosed with renal insufficiency or chronic renal failure. Therefore it cannot be ruled out that different disease states in the members of these two populations contributed to the genetic differences detected in the *E. coli* isolates derived from them.

#### ABSTRACT #12

MOLECULAR ANALYSIS OF *CLOSTRIDIUM DIFFICILE* ISOLATES FROM DOGS IN A SMALL ANIMAL INTENSIVE CARE UNIT. L Arroyo, JS Weese, JK Clooten, SA Kruth, HR Staempfli. Dept of Clinical Studies, Ontario Veterinary College, Guelph, Ontario.

*Clostridium difficile* is a recognized enteropathogen of a variety of species, including dogs. Clinical and subclinical nosocomial infection has been widely studied in humans, and a recent study investigated carriage fecal of *C. difficile* in animals in a small animal intensive care unit (Clooten et al, Unpublished data). In this study, 87 *C. difficile* isolates were obtained from 71/402 (18%) of animals. *Clostridium difficile* was isolated from 37 dogs at admission while the remaining isolates were obtained from fecal cultures taken every 3 days during hospitalization.

Isolates from the Clooten et al study were frozen for future analysis and PCR-ribotyping was subsequently performed as the basis of this study. Isolates were grown in pure culture using standard techniques. Ribotyping was performed as has been previously described and PCR products were visualized via gel electrophoresis. Isolates were classified into arbitrarily named types based on the number and location of bands.

Seventy-three *C. difficile* isolates from 61 dogs were evaluated. Six distinct ribotypes were identified: A (n=47), Aa (n=3), B (n=17), C (n=3), D (n=1) and E (n=1). Of the isolates obtained at admission, 20/31 (65%) were A, 1 (3%) was Aa, 9 (29%) were B and 1 (3%) was C. Using date of admission and date of first isolation, nosocomial infection was initially considered a possibility in 34 (56%) dogs. Ribotyping results ruled out nosocomial infection in 5 (15%) of these cases. Of the remaining potentially nosocomial cases, 21 (72%) were type A, 1 type Aa (3%) and 7 type B (24%). Interestingly, 43/47 (91%) type A isolates were toxigenic *in vitro* versus only 1/17 (6%) type B isolates (Fisher's exact  $P < 0.0001$ ). Of the minor isolates, 3/3 (100%) Aa, 2/3 (67%), 0/1 D and 0/2 E were toxigenic. Of the 11 animals from which *C. difficile* was isolated on more than 1 occasion, two different isolates were identified from 7 (64%) dogs. The second isolate identified was suspected of having been nosocomial in all instances based on concurrent hospitalization of a dog with an indistinguishable ribotype.

Ribotyping is a valuable tool in the investigation of *C. difficile* associated disease. Ribotyping can help differentiate nosocomial

versus community-acquired disease, and can be used to investigate clinical and laboratory differences between isolates. For example, the difference in toxigenicity between type A and B, the two predominant strains, was unexpected and requires further study. Comparison of ribotypes of *C. difficile* isolates from different species could be useful to help understand the potential for interspecies transmission of this potential pathogen.

#### ABSTRACT #13

EFFICACY OF FEBANTEL/PYRANTEL/PRAZIQUANTEL FOR THE TREATMENT OF *GIARDIA* INFECTION IN CATS. AV Scorza, SV Radecki and MR Lappin. From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

*Giardia* in cats can be difficult to manage medically. Currently available treatments are sometimes toxic (metronidazole; albendazole) or have < 100% efficacy (fenbendazole). The combination of febantel/pyrantel/praziquantel (Drontal Plus®, Bayer Animal Health, Merriam, KS) has been effective for treating *Giardia* infection in some dogs and is known to be safe when administered to cats.

The purpose of this study was to evaluate febantel/pyrantel/praziquantel as a treatment for giardiasis in experimentally-inoculated cats. To initiate infection, kittens were administered *Giardia* cysts isolated from a naturally-infected cat by stomach tube while sedated. Feces from each cat were collected daily and analyzed for the presence and number of *Giardia* cysts using a commercially available direct immunofluorescence test (Meridian Diagnostics, Cincinnati, Ohio). In experiment 1, five infected cats were administered febantel/pyrantel/praziquantel at the dose of one small dog tablet/cat, PO, for five days and four infected cats were used as untreated controls. In experiment 2, six infected cats were administered febantel/pyrantel/praziquantel at the dose of two small dog tablets/cat, PO, for five days and five infected cats were used as untreated controls. All cats testing negative for *Giardia* cysts on day 18 after the beginning of therapy were administered 20 mg/kg methylprednisolone acetate, IM weekly for a maximum of two injections. Mean percent positive fecal results and cyst scores were calculated for the cat groups in the intra-treatment and post-treatment periods and compared between groups using ANOVA appropriate for a repeated measure experiment (the MIXED procedure of SAS, SAS Institute, Cary, NC).

Treated cats in experiment 1 had less positive samples ( $P < 0.05$ ) and shed less cysts ( $P < 0.05$ ) than untreated cats, but *Giardia* infection was maintained. Treated cats in experiment 2 had less positive samples ( $P < 0.05$ ) and shed less cysts ( $P < 0.05$ ) than untreated cats, and *Giardia* infection was apparently eliminated in four of six treated cats even after attempted immunosuppression. Drug toxicity was not noted in either experiment.

Based on these results, the administration of two small dog tablets/cat of the febantel/pyrantel/praziquantel combination PO for five days may be an effective treatment of *Giardia* infection in some cats.

#### ABSTRACT #14

EVALUATION OF ZINC SULFATE FECAL FLOTATION AND TWO ENZYME IMMUNOASSAYS FOR THE DETECTION OF *GIARDIA DUODENALIS* IN NATURALLY INFECTED KITTENS Stanley L. Marks, Ann Melli, and Philip H. Kass. University of California, Davis, School of Veterinary Medicine, Davis, CA.

*Giardia duodenalis* is a ubiquitous enteric protozoan affecting humans and a range of domestic and wild mammals. Most clinically apparent infections in cats occur in young animals, with the most consistent sign being diarrhea. The disease is usually self-limiting; however, the chronic form may last for years and is more common in immunocompromised cats. Despite the relatively high prevalence of

*Giardia* in cats worldwide, there is a paucity of information evaluating the performance characteristics of commonly utilized diagnostic tests for the detection of *Giardia* cysts or coproantigens. The goal of this study was to compare the performance characteristics of three diagnostic tests utilized for detection of *Giardia duodenalis* cysts or antigen in feline fecal specimens; the zinc sulfate fecal flotation, and the ProSpecT *Giardia* Rapid Immunoassay, and ProSpecT *Giardia* Microplate Immunoassay (EIA, Alexon-Trend, Inc., Ramsey, MN).

All tests were evaluated on freshly collected fecal specimens obtained once daily for four consecutive days from 100 naturally infected domestic shorthair kittens, housed individually. Sensitivities, specificities, positive (PPV) and negative predictive values (NPV) were calculated for the zinc sulfate and EIA tests using the direct immunofluorescence assay as the gold standard (Merifluor Cryptosporidium/*Giardia*, Meridian Diagnostics, Cincinnati, OH). The cumulative results are documented in the table below.

	Sensitivity	Specificity	PPV	NPV
Zinc Sulfate Flotation	0.755 ± 0.142	0.841 ± 0.015	0.570 ± 0.044	0.923 ± 0.047
ProSpecT Microplate EIA	0.862 ± 0.086	0.832 ± 0.023	0.592 ± 0.044	0.953 ± 0.032
ProSpecT Rapid EIA	0.556 ± 0.141	0.975 ± 0.020	0.858 ± 0.104	0.887 ± 0.030

The number of cysts visualized via zinc sulfate flotation varied markedly from day to day in 31/100 cats, and was reflected by the wide range of sensitivities for the zinc sulfate flotation test on each of the four days, respectively (0.56, 0.74, 0.85, and 0.87).

In conclusion, the ProSpecT Microplate EIA had the highest sensitivity (86%), whereas the sensitivity of the ProSpecT Rapid EIA was unacceptably low (56%). The number of *Giardia* cysts shed in feces varies markedly from day to day, underscoring the value of performing more than one zinc sulfate fecal flotation when utilizing this method to diagnose *Giardiasis* in cats.

#### ABSTRACT #15

EFFECT OF LEVOTHYROXINE ADMINISTRATION ON HEMOSTATIC ANALYTES IN DOBERMAN PINSCHERS WITH VON WILLEBRAND'S DISEASE. Heseltine JC<sup>1</sup>, Pancieria DL<sup>1</sup>, Feldman BF<sup>2</sup>, Troy GC<sup>1</sup>, Brooks MB<sup>3</sup>. <sup>1</sup>Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA; <sup>2</sup>Department of Biomedical Sciences and Pathobiology, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA; <sup>3</sup>Diagnostic Laboratory, College of Veterinary Medicine, Cornell University, Ithaca, NY.

This study tested the hypothesis that levothyroxine supplementation acts to increase plasma von Willebrand Factor (vWF) concentration and enhance vWF function. The effects of levothyroxine administration were evaluated in 8 adult, euthyroid Doberman Pinschers with plasma vWF concentration <30%, characteristic of Type 1 von Willebrand's disease (vWD). Levothyroxine (0.04 mg/kg PO q 12 hours) and placebo were administered for 30 days in a 2-period, 2-treatment, double-blinded, crossover design with a 30-day washout between treatments. Buccal mucosal bleeding time (BMBT), vWF concentration (vWF:Ag), vWF collagen binding activity (vWF:CBA), Factor VIII coagulant activity (FVIII:C), serum total thyroxine (T4), free thyroxine (fT4), 3,5,3'-triiodothyronine (T3), and thyroid stimulating hormone were measured on days 0, 2, and 30 of each treatment period.

The 8 dogs (1 male, 7 females) had a mean age of 3 years, with marked reduction in baseline plasma vWF:Ag and a proportional lack of vWF:CBA (mean vWF:Ag = 8.9%, mean vWF:CBA = 11.1%; reference range > 70%). In contrast, all dogs had FVIII:C within reference range. The BMBT varied among dogs, with 4 of 8 having values < 5 minutes. The response to placebo versus active levothyroxine treatment revealed no significant differences between groups at days 0, 2, or 30 for BMBT, vWF:Ag, vWF:CBA, and

FVIII:C. Serum total thyroxine, fT4, and T3 were significantly higher in the levothyroxine-treated group compared to the placebo group at days 2 and 30. Thyroid stimulating hormone was significantly lower in the levothyroxine-treated group compared to the placebo group at days 2 and 30. No clinical signs of hyperthyroidism were observed. Levothyroxine at 0.04 mg/kg caused laboratory evidence of hyperthyroidism but did not affect plasma FVIII and vWF concentrations or the vWF-dependent functional parameters of collagen binding and BMBT. The results of this study do not reveal a direct action of levothyroxine supplementation on plasma vWF concentration or activity in euthyroid Doberman Pinschers with Type 1 vWD.

#### ABSTRACT #16

PREDNISONE AND CYCLOSPORINE VS. PREDNISONE ALONE FOR TREATMENT OF CANINE IMMUNE MEDIATED HEMOLYTIC ANEMIA (IMHA). B. Husbands, D. Polzin, P.J. Armstrong, L. Cohn, N.E. Patterson. University of Minnesota, Veterinary Medical Center, Saint Paul, MN.

Canine IMHA has an unacceptably high mortality rate. Cyclosporine is reported as an effective therapy for immune-mediated diseases in human patients. We hypothesized that cyclosporine would improve survival in dogs with IMHA.

A double-masked, randomized, controlled clinical trial was performed on 38 dogs (17 males; 21 females). Dogs admitted into the study had idiopathic immune mediated hemolytic anemia as evidenced by hemolytic anemia and a positive Coomb's test, autoagglutination, and/or spherocytosis. Dogs were excluded if they had concurrent disease or had received therapy with any immunosuppressive drug or more than 3 days of prednisone therapy. Two treatment protocols were compared. In addition to prednisone at 2-4 mg/kg/day PO, Group I dogs received cyclosporine (3-5 mg/kg/every 12 hours PO) and Group II received placebo. Prednisone at the initial dose and cyclosporine or placebo were administered for 28 days and all dogs were monitored for 1 year. All dogs received heparin (200 U/kg q 8 hrs SQ) until the PCV exceeded 30%. Supportive therapies including transfusions were provided, as indicated.

There was no significant difference in survival between treatment groups by Kaplan Meier survival analyses. 8/19 Group I dogs and 7/19 Group II dogs died of disease-related causes in the first 28 days. Thereafter, there was only one disease-related death (relapse - Group II). Three additional Group II dogs experienced a relapse and survived. A clinical diagnosis of thromboembolism was made in 11/19 Group I dogs and 10/19 Group II dogs. No adverse effects attributable to cyclosporine therapy were observed.

#### ABSTRACT #17

THE USE OF LITHIUM CARBONATE TO PREVENT CARBOPLATIN-INDUCED THROMBOCYTOPENIA IN DOGS. Amélie Leclerc, Anthony Abrams-Ogg, Stephen Kruth, Dorothee Bienzle; Ontario Veterinary College, Guelph, Ontario, CANADA.

Thrombocytopenia is a side effect of carboplatin treatment in dogs. It usually does not cause clinical bleeding, but may be sufficiently severe to delay further treatment or necessitate selection of another chemotherapeutic agent. Thrombopoietic cytokines such as IL-11 have been used in humans to prevent thrombocytopenia associated with chemotherapy, but the cost of the drug, lack of wide availability, and antibody formation have made its use impractical in dogs. Lithium carbonate ameliorated neutropenia associated with certain chemotherapeutic agents, and stimulated GM-CSF production, in dogs. Experience in other species indicated conflicting benefits of lithium carbonate to attenuate thrombocytopenia induced by chemotherapy or radiation therapy. The purpose of this study was to

determine whether lithium carbonate prevents thrombocytopenia induced by carboplatin in healthy dogs.

Eighteen healthy beagles were randomly assigned to one of 3 groups: lithium (10 mg/kg PO BID, days 2 – 21), carboplatin (300 mg/m<sup>2</sup> IV, day 1), or carboplatin and lithium (same doses). Blood platelet counts and lithium levels were determined on day 1 and every 2 days thereafter. Megakaryocytes were enumerated in bone marrow biopsies, and CD34+ cells were identified in bone marrow aspirates by flow cytometry on days 1, 7, 14 and 21.

Platelet counts were significantly higher ( $p < 0.05$ ) in the group that received lithium alone compared to groups receiving carboplatin or carboplatin with lithium. A significant progressive increase in the platelet count over 21 days was noted in the group receiving lithium alone while a significant decrease (nadir day 11) was noted in the groups receiving carboplatin, with or without lithium. There was no significant difference between platelet counts of groups receiving carboplatin or carboplatin and lithium. There was no significant difference between groups for plasma lithium concentration, megakaryocyte count, and CD34+ bone marrow cells.

We concluded that carboplatin induced thrombocytopenia and that administration of lithium carbonate did not prevent thrombocytopenia from occurring. Lithium carbonate induced a significant increase in the platelet count of normal dogs not receiving chemotherapy, which may indicate that lithium stimulates platelet production. Based on these results, further studies using a different schedule of lithium administration are warranted.

#### **ABSTRACT #18**

EFFECT OF ORAL ADMINISTRATION OF UNFRACTIONATED HEPARIN ON COAGULATION PARAMETERS AND PLASMA, URINE, AND FECAL HEPARIN LEVELS IN THE DOG. M. Sivasankar<sup>1</sup>, A.P. Carr<sup>1</sup>, J.D. Stachnik<sup>1</sup>, L.M. Hiebert<sup>2</sup>, and S.M. Wise<sup>2</sup>. Departments of Small Animal Clinical Sciences<sup>1</sup> and Veterinary Biomedical Sciences<sup>2</sup>, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada.

Heparins are naturally occurring glycosaminoglycans, with variations in molecular weight and numbers of sulphur bonds between molecules affecting their activity. Heparin acts by binding with antithrombin III (ATIII) and causing accelerated neutralization of serine proteases XIIa, XIa, and thrombin by ATIII. Heparin can also act on heparin cofactor II (HCII), directly on thrombin, or bind to platelets. Heparin has been utilized in treatment protocols for disseminated intravascular coagulation and its use may be warranted in other diseases associated with thrombosis such as cardiomyopathies, renal disease, immune mediated disease, and peripheral vein thrombosis. Alternate administration routes that have been explored include oral, nebulization, intrapulmonary, and topical. Rat thrombosis models have demonstrated that oral heparins are quickly routed to the endothelium resulting in minimal alterations in coagulation parameters yet detectable reductions in thrombus formation. The oral route may be an effective and less invasive mode of therapy available to practitioners in the management of thrombotic disorders.

Six healthy dogs were used in the study. Plasma and urine samples were collected at 0, 3, 6, 20, 60 minutes and 4, 8, 12, 24, 48, and 72 hours after oral administration of unfractionated heparin (Beef Lung Heparin, Scientific Protein Laboratories, Waunakee, WI) at 7.5 mg/kg. Fecal samples were collected at 0, 24, 72 hours after heparin administration. The change in plasma and urine antiIIa, plasma and urine antiXa, plasma ATIII, and plasma and urine heparin concentrations over time were examined using generalized estimating equations with a marginal model to account for multiple observations from individual animals (SAS v.8.2 for Windows (PROC GENMOD): SAS Institute, Cary, North Carolina, USA). The median and range were calculated for each parameter at each time point. Although none of the outcomes varied significantly over time

( $p < 0.05$ ), there were differences noted in each of the variables as compared to levels prior to drug administration. No clinical evidence of coagulation abnormalities were detected in any of the dogs during the study. The small number of subjects limited the power of this study but oral heparin therapy may have applicability in therapy for animals prone to thromboembolic disease. Further studies involving more subjects are warranted prior to clinical use of oral heparin. Studies are also warranted to investigate the effects of oral heparin at the level of the endothelium in the dog.

#### **ABSTRACT #19**

THE USE OF A LOW MOLECULAR WEIGHT HEPARIN IN 6 DOGS. M. Dunn, V. Charland, C. Thorneloe, University of Montreal, St-Hyacinthe, Quebec, Canada.

Low molecular weight heparins (LMWH) are derived from standard heparin (MW 15 000 Daltons) and possess an average molecular weight of 5000 Daltons. In dogs, LMWHs have been shown to have a higher bioavailability, a longer half-life and are less protein-bound as compared to standard heparin. LMWHs exert their anticoagulant activity by binding to antithrombin III and preferentially inhibiting factor X. This preferential inhibition results in inhibition of hemostasis with minimal prolongation of PT and PTT. These characteristics make LMWHs an interesting therapeutic alternative to standard heparin therapy.

The goal of this pilot study was to evaluate the anticoagulant activity of a LMWH in 6 dogs suffering from diseases associated with hypercoagulable states and evaluate the repercussions of this therapy on various hemostatic parameters. A prospective randomized clinical study comparing a LMWH to standard heparin in dogs presenting with diseases associated with a hypercoagulable state is presently underway.

The LMWH, dalteparin, was chosen as its pharmacokinetics have been described in healthy dogs. Baseline CBC, PT, PTT, FDP, d-dimer, AT III, BMBT and anti-Xa activity were measured. Dogs then received 150 U/kg of dalteparin SC BID for 3 days. An Hct, platelet count, PT, PTT, FDP, d-dimer, At III, anti-Xa activity and BMBT were assessed on day 1, 3 and 12 hours following dalteparin administration. These parameters were also assessed on days 2 and 3, 3 hours following dalteparin administration. A Wilcoxon test was used to assess the effect of dalteparin on coagulation parameters.

Six dogs were enrolled in the study. Two dogs had pancreatitis, 1 gastric torsion, 1 pulmonary abscess, 1 IMHA and 1 splenic mass. All dogs tolerated the dalteparin well and none showed clinical bleeding. Two dogs died related to their illness and 4 dogs survived. No significant differences following dalteparin therapy were observed in the following parameters: Hct, platelet count, PT, PTT, At III and BMBT. Dogs showed a significant increase in anti-Xa activity 3 hours following dalteparin administration ( $p = 0.03$ ). Anti-Xa activity levels 3 hours following dalteparin administration varied from 0.6 – 1.1 U/ml and at 12 hours varied from 0.1 – 0.3 U/ml. No dogs showed any clinical evidence of thrombosis during the study.

In human medicine, an anti-Xa activity level of 0.3 – 0.6 U/ml is sufficient to prevent thromboembolic disease. It has been suggested that anti-Xa activity values in this range are also necessary in other species in order to prevent thrombosis. All dogs in this study had anti-Xa activity levels in this range at T3 without showing any prolongation of bleeding times or adverse effects. Anti-Xa activity levels dropped considerably in all dogs at 12 hours (2 dogs were no longer in therapeutic range).

In this study, dalteparin provided anti-Xa activity levels in the range necessary for thromboprophylaxis without increasing bleeding times. Dalteparin's twice a day administration and lack of side effects makes it a convenient and safe choice for thromboprophylaxis in the dog. A study comparing dalteparin and standard heparin is currently underway.

## ABSTRACT #20

FACTOR VIII MARKER GENOTYPING FOR HEMOPHILIA A CARRIER DETECTION IN DOGS. MB Brooks, JL Barnas, JJ Fremont, J. Ray. Cornell University, College of Veterinary Medicine, Ithaca, NY.

Hemophilia A is a common X-linked recessive bleeding disorder of dogs. The defect occurs in mixed breed and purebred dogs, apparently arising through frequent *de novo* mutations in the coagulation Factor VIII (FVIII) gene. Affected dogs are readily diagnosed by measuring FVIII coagulant activity (FVIII:C), however these assays do not accurately identify carrier females. Direct mutation detection is difficult, due to FVIII's large size (186 kb) and hemophilia's molecular heterogeneity. Our study tested the feasibility of linkage analyses, using a single intragenic FVIII marker, to enhance the accuracy of carrier detection in different breed-variants of canine Hemophilia A.

Newly diagnosed cases of hemophilia A and their relatives were recruited in a 2-year study period. Each pedigree consisted of at least 1 affected male and obligate carrier dam, with inclusion of available siblings, offspring, and mates of these index patients. Referral veterinarians submitted citrate plasma and EDTA blood samples for biochemical and molecular analyses. Plasma FVIII:C was measured in a clotting time test and von Willebrand Factor concentration (vWF:Ag) was measured in an ELISA. Genotyping for a highly polymorphic FVIII marker (cf8ms) was accomplished using cell lysates as template and fluorescent primers in a PCR to amplify the FVIII gene region containing the marker sequence. Marker allele size was assigned using an ABI 310 analyzer.

A total of 66 dogs were enrolled in the study, comprising 9 breed-variants of Hemophilia A: Border terrier, Boxer, Golden retriever, Irish setter, Jack Russell terrier, Labrador retriever, Maltese, Portuguese water dog, and Shih Tzu. Pedigree size ranged from 3 dogs to an extended pedigree of 23 Golden retrievers. Eight breeds were affected with severe Hemophilia A (FVIII:C < 2%) and Golden retrievers had mild Hemophilia (FVIII:C 5 to 8%). Factor analyses (FVIII:C and ratio of FVIII to vWF) correctly predicted carrier status for 9 of 11 obligate carrier females i.e. dams of affected males. Genotyping revealed 14 different-sized cf8ms alleles and an overall heterozygosity of 0.67 in the study population. In 1 of 9 pedigrees (Boxer), the obligate carrier dam was homozygous at the marker locus. In all other pedigrees, carrier dams were heterozygous and the marker allele associated with the Hemophilia A phenotype could be used to differentiate carrier from clear daughters. In these combined pedigrees, marker genotype classified 8 suspect females as clear and 9 suspect females as carriers. Two of the nine predicted carrier females were homozygous at the marker locus.

We conclude that carrier detection incorporating linkage analyses can be successfully applied to prevent the propagation of Hemophilia A in many breeds. The strategy could be enhanced through the development of additional informative FVIII markers.

## ABSTRACT #21

CHARACTERIZATION OF THE ANEMIA OF INFLAMMATORY DISEASE IN 21 CATS WITH ABSCESSSES, PYOTHORAX, OR FAT NECROSES. B. Kohn and M. Ottenjann. Small Animal Clinic, Free University of Berlin, Germany.

Anemia of inflammatory disease (AID) is the most common anemia in humans and animals. It is also known as anemia of chronic disease and is associated with inflammatory processes, chronic infections, post-traumatic conditions, and various neoplastic diseases. The pathogenesis is multifactorial, resulting from decreased iron availability, a decline in erythrocyte survival, and decreased response to anemia.

We describe here the hematologic features of 21 anemic cats with abscesses (12), pyothorax (6), and fat necroses (3). We excluded cats with a positive FeLV/FIV test result, neoplasias, nephro- and

endocrinopathies as well as blood loss anemia in order to avoid confounding factors. A complete blood cell count, clinical chemistry, the serum concentration of erythropoietin (EPO), iron, total iron binding capacity (TIBC), ferritin, acute-phase-proteins and Coombs' tests were performed. The Hct was monitored between 3-41 days (median=11).

Nine cats were anemic on presentation, whereas the others developed anemia during hospitalization. A Hct decline of 1-28% (m=10) was noted in 18 cats within 3-16 days (m=8). The anemia was mild (Hct 24-28%, n=11), moderate (17-22%, 7) to severe (12-13%, 3). At least on one occasion the MCV and MCHC values were slightly below the normal range in 2 and 8 cats, resp. The agg. reticulocyte counts were <40,000/ $\mu$ l in 18 of 21 anemic cats, and the anemia was mildly regenerative (43,000-85,000/ $\mu$ l) in the other 3 cats. A leukocytosis (18,900-100,000/ $\mu$ l, m=33,900) was observed in 17 cats, one cat had a leukopenia (2,730/ $\mu$ l). Mild hyperbilirubinemia (0.4-1.6mg/dl) was seen in 5 cats. Hypoalbuminemia (n=18; 11-29g/l, m=21) and hyperglobulinemia (n=16; 44-74g/l, m=56) resulted in a lower albumin/globulin-ratio in 19 cats (0.30-0.79, m=0.43). Iron (ref. range 33-134 $\mu$ g/dl) and TIBC (ref. 169-325 $\mu$ g/dl) concentrations were low in 2 (21 $\mu$ g/dl) and 6 (148-168 $\mu$ g/dl, m=167) cats, resp. Ferritin (ref. 31-144ng/ml) values were normal in 7 and mildly to severely elevated in 12 cats (190-5586ng/ml, m=393). The acute-phase proteins  $\alpha_1$ -acid-glycoprotein (ref. 0.1-0.48g/l) and haptoglobin (ref. 0.04-3.84g/l) were elevated in 15/15 (0.6-2.3, m=1.3) and 13/15 (5.3-13, m=9) cats, resp. EPO (ref. 0-20U/l, n=12) was normal in 4 (4-8U/l), mildly raised in 7 (23-74U/l) and severely (716U/l) elevated in 1 anemic cat. The Coombs' test was negative in 8 cats and positive for IgG in 1 cat. Two cats were euthanized due to the underlying disease, and 3 cats needed fresh whole blood transfusions.

In conclusion, AID in cats with abscessation, pyothorax, or fat necrosis was usually mild to moderate, nonregenerative, and normocytic normochromic. However, AID can be of clinical significance causing severe and transfusion-dependent anemia. AID seems to be multifactorial as in other species since there was evidence for inflammation, iron sequestration, insufficient EPO concentration and bone marrow response, and decreased RBC survival. Specific and supportive medical interventions including transfusions can reverse these processes in cats.

## ABSTRACT #22

ECCENTROCYTOSIS AND ASSOCIATED DISEASES IN THE DOG: REVIEW OF 60 CASES. M. Caldin<sup>1</sup>, T. Furlanello<sup>1</sup>, E. Carli<sup>1</sup>, S. Tasca<sup>1</sup>, C. Patron<sup>1</sup>, G. Lubas<sup>2</sup>. <sup>1</sup>Veterinary Clinic "San Marco", Padova, Italy; <sup>2</sup>Dipartimento Clinica Veterinaria, Università di Pisa, Pisa, Italy.

The eccentrocyte or erythrocyte hemighost is a red blood cell that appears, at light microscopy, as its hemoglobin has been confined to one side of the cell, leaving the remaining part quite pale and clear. This morphologic abnormality is induced by oxydation of both the cell membrane and its cytoskeleton. When the oxydation is directed toward hemoglobin it induces Heinz bodies formation which have been more commonly described in the cat. Eccentrocytes in dogs have been rarely reported and associated to food addition of onion and/or garlic and selected drugs administration. This abstract reports the prevalence of eccentrocytosis in dogs, attempting to correlate this finding to various clinical presentations.

In a period of 2.5 years we performed a total of 5,086 CBCs on 4,251 canine patients admitted to the Veterinary Clinic for clinical consultation. Each sample was processed both with ADVIA 120<sup>®</sup> Bayer laser cell counter and peripheral blood smear microscopic evaluation, stained with a modified Wright technique (Aerospray slide stainer<sup>®</sup> 7120, Wescor). Eccentrocytes were semiquantitatively assessed in several 100x microscopic field graded 1+ (1-2 eccentrocytes), 2+ (3-8), 3+ (9-20), and 4+ (over 20). Full clinical



and laboratory data were available and evaluated in order to assess the presumptive etiopathogenesis of eccentrocytosis cases.

Eccentrocytosis has been observed in 60 dogs (1.4%). These findings were associated to: onion and/or garlic food addition (9), untreated vitamin K antagonist poisoning [VKAP] (9), ketoacidotic diabetes mellitus (7), drug administration (NSAID 3, azathioprine 1, propofol 1, cyclosporine 1), T multicentric lymphoma (5), other neoplasias (3). 19 dogs were affected by a miscellanea of various diseases, often medically and/or surgically treated. In only 2 cases the eccentrocytosis has been reported as an occasional finding, in otherwise healthy animal. 40/60 dogs (66.6%) were affected by various grade of anemia: 1 very severe, 3 severe, 19 moderate, and 17 mild. At the initial presentation 20 dogs were showing eccentrocytosis without anemia, but 3 dogs became severely anemic in the following days. Most of the cases had a 1/2+ grading and very few 3/4+ grading.

From our data, it seems that many diseases causing Heinz body formation in cats can induce eccentrocytosis in dogs instead. Furthermore, many other pathologic conditions are associated to this red blood cell abnormality. The reported prevalence of anemia could be linked to an hemolytic process secondary to the erythrocyte oxidation, although other causes of anemia should be considered. Hence, eccentrocytosis could be considered an important sign of ongoing hemolysis. The association between VKAP and eccentrocytosis in naïve dogs should also be pointed out, and hemolysis could be a contributing factor to the anemia in poisoned dogs.

#### ABSTRACT #23

EFFECT OF DESMOPRESSIN ON VON WILLEBRAND FACTOR MULTIMERS IN DOBERMAN PINSCHERS WITH TYPE 1 VON WILLEBRAND DISEASE: PLASMA COLLAGEN BINDING ACTIVITY AND MULTIMER ANALYSIS. MB Callan<sup>1</sup>, U Giger<sup>1</sup>, JL Catalfamo<sup>2</sup>. <sup>1</sup>School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA and <sup>2</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY.

Desmopressin (DDAVP) has been used to control or prevent mild bleeding in dogs with type 1 von Willebrand disease (vWD). Despite clinical evidence of improved hemostasis and marked shortening of the buccal mucosal bleeding time and ADP closure time as assessed by the Platelet Function Analyzer (PFA-100, Dade Behring) following DDAVP administration to dogs with vWD, there is only a modest rise in plasma von Willebrand factor antigen concentration (vWF:Ag), in contrast to the typical 3 to 5-fold increase in vWF:Ag in human patients. It has been postulated that DDAVP leads to a preferential increase in the high molecular weight (HMW) vWF multimers in dogs, as has been documented in humans. A greater increase in vWF collagen binding activity (vWF:CBA) compared to quantity (vWF:Ag), i.e., a decrease in the vWF Ag:CBA ratio, following DDAVP is attributed to a disproportionate increase in HMW multimers.

The purpose of this study was to assess the effect of DDAVP on HMW vWF multimers. We evaluated plasma vWF:Ag, vWF:CBA, a functional assay dependent on the presence of HMW vWF, and vWF multimer size following administration of DDAVP (1 µg/kg SC) to 16 Doberman Pinschers with type 1 vWD and 4 healthy control dogs. Blood samples anticoagulated with 3.8% sodium citrate were collected pre- and 1 hour post-DDAVP administration. Plasma vWF:Ag and vWF:CBA were assayed by ELISAs, and vWF multimers were separated by a stacked SDS-agarose gel electrophoresis, electrophoretically transferred to nitrocellulose membranes, and detected by chemiluminescence.

Mean plasma vWF:Ag and vWF:CBA in dogs with type 1 vWD increased from a baseline of 11.5 to 21.8% and 11.3 to 20.3%, respectively, 1 hour post-DDAVP. The vWF Ag:CBA ratios at baseline (0.98) and post-DDAVP (1.01) indicate a concordant

increase in quantity and functional activity, rather than a disproportionate increase in vWF:CBA due to a preferential increase in HMW multimers. In normal control dogs mean plasma vWF:Ag and vWF:CBA increased from a baseline of 71.3 to 119.3% and 61.5 to 114.5%, respectively. The pre- and post-DDAVP vWF Ag:CBA ratios were also not significantly different (1.16 vs. 1.04). Plasma vWF multimer analysis revealed proportional increases in band intensity for all multimer sizes in the post-DDAVP samples in comparison to the baseline samples from both the control dogs and dogs with vWD, consistent with the vWF Ag:CBA ratios of approximately 1.

We conclude that DDAVP's beneficial effect on primary hemostasis in many dogs with type 1 vWD cannot be explained by a preferential increase in HMW vWF multimers in dogs as has been suggested in humans, and, thus, its mechanism of action remains elusive.

#### ABSTRACT #24

CLINICAL ASSESSMENT OF ACID-BASE STATUS IN DOGS: CALCULATION OF PLASMA ATOT AND KA VALUES FOR USE IN THE STRONG ION AND SIMPLIFIED STRONG ION MODELS. Peter D. Constable,<sup>1</sup> Henry R. Stämpfli.<sup>2</sup> <sup>1</sup>Dept. of Veterinary Clinical Medicine, University of Illinois, Urbana-Champaign, IL; <sup>2</sup>Dept. of Clinical Studies, University of Guelph, Guelph Ontario, Canada.

Acid-base abnormalities are frequently present in sick dogs. Two quantitative mechanistic acid-base models are available for the clinical assessment of acid-base status: the strong ion model (*Can J Physiol Pharmacol* 61:1444, 1983) and the simplified strong ion model (*J Appl Physiol* 83:297, 1997). Both strong ion approaches require species-specific values for Atot (the total concentration of non-volatile buffers in plasma) and Ka (the effective dissociation constant for weak acids in plasma), and we have experimentally determined Atot and Ka values for horse, cattle, cat, and human plasma. The aim of this study was to determine Atot and Ka values for dog plasma.

Plasma was harvested from 10 healthy adult dogs and tonometered with CO<sub>2</sub> at 37 C. Plasma pH, Pco<sub>2</sub>, and plasma concentrations of quantitatively important strong cations (Na, K, Ca, Mg), strong anions (Cl, L-lactate), and non-volatile buffer ions (total protein, albumin, phosphate) were measured over a pH range of 6.9 to 7.8. Strong ion difference (SID) was estimated from the measured strong ion concentrations and nonlinear regression was used to calculate Atot and Ka from the measured pH and Pco<sub>2</sub> and estimated SID; the calculated Atot and Ka values were then validated using data (*Pflugers Arch.* 363:141, 1976) from in vitro HCl/NaOH titration of plasma from 6 dogs and data (*Scand J Clin Lab Invest* 14 Suppl 66:1, 1962) from in vivo studies in 12 dogs.

Mean (± SD) values for dog plasma were: Atot = (17.4 ± 8.6) mmol/l (equivalent to 0.27 mmol/g of total protein or 0.47 mmol/g of albumin); Ka = (0.17 ± 0.11) × 10<sup>-7</sup>; pKa = 7.77. The calculated SID for normal dog plasma (pH = 7.40; Pco<sub>2</sub> = 37 mm Hg; [total protein] = 64 g/l) was 27 mEq/l. The calculated values for Ka and SID differed from those empirically assumed for the dog (Ka = 3.0 × 10<sup>-7</sup>; SID = 40 mEq/l) and indicated that dog plasma proteins have a large net strong anion charge.

These results can be used to identify the mechanism for an acid-base disturbance in dogs, because acid-base changes are caused by a strong ion acidosis or alkalosis (change in SID), respiratory acidosis or alkalosis (change in P<sub>CO2</sub>), or a non-volatile buffer ion acidosis or alkalosis (change in Atot). At normal pH, a 1 mEq/l decrease in SID (due to a 1 mmol/l increase in L-lactate concentration) will decrease pH by 0.018, a 1 mm Hg increase in P<sub>CO2</sub> will decrease pH by 0.010, and a 1 g/l increase in total protein concentration will decrease pH by 0.001. The concentration of unmeasured strong anions (such as L-lactate) in dog plasma can be most accurately estimated by calculating the strong ion gap (SIG, in mEq/l) from the net strong

anion charge of plasma proteins, the net non-volatile buffer ion charge of plasma, and the anion gap ( $[\text{Na}] + [\text{K}] - [\text{Cl}] - [\text{HCO}_3]$  in mEq/l), whereby:  $\text{SIG} = [\text{total protein in g/l}] \{0.23 + 0.27 / (1 + 10^{(7.77 - \text{pH})})\} - \text{anion gap}$ .

#### ABSTRACT #25

IMMUNOHISTOCHEMICAL DETECTION OF CYCLIN-DEPENDENT KINASE INHIBITOR p27 IN MALIGNANT AND BENIGN CANINE MAMMARY TUMORS. Sébastien Overvelde, Michel Desnoyers, Christiane Girard, Pierre Hélie. Department of Pathology, College of Veterinary Medicine, University of Montréal, St-Hyacinthe, Québec, Canada.

Purpose of the study: Mammary tumors are frequent, representing the most common type of neoplasia in the bitch. Histopathological classification of those tumors as either benign or malignant can sometimes be difficult as criteria used to define malignancy vary considerably among pathologists. More and more, immunohistochemistry is used as a diagnostic tool for better classification of neoplasia. In human medicine, the determination of expression of cyclin-dependent kinase inhibitor (CDKI) p27 in breast cancer has shown good correlation with degree of malignancy, prognosis and survival time. To our knowledge, the expression of p27 in canine mammary tumors has never been evaluated.

Methods: Twenty-eight malignant and twenty-eight benign canine mammary tumors were examined. In each, the level of expression of p27 was determined and compared with the expression of a cellular proliferation marker (Ki-67). Prior to immunohistochemical staining, all tumors were reviewed by two board-certified anatomical pathologists to ensure diagnostic agreement. All tumors were stained with monoclonal antibodies for p27 and Ki-67. A scoring system was used for p27 looking at both the number and intensity of staining cells. This scoring system was also compared to the percentage of neoplastic cells staining for Ki-67.

Results: When using the scoring system for p27 to differentiate benign and malignant tumors, the latter expressed significantly less p27 ( $p < 0.0001$ ) and significantly more Ki-67 ( $p = 0.026$ ) than benign tumors. The specificity and sensitivity for diagnosing a mammary tumor as malignant when using a p27 score of 6 or less were 85.7% and 89.3% respectively.

Conclusion: The level of expression of p27 and Ki-67 are useful adjuncts to histopathology for a better classification of canine mammary tumors. Prospective studies looking at survival time and recurrence of neoplasia will be required to determine if expression of p27 and Ki-67 have a prognostic value in canine mammary tumors.

#### ABSTRACT #26

INHIBITION OF CELL DIVISION AND INDUCTION OF APOPTOSIS IN CANINE OSTEOSARCOMA CELLS BY MYCOBACTERIAL CELL WALL-DNA COMPLEX (MCC).

Mario C. Filion, Benoit Filion, and Nigel C. Phillips. Bioniche Therapeutics Division, Bioniche Life Sciences Inc, Montréal, Québec, Canada.

Mycobacterial cell wall-DNA complex (MCC<sup>1</sup>), a mycobacterial cell wall composition prepared from the non-pathogenic microorganism *Mycobacterium phlei*, is a bifunctional anticancer agent that induces apoptosis of cancer cells and stimulates cytokine synthesis by immune cells. MCC is currently being evaluated for the treatment of bladder cancer in humans. MCC also induces the synthesis of IFN- $\gamma$  by canine peripheral blood mononuclear cells (PBMC) in a concentration-dependent manner. In the present study, we have determined whether MCC has anticancer activity against two canine osteosarcoma cancer cell lines: D17 cells, isolated from a poodle, and D22 cells, isolated from a collie. MCC, in the concentration range 0.01-100  $\mu\text{g/ml}$ , directly inhibited the division of both osteosarcoma cell lines. The IC<sub>50</sub> for D17 cells was 3.9  $\mu\text{g/ml}$

and for D22 cells 44.4  $\mu\text{g/ml}$ . Inhibition of division was associated with the presence of activated caspase-3 and cleaved poly (ADP-ribose) polymerase (PARP), both detected by flow cytometry, and condensed nuclear DNA, determined by Hoescht 33258 staining, all of which are characteristics of cells undergoing apoptosis. MCC was also found to potentiate the antiproliferative activity of the bisphosphonates alendronate and pamidronate against these osteosarcoma cell lines. In conclusion, our results show that MCC exerts growth inhibition and apoptosis-inducing activity against two osteosarcoma cell lines *in vitro*. MCC may have potential in the treatment of canine osteosarcoma.

<sup>1</sup>US patent # 6,326,357 and 6,329,347

#### ABSTRACT #27

ELEVATED 15F<sub>2t</sub>-ISOPROSTANE CONCENTRATIONS IN THE URINE OF DOGS WITH LYMPHOMA. Brown MR, McMichael M, Rogers K, Barton C, Ruaux C, Williams DA. College of Veterinary Medicine, Texas A&M University, College Station, Texas.

Isoprostanes are a by-product of the action of reactive oxygen species on arachidonic acid, and are considered to be an accurate marker for oxidative stress *in vivo*. In the presence of oxidative stress, human B lymphoma cells are unable to undergo apoptosis and instead, die by a form of necrosis. Unlike apoptotic cells which are removed by phagocytosis and thus do not induce an inflammatory reaction, necrotic cells lyse, release their cellular contents into the extracellular space, and induce inflammation. The purpose of this study was to compare the concentrations of 15F<sub>2t</sub>- isoprostane, in the urine of clinically healthy control dogs and dogs with newly diagnosed, untreated lymphoma.

Free catch urine samples were obtained from 8 clinically healthy control dogs and frozen immediately at -80°C. Additionally, a free catch urine sample was obtained from 8 dogs with newly diagnosed, untreated lymphoma on initial presentation and frozen immediately at -80°C. Urinary creatinine concentration was measured in all samples. 15F<sub>2t</sub>- isoprostane was measured using a commercial competitive enzyme immunoassay kit (Cayman Chemical Company).

The mean 15F<sub>2t</sub>- isoprostane/creatinine ratio (pg/ml/mgCr/dl) was significantly higher in the lymphoma dogs (mean = 12.44, SD = 2.708) than in the clinically healthy control dogs (mean = 4.632, SD = 1.013,  $p = 0.007$ ). These results suggest that significant oxidative injury occurs in dogs with lymphoma. Further studies are needed to determine a potential relationship between oxidative stress and clinical outcomes in dogs with lymphoma. Additionally, there is a need to evaluate whether chemotherapy or antioxidant medications may decrease the concentrations of 15F<sub>2t</sub>- isoprostane in dogs with lymphoma.

#### ABSTRACT #28

DETECTION OF MINIMAL RESIDUAL DISEASE IN CANINE B CELL LYMPHOMA PATIENTS USING RT-PCR. Heather L. Donahue, Susan E. Lana, Susan S. Plaza, Robert Burnett, Anne Avery, Colorado State University, Fort Collins, CO.

In dogs with B cell lymphoma, response to treatment is assessed by monitoring peripheral lymph node dimension and clinical signs. This study investigates detection, of minimal residual disease (MRD) in peripheral blood using real time PCR methodology (RT-PCR). The RT-PCR technique is potentially capable of detecting and quantifying as few as 6 neoplastic lymphocytes per reaction. In human medicine, minimal residual disease is routinely monitored in lymphoma and leukemia patients. Several studies have shown molecular remission to be a significant prognostic factor in these patients.

We have developed a real time PCR method for detecting neoplastic populations of lymphocytes in dogs. We hypothesized that this method could detect tumor cells in the peripheral blood of

lymphoma patients after they had achieved clinical remission. The availability of such an assay would allow ready monitoring of response to therapy.

DNA was isolated from the peripheral blood of dogs diagnosed with stage IIIa or IVa lymphoma undergoing a 9 week ultra-short Madison- Wisconsin chemotherapy protocol. Samples were obtained at diagnosis, week 4, week 6, and week 9 of chemotherapy, and at subsequent six week intervals which corresponded with clinical recheck appointments following chemotherapy. Finally, samples were obtained when the dog was clinically determined to be out of remission. Neoplastic lymphocyte populations were amplified using primers specific for canine B cell lymphoma. Amplified populations were detected and analyzed using RT-PCR methodology.

Preliminarily, we have detected tumor cells in the blood from seven of eight patients with B cell lymphoma at presentation. Blood samples taken from patients during chemotherapy and through remission showed that in most cases the neoplastic population of cells could be detected after the patients were judged to be in clinical remission, but all patients achieved a "molecular" remission in their blood at some point in the course of their disease. Our results indicate that neoplastic cells persist in the circulation during and after chemotherapy, and that it is feasible to detect such cells using this assay. This assay is currently being applied to the study of a larger population of patients in order to correlate molecular remission with the length of clinical remission and prognosis. This study will clarify if using this technique to detect the presence of MRD can alter staging, outcome, and individualize cancer therapy.

#### ABSTRACT #29

ELEVATIONS IN SERUM GAMMA GLUTAMYL TRANSFERASE VALUES IN HORSES WITH RIGHT DORSAL DISPLACEMENTS OF THE LARGE COLON. RB Gardner, DV Nydam, HO Mohammed, NG Ducharme, TJ Divers. Cornell University, Ithaca, NY.

Anecdotal reports have suggested that horses with right dorsal displacements of the large colon (RDDLC) may have concurrent elevations in the serum hepatic enzyme gamma glutamyl transferase (GGT). Elevations in GGT, and possibly direct bilirubin, may result in incorrect diagnosis of hepatic disease as the cause of colic. This retrospective study was designed to test the hypothesis that horses with RDDLC have elevations in serum GGT values.

Medical records from 37 horses with RDDLC and 48 horses with left dorsal displacements of the large colon (LDDLC) that presented to the Cornell University Hospital for Animals between 1991 and 2003 were reviewed. Cases were included for study only if the RDDLC or LDDLC was confirmed by exploratory laparotomy or necropsy, and if a serum GGT measurement was obtained within 24 hours prior to surgery. Horses with LDDLC were used as a comparison group due to similar presentation, cardiovascular and hematologic status. Data collection included signalment, history, physical examination findings, serum biochemistry results, findings at surgery or necropsy, case outcome and results of liver histopathology. During the study period, GGT values were obtained using 2 different chemistry analyzers with differing reference ranges. Horses were determined to have a GGT value within or outside of the reference range and the proportion of those with elevated GGT values were evaluated with the exact binomial 95% confidence interval.

An elevated pre-surgical GGT value was identified in 18 of 37 (48.6%; 31.9-65.6%) horses with RDDLC and 1 of 48 (2.1%; 0.05-11.1%) horses with LDDLC. For values obtained with chemistry analyzer # one, 5 of 10 (50.0%; 18.7-81.3%) horses with RDDLC and 1 of 36 (2.8%; 0.07-14.5%) horses with LDDLC had elevations in GGT. With chemistry analyzer # two, 13 of 27 (48.1%; 28.7-68.1%) horses with RDDLC and 0 of 12 (0%; 0-26.5%) horses with LDDLC had elevations in GGT. GGT values within 4 days post-surgery were unchanged or decreased in all 11 (100%) horses with

RDDLC in which they were measured. Direct bilirubin concentration was elevated in 8 of 24 (33.3%) horses with RDDLC, while it was normal in the 2 horses with LDDLC in which it was measured. Liver biopsies obtained in 4 horses with RDDLC all had histopathologic evidence of cholangitis or cholangiohepatitis. Thirty-six of 37 (97.2%) horses with RDDLC were discharged with a good prognosis and none returned due to hepatic disease.

Evaluation of surgical and necropsy cases has revealed that abnormal positioning of the colon in horses with RDDLC resulted in stretching of the mesoduodenum and compression of the bile duct. This compression may result in extra-hepatic bile duct obstruction resulting in an elevation in serum GGT values.

When compared to horses with LDDLC, horses with RDDLC have elevations in serum GGT values. Horses with physical and rectal examination findings consistent with RDDLC and without a history of hepatic disease should not have surgery delayed due to mild elevations in GGT.

#### ABSTRACT #30

DEMONSTRATION OF UREASE ACTIVITY IN THE EQUINE STOMACH AND APPLICATION OF THE <sup>13</sup>C-UREA BLOOD TEST TO THE HORSE. Hepburn RJ<sup>1</sup>, Murray MJ<sup>2</sup>, Furr MO<sup>1</sup>, McKenzie HM<sup>1</sup> and Ward DL<sup>3</sup>. <sup>1</sup>Marion DuPont Scott Equine Medical Center, VA-MD Regional College of Veterinary Medicine, VPI & SU, Leesburg, VA, <sup>2</sup>Merial Ltd., Duluth, GA, <sup>3</sup>VA-MD Regional College of Veterinary Medicine, VPI & SU, Blacksburg, VA.

Evidence for the presence of *Helicobacter* in the equine gastric antrum has recently been shown by PCR amplification of the *16s rRNA*, immunohistochemistry of the *UreI* channel and the *UreA* subunit of *Helicobacter* urease, serology, and histology of antral mucosa. Gastric *Helicobacter* species regulate internal pH via the cytoplasmic enzyme urease. Detection of gastric urease activity is used by several methods to diagnose *Helicobacter* infection. In humans the <sup>13</sup>C urea blood test (UBdT) provides for simple, accurate assessment of gastric urease activity. In the presence of urease, <sup>13</sup>C-urea is hydrolyzed to ammonia and <sup>13</sup>CO<sub>2</sub>, which is rapidly absorbed across the gastric epithelium. A blood sample is analyzed for <sup>13</sup>C-bicarbonate by gas isotope mass ratio spectrometry and compared to a <sup>13</sup>C international standard. The result is reported as Δ<sup>13</sup>C over the t<sub>0</sub> baseline (ΔCOB). Increased ΔCOB indicates urease activity, and in human beings this is highly correlated with *Helicobacter* infection. The purpose of this study was to identify equine gastric urease activity using the <sup>13</sup>C-urea blood test (UBdT).

Eight adult horses without gastric ulcers were used in a cross over study. Feed was withheld for 12 hours prior to testing and throughout sampling. Detomidine (0.03mg/kg IV) was administered to slow gastric emptying (D+ group). All horses received 500mg of C<sup>13</sup>-urea as a 1% solution in water by nasogastric intubation. Blood samples were taken at baseline and at 30 minute intervals for 2 hours and submitted to a reference laboratory (Metabolic Solutions, Nashua, NH) for detection of <sup>13</sup>C-bicarbonate. After a 48 hour wash out period the protocol was repeated without detomidine (D- group).

Increased ΔCOB values were found at all time points in both groups. Statistical analysis by RMANOVA showed the increases in ΔCOB to be significant (p<0.001) at all time points in both the D+ group and D- group, indicating the presence of urease activity. Peak increases were at t<sub>120</sub> (D+) and t<sub>60</sub> (D-). The timing of peak activity and the increased ΔCOB at t<sub>90</sub> and t<sub>120</sub> suggested that cecal urease activity may affect results. The D+ protocol was repeated with the <sup>13</sup>C-urea administered directly into the cecum under laparoscopic guidance. RMANOVA showed significantly increased ΔCOB at all time points, reflecting cecal urease activity. Because the T<sub>50%</sub> for fluid phase gastric emptying in the horse is 30-35 minutes, we interpret the significant increase in ΔCOB in both groups at t<sub>30</sub> and t<sub>60</sub> as demonstrating gastric urease activity. Interference by cecal

ureolytic bacteria is likely to be present after 60 minutes. Upon confirmation of *Helicobacter* as an etiology for equine gastric glandular ulceration, refinement of the  $^{13}\text{C}$ -urea blood test may provide for a useful diagnostic tool in the horse.

#### ABSTRACT #31

**IN VIVO** PRETREATMENT WITH PGG-GLUCAN, A NOVEL IMMUNOMODULATOR, FAILS TO ALTER CYTOKINE MRNA EXPRESSION OF EQUINE PERIPHERAL BLOOD MONONUCLEAR CELLS EXPOSED TO ENDOTOXIN *EX VIVO*. Benjamin W. Sykes<sup>1</sup>, Steeve Giguère<sup>2</sup>, Martin O. Furr<sup>3</sup>. <sup>1</sup>Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland. <sup>2</sup>College of Veterinary Medicine, University of Florida, Gainesville, FL. <sup>3</sup>Marion duPont Scott Equine Medical Center, Virginia Polytechnic Institute, Leesburg, VA.

Endotoxemia is a leading cause of death in the horse and results in major losses to the equine industry annually. Despite its significance, limited therapeutic options are available. Current treatment is largely reliant on supportive care and inhibition of metabolites of the arachidonic acid cascade. Interest in the evaluation of immunomodulator agents as alternative therapeutic options has increased lately with recognition of the importance of various mediators in the inflammatory response. PGG-Glucan, a soluble  $\beta$ -glucan, derived from the yeast *Saccharomyces cerevisiae*, is one such agent. It has been evaluated in experimental animals and humans, and shown to decrease the severity of infection in a variety of studies. *In vivo* pretreatment of mice with PGG-Glucan has been shown to decrease the release of tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), and to enhance the release of interferon  $\gamma$  (IFN $\gamma$ ), from isolated lymphocytes and monocytes subsequently exposed to endotoxin (LPS), staphylococcal enterotoxin B or toxic shock syndrome toxin 1 *ex vivo*.

We evaluated the effects of *in vivo* pretreatment with PGG-Glucan on the cytokine messenger RNA (mRNA) expression of isolated equine peripheral blood mononuclear cells (PMBCs) subsequently exposed to LPS *ex vivo*. Twelve horses were divided into treatment and control groups. Treatment horses received PGG-Glucan (1 mg/kg, IV) 24 hours prior to PMBC isolation. Peripheral blood mononuclear cells were isolated by ficoll-hypaque separation and incubated in the presence of LPS. Cell cultures were resuspended and mRNA extracted using a commercial isolation kit at 0, 6, 12, 24 and 48 hours. Reverse transcription polymerase chain reaction (PCR) was performed and cytokine mRNA expression for TNF $\alpha$ , interleukin 1 $\beta$  (IL-1 $\beta$ ), interleukin-10 (IL-10) and IFN $\gamma$  determined using real time PCR. Determination of TNF $\alpha$ , IL-1 $\beta$  and IL-10 expression was performed at 0, 6, 12 and 24 hours while IFN $\gamma$  expression was determined at 0, 12, 24 and 48 hours. Results were expressed relative to glyceraldehyde-3-phosphate dehydrogenase.

A significant effect of LPS stimulation over time was seen on TNF $\alpha$ , IL-1 $\beta$ , IL-10 and IFN $\gamma$  production. No significant difference was observed between the PGG-Glucan treatment group and control group at any time point although a large amount of variance was present and may have masked some potentially beneficial effects. Based on this study, pretreatment with PGG-Glucan at 1 mg/kg, 24 hours prior to exposure to LPS is unlikely to have a significant effect on equine endotoxemia.

#### ABSTRACT#32

**ESSENTIAL ROLE FOR THE MAP KINASE P38 IN EQUINE NEUTROPHIL MIGRATION.** Clayton D. Chilcoat, Jennifer Trujillo, Yousuf Sharief, Samuel L. Jones. Department of Clinical Sciences, North Carolina State University, Raleigh, NC.

Neutrophils have an important role in the pathophysiology of inflammatory diseases. Thus, the mechanism of neutrophil migration into inflamed tissues is a target for anti-inflammatory drug

development. Neutrophils are recruited to sites of inflammation by chemoattractant molecules such as leukotriene (LT) B<sub>4</sub> and platelet activating factor (PAF). Chemoattractants induce a polarized phenotype with a leading edge where actin-based membrane protrusions and new substrate attachments are formed and a trailing edge where de-adhesion occurs. However, the signaling mechanism regulating neutrophil polarity is poorly understood. The MAP kinase family member p38 is involved in chemoattractants receptor signaling and neutrophil migration. Our hypothesis is that p38 is an essential regulator of neutrophil polarity during equine neutrophil migration. We first determined whether p38 is activated in chemoattractant-stimulated equine neutrophils. The p38 enzyme is activated by dual phosphorylation on Thr180 and Tyr182. Western blot analyses using a specific antibody that recognized activation-associated phosphorylation demonstrated that LTB<sub>4</sub> and PAF stimulation activated p38 in equine neutrophils within minutes. Treatment of equine neutrophils with the specific p38 inhibitor SB203580 abolished LTB<sub>4</sub>- and PAF-induced migration. The IC<sub>50</sub> for inhibition of migration was 2-3  $\mu\text{M}$ , comparable to published IC<sub>50</sub> values for inhibition of p38 dependent functions in neutrophils from other species. To begin to determine the role for p38 in the mechanism regulating neutrophil migration, we examined whether p38 was required for polarization and integrin-mediated adhesion. SB203580 treatment abolished LTB<sub>4</sub>-induced equine neutrophil polarization. However, SB203580 had no effect on LTB<sub>4</sub>- or PAF-induced adhesion or CD18 integrin adhesion receptor expression. In contrast, SB203580 inhibited TNF $\alpha$ -induced adhesion in a dose dependent manner. Our data demonstrate that p38 activation is essential for equine neutrophil migration. Moreover, p38 has a role in establishing neutrophil polarity, but does not regulate adhesion activation by chemoattractants. Further studies will determine whether p38 regulates polarity via a mechanism involving the actin cytoskeleton.

#### ABSTRACT #33

**INFARCTIVE PURPURA HEMORRHAGICA IN FIVE HORSES.** HJ Kaese, DW Hayden and SJ Valberg, University of Minnesota, St Paul, MN.

Infection with *Streptococcus equi equi* has up to a 20% complication rate. One complication, equine purpura hemorrhagica (EPH), is a hypersensitivity reaction where antigen-antibody deposition leads to cutaneous vasculitis presenting as cutaneous edema, and petechial or ecchymotic mucosal hemorrhages. The purpose of this report is to characterize an unusually severe presentation of EPH that involved infarctive lesions of skeletal muscle, gastrointestinal and pulmonary tissues. This presentation closely resembles anaphylactoid purpura or Henoch-Schönlein disease that is well described in human literature but not in veterinary medicine.

Nineteen horses that presented to the University of Minnesota Veterinary Medical Center between 1994 and 2003 were identified from medical records that had stranglers or EPH and abnormally elevated creatine kinase (CK) and aspartate transaminase (AST). Of these, 4 Quarter Horses and one Morgan horse, 5 to 13 years of age, were identified with infarctive lesions of skeletal muscle and/or the gastrointestinal tract and lungs. Four horses presented with signs of colic and muscle stiffness and 1 with muscle swelling and stiffness alone. Pitting limb edema was present in 3 and ventral abdominal swellings in 2 horses. Four horses were diagnosed with Stranglers within three weeks of presentation. The fifth horse had an upper respiratory infection of unknown etiology 1 month prior to presentation whereupon a markedly elevated *S equi* M protein titer (1:25,600) was identified. Common clinical pathologic changes included neutrophilia with a left shift and toxic change, hypoproteinemia, hypoalbuminemia and elevations in CK (47,360-280,000U/L) and AST (964-6,960U/L). Three horses were

euthanized within 24 hours of admission due to unrelenting pain. One horse, treated with penicillin, phenylbutazone and dexamethasone (40 mg/day three times), improved for 3 days, then developed colic and was euthanized after celiotomy revealed inoperable intestinal infarcts. The fifth horse was treated for 2 weeks with penicillin, 3 weeks with dexamethasone (>40 mg for 7 days) and then a 10 week tapering course of oral prednisolone. Monitoring serum CK activity was necessary before tapering the dose of corticosteroids. At necropsy, 4 horses had infarcts of the skeletal musculature, gastrointestinal tract and lungs; *S equi*, was isolated from all of the horses. Histopathologic lesions included leukocytoclastic vasculitis and vascular fibrinoid necrosis with acute coagulative necrosis resembling infarcts. Infarctive EHP appears to be a highly fatal complication of *S equi* infection presenting with signs of focal muscle swelling, stiffness and colic. Successful treatment of one case required early recognition, antibiotic therapy and, of critical importance, prolonged high doses of corticosteroids to reduce the immune-mediated inflammatory response.

#### ABSTRACT #34

IMMUNOLOGICAL RESPONSES TO WEST NILE VIRUS IN VACCINATED AND CLINICAL EQUINE CASES. A Davidson<sup>1</sup>, J Traub-Dargatz<sup>1</sup>, R Rodeheaver<sup>1</sup>, E Ostlund<sup>2</sup>, D Pederson<sup>2</sup>, R Moorhead<sup>3</sup>, J Stricklin<sup>1</sup>, B Young<sup>1</sup>, R Long<sup>1</sup>, R Dewell<sup>1</sup>, S Roach<sup>1</sup>, R Forde<sup>4</sup>, S Albers<sup>2</sup>, S Kaldenberg<sup>2</sup>, R Callan<sup>1</sup>, M Salman<sup>1</sup>. <sup>1</sup>James L. Voss Veterinary Teaching Hospital and Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. <sup>2</sup>National Veterinary Services Laboratories, Ames, IA. <sup>3</sup>Animal Clinic and Pharmacy, Ogallala, NE. <sup>4</sup> Rocky Mountain Regional Animal Health Laboratory, Denver, CO.

This study was organized in an effort to evaluate short and long term neutralizing antibody responses of horses to vaccination against West Nile Virus (WNV) and to compare this to responses of clinically affected horses.

The two groups of equids utilized in this study included horses immunized against WNV and clinical cases of WNV. Vaccinated horses received two doses of the killed adjuvanted WNV product per the manufacturer's recommendations. Serum was collected from vaccinated horses in the fall of 2002 prior to their first vaccine and 3 to 6 weeks after the second vaccination. Serum was also collected from confirmed clinical cases approximately 6 weeks after disease onset. Follow up serum samples were obtained on enrolled horses five to seven months later prior to vaccination against WNV in spring of 2003. Neutralizing antibodies (IgG) were determined utilizing a plaque reduction neutralization assay (PRNT). Any vaccinated horse that tested positive or non-specific for IgM was excluded from the PRNT analysis.

There were 224 horses enrolled in the study, with 187 in the vaccinated group. 47 horses were eliminated from the study (19 were IgM positive or non-specific for IgM, 28 horses had serum obtained at only one time period during the sampling). There were 37 clinical cases enrolled in the fall of 2002. Follow up samples prior to spring 2003 vaccination for WNV were obtained for 84 of the vaccinated horses and for 20 of the clinical cases.

Preliminary results indicated that clinically affected horses mounted and maintained a high antibody response as determined by PRNT. In the fall of 2002, all of the clinical cases and 67% (94/140) of the vaccinated horses had a PRNT result of >1:100. Follow-up sampling in spring of 2003 revealed approximately 90% (18/20) of the clinical cases and 33% (28/84) of the vaccinated horses having a PRNT of >1:100. Approximately 14% (19/140) of vaccinated horses in the fall of 2002 and 27% (24/84) of horses in the spring of 2003 did not develop a neutralizing antibody response. It appears that a portion of the vaccinated horses failed to make a detectable neutralizing

antibody response. The relationship of this finding to susceptibility to WNV is unknown at this time.

#### ABSTRACT #35

THE FEBRILE RESPONSE AFTER CERVICAL MYELOGRAPHY IN THE HORSE: A RETROSPECTIVE ANALYSIS AND EXPERIMENTAL STUDY. Jérôme Van Biervliet, Julia Flaminio, Thomas Divers, Alan Nixon, Brian Summers, Daryl Nydam. College of Veterinary Medicine, Cornell University, Ithaca, New York.

The purpose of the retrospective study was to report the incidence, duration and extent of the transient febrile response after cervical myelography in the horse, to document other complications and to study any possible relations. The experimental study was intended to characterize the hypothesized inflammatory response after intrathecal iohexol injection.

The records of all horses on which a myelogram was performed from 1990-2003 were studied. The iohexol dose (volume and concentration), the CSF cytology and protein content, the final clinical diagnosis, other complications of the procedure, NSAID use, degree of neurologic deficits prior and after the procedure, rectal temperatures (0, 24, 48, 72 and 96 hrs) were noted. The occurrence of fever, defined as a rise of the rectal temperature above 101.8° F, and of other complications was calculated. The duration and extent of the fevers was analyzed in this population of 76 horses and possible relationships to the dose of iohexol, pre-existing CSF abnormalities, final clinical diagnosis and NSAID use were evaluated.

After taking an AO CSF sample, 12 neurologically normal horses were randomly assigned to a treatment group in which Iohexol (75 ml, 240 mg/ml) was injected in the sub-arachnoid space and a control group in which sterile saline was injected (75 ml). A radiograph was taken to ensure proper placement of the contrast material/needle, after which the horses were recovered. During a second anesthesia 24 hrs later for 8 horses (5 treated and 3 control) and 48 hrs later for 4 horses (3 treated and 1 control), another AO CSF sample was taken and the horses were euthanized. Temperature, heart rate, respiratory rate and gait were monitored Q 6 hrs. The spinal cords with corresponding meninges were examined by a blinded pathologist at the level of each exiting spinal nerve. Fresh tissue samples of the dura and pia mater were taken at the level of the 1<sup>st</sup> through the 5<sup>th</sup> spinal nerve. Analyses performed on pre- and post-treatment CSF included: cytology, Il-6, TNF- $\alpha$  and PGI-2 levels. m-RNA expression of Il-1 $\alpha$ , Il-1 $\beta$ , Il-6 and TNF- $\alpha$  was measured in both dura and pia mater at the level of the 1<sup>st</sup>, 2<sup>nd</sup> and 5<sup>th</sup> spinal nerve using real time qPCR (Taqman<sup>®</sup>).

25% of the horses in the pilot study developed a fever (median 102.0 at 24 hrs) of 24-to-48 hrs duration. No correlations were found with other parameters. None of the experimental horses developed a fever; one horse showed mild tetra-ataxia/paresis at 24 hrs. Although no statistically significant differences were found, individual treated horses showed a minimal-to-mild non-suppurative meningitis centering around blood vessels and spinal nerves, an Il-6 and PGI-2 response in the CSF peaking at 24 hrs, and Il-1  $\alpha$  and  $\beta$ , Il-6 and TNF  $\alpha$  mRNA-expression at 24 hrs peaking at the 2<sup>nd</sup> spinal nerve. In conclusion, fever is a common complication of myelography in horses and iohexol induces a minimal-to-mild non-suppurative pachymeningitis and epineuritis, possibly mediated by Il-1  $\alpha$  and  $\beta$ , Il-6 and PGI-2.

### ABSTRACT #36

JUVENILE HEMANGIOSARCOMA IN HORSES. Imogen Johns, Jennifer O. Stephen\*, Dean W. Richardson and Pamela A. Wilkins. University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square, PA and \*The Equine Hospital, Royal Veterinary College, Hertfordshire, England.

Hemangiosarcoma is a rare neoplasm of horses, affecting predominately middle aged to older horses, although it has been reported in younger horses. Clinical experience has suggested that hemangiosarcoma in young horses behaves differently than in mature patients. The purpose of this study was to identify the clinical and pathological characteristics of juvenile hemangiosarcoma.

Medical records from 1986 to 2003 at the University of Pennsylvania New Bolton Center were searched for horses less than three years of age with a histopathological diagnosis of hemangiosarcoma in. Nine records were identified. Breeds affected were Thoroughbred (6), Thoroughbred-cross (1), Standardbred (1) and Rocky Mountain Horse (1). Age at presentation ranged from 9 days to 3 years. In 2 cases, involving the mandible and/or maxilla, the masses were known to have been present since birth. All horses presented with cutaneous or leg swellings. Masses were located in the thoracic body wall (2), mandible/maxilla (2), limbs (3) or were more disseminated, primarily in muscle tissue (2). Physical examination findings included tachycardia (4), fever (2) and depression (2). Hematologic and serum biochemical analyses revealed anemia (4), hyperfibrinogenemia (2), hypofibrinogenemia (3), thrombocytopenia (1) and neutrophilic leukocytosis (1). Ultrasonographic (6) and radiographic (7) evaluation was used to assess lesion characteristics, involvement of surrounding tissues or evidence of metastasis, but was not diagnostic in any case. Ante-mortem histopathologic diagnosis was obtained in all cases in which it was attempted (8). Multiple biopsy samples were necessary in one horse before definitive diagnosis was made. Fine needle aspirates, arthrocentesis (1) and abdominocentesis (1) were not diagnostic. Six of 9 horses were euthanized. Euthanasia without treatment was performed in 3 cases. Medical management (antimicrobial, non-steroidal antiinflammatory drug, and intravenous fluid therapy, in addition to whole blood transfusion) was attempted in one case before definitive diagnosis. Surgical resection of masses was performed in 3 horses, 2 of which were later euthanized due to tumor re-occurrence. Diagnosis was confirmed histologically at post mortem in all euthanized cases. Disseminated hemangiosarcoma was diagnosed in 2 cases, and the musculoskeletal system was most commonly involved.

Juvenile hemangiosarcoma carries a poor prognosis. However, 2 cases resolved spontaneously without specific therapy and returned to racing (1) or produced normal healthy foals (1). Early histopathological diagnosis may be beneficial if the mass is localized and amenable to surgical resection, which may potentially be curative. In cases where the horse is medically stable, and identified masses are not substantially interfering with quality of life or use, a period of observation may be warranted.

### ABSTRACT #37

USE OF HUMAN ALBUMIN AS A COLLOIDAL THERAPY IN THE HYPOPROTEINEMIC EQUINE. Shane F DeWitt, Mary Rose Paradis. Tufts' University School of Veterinary Medicine, North Grafton, MA.

The purpose of the investigation was to determine the safety and efficacy of human albumin use as a potential colloidal therapy in the hypoalbuminemic or hypoproteinemic equine patient. Human albumin has been successfully utilized in human, canine and feline patients as a colloidal therapy. Historically, Hetastarch® or plasma have been the most commonly used colloidal products in the equine patient. However, their effect is minimal and short lived as they contain little or no protein and the colloid oncotic pressure (COP) is low in comparison to 25% human albumin solution. The data

obtained from patients in which albumin was utilized was then compared to Hetastarch®. This investigation also included a review of the medical records to identify other patients who fit the criteria of inclusion. In order to be included, horses had to have a serum albumin concentration of less than 1.8 g/dl, a COP less than 10 mm Hg or in imminent danger of developing severe complications from hypoproteinemia. Patients also needed to have a COP, serum biochemistry and packed cell volume / total solids performed before and after transfusion. Plasma and albumin were administered slowly, and during transfusion, patient's vital signs were monitored regularly. Hetastarch® was administered as a bolus.

In total, 19 horses were identified that fit the criteria of inclusion (7 treated with albumin, 10 with Hetastarch® and 2 with plasma). The 2 horses treated with plasma were not included in the statistical analysis due to the small sample size. Horses treated with albumin had a mean packed cell volume and total protein of 43.9% +/- 10.5, 3.2 g/dl +/- 1.0 and 35.0% +/- 6.9, 3.7 g/dl +/- 0.7 (P=0.008) pre and post treatment, respectively. Horses treated with Hetastarch® had a mean packed cell volume and total protein of 38.6% +/- 9.2, 3.9 g/dl +/- 0.8 and 33.8% +/- 10.1, 3.8 g/dl +/- 0.9 (P=0.013) pre and post treatment, respectively. Mean COP of albumin treated horses was 9.7 mm Hg +/- 2.3 and 16.6 mm Hg +/- 2.4 pre and post transfusion, respectively (P=0.002). Mean COP of Hetastarch® treated horses was 11.4 mm Hg +/- 2.8 and 13.3 mm Hg +/- 3.6 pre and post treatment, respectively (P=0.018). Albumin levels in horses treated with albumin were 1.4 g/dl +/- 0.3 and 2.2 g/dl +/- 0.3 (P=0.001) pre and post treatment, whereas horses treated with Hetastarch® had albumin concentrations of 1.8 g/dl +/- 0.6 and 1.6 g/dl +/- 0.4 (P=0.022) pre and post treatment, respectively. There were no significant changes in osmolality or electrolyte concentrations. No changes in vital signs were observed during either treatment.

These results suggest that 25% human albumin solution is a safe and effective colloid for administration to equine patients. The increase in serum albumin concentrations with albumin therapy may provide a greater and more prolonged effect than treatment with Hetastarch®.

### ABSTRACT #38

PHARMACOKINETICS AND PHARMACODYNAMICS OF FUROSEMIDE AFTER ORAL ADMINISTRATION IN HORSES. Johansson A.M., Gardner S.Y., Levine J.F., Papich M.G., LaFevers D.H., Goldman R.B., Sheets M.K., Atkins C.E. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Furosemide is the most common diuretic drug used in horses suffering from edema. Furosemide is routinely administered as intravenous or intramuscular bolus doses 3 - 4 times a day in horses. Oral administration is often suggested as an alternative, even though documentation of absorption and efficacy in horses is lacking. The purpose of this study was to determine systemic availability and diuretic efficacy of oral furosemide (1 mg/kg) in horses.

This study was carried out in a randomized, crossover design and compared 8-hour urine volume between control horses that received placebo, horses that received oral (PO) furosemide (1mg/kg) and horses that received intravenous (IV) furosemide (1 mg/kg). Blood samples for analysis of plasma furosemide concentrations, packed cell volume, and total solids were obtained at specific time-points. Furosemide concentrations were determined by reverse-phase high performance liquid chromatography with fluorescent detection.

Systemic availability of furosemide after PO administration was poor, erratic, and variable between horses. Median systemic availability was 5.4 % (25<sup>th</sup> percentile: 3.5, 75<sup>th</sup> percentile: 9.6). Horses that received furosemide IV produced 7.4 L (7.1, 7.7) urine over the 8-hour period. The maximum plasma concentration of 0.03 µg/ml after PO administration was not sufficient to increase urine volume compared to control horses [1.2 L (1.0, 1.4) for PO versus 1.2 L (1.0, 1.4) for control]. There was a mild decrease in urine specific

gravity within 1-2 hours after administration of furosemide PO, and urine specific gravity was significantly lower in horses treated with furosemide PO compared to control horses at the two-hour time-point.

In conclusion, systemic availability of PO furosemide (Salix) was poor and variable. Furosemide at 1 mg/kg PO did not induce diuresis in horses.

#### ABSTRACT #39

DISPOSITION OF FLUNIXIN MEGLUMINE INJECTABLE PREPARATION ADMINISTERED ORALLY TO HORSES. A. Pellegrini-Masini<sup>§</sup>, R. H. Poppenga\*, R. W. Sweeney<sup>§</sup>. <sup>§</sup>Department of Clinical Studies-New Bolton Center, \*Department of Pathobiology, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA.

Flunixin meglumine is used in equine medicine for the preventive treatment of endotoxic shock, in the management of colic patients, musculoskeletal injuries, and ocular diseases. There have been reports of localized swelling, stiffness and sweating, and less frequently of bacterial myositis, following intramuscular administration of flunixin meglumine. Repeated intramuscular administration of flunixin is not advisable due to the side effects of muscle soreness, as well as the possibility of severe complications (abscess, myositis). Oral administration of flunixin meglumine often is most desirable, particularly when the treatments are to be administered by the owner. Recently, the granular and paste oral formulations of flunixin meglumine have had intermittent commercial availability. Many practitioners have resorted to oral administration of injectable flunixin meglumine preparation. While the bioavailability of granular and paste formulations is good, the disposition of orally administered injectable form of flunixin meglumine has not been reported. The purpose of this study was to evaluate the pharmacokinetics, including bioavailability, of flunixin meglumine following oral administration of the injectable preparation.

An injectable preparation of flunixin meglumine was administered orally and intravenously at a dose of 1.1 mg/kg to six healthy adult horses in a random cross-over design. Injectable flunixin meglumine (50 mg/ml) was mixed with molasses and administered into the mouth by syringe, to mimic clinical use. Following intravenous administration, flunixin meglumine disposition conformed to a two-compartment model, with elimination half-life of 2.1 hours, similar to previous studies. Following oral administration of injectable flunixin meglumine, the drug was detected in plasma within 15 minutes of administration and peak plasma concentrations were observed 45 to 60 minutes after administration. Mean bioavailability of the oral drug was  $71.9 \pm 26.0\%$ , with an absorption half-life of 0.76 hours. The apparent elimination half-life after oral administration was 2.4 hours. Drug concentration rapidly reached the therapeutic range and remained there for approximately 12 hours. The injectable preparation of flunixin meglumine appears suitable for oral administration in horses.

#### ABSTRACT #40

THE INFLUENCE OF ANESTHESIA AND DISEASE ON SERUM LIDOCAINE CONCENTRATION IN HORSES. DJ Feary, KR Mama, AE Wagner. Colorado State University, Department of Clinical Sciences, Fort Collins, CO.

Intravenous lidocaine infusion has become an increasingly utilized therapy in horses for a variety of clinical conditions. This study was designed to determine the influence of anesthesia on serum lidocaine concentration in two groups of clinically normal, fasted, healthy horses, and then to further determine the influence of gastrointestinal disease on serum lidocaine concentration in anesthetized horses. Eight horses ( $10 \pm 4.0$  yrs [ $X \pm SD$ ],  $546 \pm 63$  kg) in the standing

(ST) drug-free group, 8 horses ( $2.3 \pm 0.4$  yrs,  $424 \pm 31.5$  kg) in the anesthetized healthy (AN-healthy) group, and 10 horses ( $10 \pm 8$  yrs,  $536 \pm 38$  kg) in the anesthetized diseased (AN-colic) group were studied. AN-healthy horses were anesthetized for elective arthroscopic surgery. AN-colic horses were equine patients anesthetized for emergency exploratory abdominal surgery for colic. The AN horses received xylazine, guaifenesin ( $\pm$  benzodiazepine), and ketamine for anesthetic induction and were maintained with sevoflurane. Horses in all groups received a clinically utilized infusion of 2% lidocaine; loading dose (1.3 mg/kg IV over 15 mins), followed by a continuous infusion (0.05 mg/kg/min for an additional 90 mins). Arterial blood was collected prior to lidocaine administration (pre-drug), at 15 min intervals during administration, and at fixed time points for 6 hrs post-infusion. Serum was frozen and stored at  $-40^{\circ}\text{C}$  for analysis using liquid chromatography/mass spectrometry. Selected cardiopulmonary parameters (heart rate, mean arterial pressure,  $\text{PaO}_2$ ,  $\text{PaCO}_2$ ) and temperature were recorded at fixed intervals during lidocaine infusion. All horses were observed for signs of lidocaine toxicity (e.g., muscle fasciculations). Drug concentration data for ST and AN-healthy groups were analyzed using likelihood-based-mixed-effect model to assess period and treatment differences ( $p < 0.05$ ). Significantly higher serum lidocaine concentrations were reached in AN-healthy ( $3672 \pm 568$  ng/mL) compared with ST horses ( $1849 \pm 384$  ng/mL) during loading dose and continuous infusion. Serum lidocaine concentrations in AN-colic horses ( $1923 \pm 425$  ng/mL) were lower than AN-healthy horses and were comparable to ST horses. Serum lidocaine concentrations paralleled the amount administered and returned to pre-drug levels within 4 hrs of discontinuation of the infusion in all groups. Cardiopulmonary values differed between groups but were within normal limits for study and clinical conditions. No signs of lidocaine toxicity were observed. Serum lidocaine concentrations were influenced by general anesthesia in healthy horses, suggesting a dose modification may be appropriate in anesthetized horses. Serum lidocaine concentrations were further influenced by gastrointestinal disease in anesthetized horses, suggesting a likely multi-factorial effect of systemic status, amount of anesthetic agents administered, and cardiovascular support employed. Further studies directed at determining the effect of specific drugs and cardiac output on serum lidocaine concentrations in horses will ultimately allow for appropriate dosing of intravenous lidocaine in a variety of clinical circumstances.

#### ABSTRACT #41

PRE-EXERCISE HYPERVOLEMIA DOES NOT CAUSE ARTERIAL HYPOXEMIA IN THOROUGHBREDS DURING EXERCISE SIMULATING THE SECOND DAY OF A 3-DAY EQUESTRIAN EVENT. B. S. Tennent-Brown, T. E. Goetz, M. Manohar, A. S. Hassan, D. E. Freeman, J. Bundy, M. Evans. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Recently, Sosa Leon *et al* (*Equine Vet. J. Suppl.* 34: 425-429, 2002) reported that hyperhydration of horses prior to an exercise test simulating the second day of a 3-day equestrian event induced arterial hypoxemia which could be detrimental to performance. It was suggested that the likely cause of hypoxemia in the hyperhydrated horses was some degree of pulmonary edema. To further investigate this phenomenon, we examined the effects of pre-exercise hypervolemia on arterial oxygenation in horses performing the same exercise protocol as Sosa Leon *et al* during which work in phases B and D elicited 81% and 69% of the maximal heart rate, respectively.

Using a cross-over experimental design, blood-gas studies were carried out on 7 exercise-trained horses in the control and hyperhydration treatments. The sequence of treatments was randomized for each horse and 7 days were allowed between studies. Hyperhydration was induced by administering 0.425 g/kg NaCl via nasogastric tube (5 hrs pre-exercise) followed by free access to water.

NaCl administration induced a pre-exercise increase of 16.4 ± 2.4% in plasma volume. Throughout exercise, plasma volume in the hyperhydrated horses significantly exceeded that in the control horses.

In standing horses, plasma volume expansion subsequent to NaCl administration resulted in significant decreases in total plasma protein concentration, hemoglobin concentration, and arterial O<sub>2</sub> content, but the arterial to mixed venous O<sub>2</sub> content gradient remained unchanged. During exercise in phases B and D, total plasma protein concentration, hemoglobin concentration, core temperature, heart rate, total arterial O<sub>2</sub> content, and the arterial to mixed venous O<sub>2</sub> content gradient increased significantly in both treatments. There were no differences in arterial O<sub>2</sub> tension or hemoglobin-O<sub>2</sub> saturation between treatments during phases B and D. Mean arterial O<sub>2</sub> tension in the hyperhydrated vs. control horses, respectively, was: phase B 102 vs. 100 mm Hg; phase D 104 vs. 99 mm Hg. Mean arterial hemoglobin-O<sub>2</sub> saturation in the hyperhydrated vs. control horses, respectively, was: phase B 98.4% vs. 98.3%; phase D 98.7% vs. 98.1%.

In conclusion, our data indicate that induction of hyperhydration prior to a simulated second day of a 3-day equestrian event did not cause arterial hypoxemia or desaturation of arterial hemoglobin.

#### ABSTRACT #42

EVIDENCE FOR INCREASED RELIANCE ON GLYCOLYSIS IN THE EQUINE FOOT THAT IS NOT OXYGEN SUPPLY LIMITED. Cornelis J. Cornelisse, Robert M. Bowker\*, Harold C. Schott II. Departments of Large Animal Clinical Sciences and \*Pathobiology and Diagnostic Investigation. College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Skin and cartilage serve different functions but derive a significant amount of their energy production from nonoxidative metabolism of glucose (glycolysis) with lactate as a byproduct. Because epidermal tissue and cartilage make up a large portion of the active metabolic mass in the equine foot, we hypothesized that glycolysis is an important pathway for energy production in the equine foot.

Ten quietly standing horses were studied. Blood was collected from the transverse facial artery (TFA), jugular vein (JUGLR), a cephalic and a femoral vein, and a digital vein from each foot. Blood was analyzed (all sites at 37°C) for pO<sub>2</sub>, pCO<sub>2</sub>, O<sub>2</sub>-content, and CO<sub>2</sub>-content and hemoglobin (Hb), glucose and lactate concentrations. The means from cephalic and femoral venous samples (PVEIN) as well from digital venous samples (FOOT) were used in the final analysis. Lactate to glucose ratios (LG) were calculated for the arteriovenous differences between TFA blood and blood of JUGLR, PVEIN and FOOT origin. An analysis of variance for repeated measures was used to test the data for statistical significance (p<0.05).

The pO<sub>2</sub> was significantly greater in FOOT [57.4 ± 2.9 mmHg] than in PVEIN [44.3 ± 2.2 mmHg] or JUGLR [37.6 ± 1.0 mmHg] but significantly lower than in TFA [98.6 ± 3.1 mmHg]. Similarly, the O<sub>2</sub>-content was significantly greater in FOOT [15.2 ± 0.5 ml/dl] than in PVEIN [13.8 ± 0.5 ml/dl] and in JUGLR [12.9 ± 0.5 ml/dl] but significantly lower than TFA [16.7 ± 0.5 ml/dl]. Hb was significantly different between PVEIN [12.7 ± 0.5 g/dl] and TFA [11.8 ± 0.5 g/dl]. The pCO<sub>2</sub> was significantly lower in TFA [41.2 ± 0.9] than in PVEIN [44.3 ± 0.3 mmHg] but the CO<sub>2</sub>-content was not significantly different amongst vessels. Lactate concentration was not different between TFA [0.7 ± 0.1 mmol/l] and JUGLR [0.6 ± 0.1 mmol/l] but FOOT [1.8 ± 0.2 mmol/l] was significantly greater than in blood collected from all other sites. Glucose concentration was significantly lower in FOOT [89.9 ± 2.7 mg/dl] and PVEIN [89.5 ± 2.7 mg/dl] than in TFA [106.7 ± 2.8 mg/dl] or JUGLR [100.3 ± 2.7 mg/dl]. The LG was significantly greater in the FOOT [1.4 ± 0.1] than in PVEIN [0.9 ± 0.1], which was in turn greater than in JUGLR [0.1 ± 0.2].

Reanalysis of blood gases corrected for estimated blood temperatures (from literature) reconfirmed the absence of hypoxia in the foot.

These data reveal that, despite an apparent adequate oxygen supply, substantial energy production via anaerobic glycolysis occurs in the equine foot. A greater dependence on glycolysis could be a risk factor for diseases of the equine foot when substrate availability or uptake is altered.

#### ABSTRACT #43

EFFECT OF ORAL PROBIOTICS ON CALF DIARRHEA: CLINICAL TRIALS PUBLISHED BETWEEN 1973-2003. A Rodriguez<sup>1</sup>; JS Weese<sup>1</sup>; T Duffield<sup>2</sup> & H Staempfli<sup>1</sup>. Departments of Clinical Studies<sup>1</sup>, and Population Medicine<sup>2</sup>, Ontario Veterinary College, University of Guelph, Canada.

Probiotic products have traditionally been claimed to have therapeutic properties for a wide variety of gastrointestinal disorders in cattle. Although numerous studies are available assessing the effect of probiotics in calves, there is still lack of conclusive data to support their use. The objective of this study was to review available literature and to identify whether there was a trend regarding the effect of probiotics on diarrhea in calves. Clinical trials investigating the effect of probiotics on prevention/treatment of calf diarrhea published between January 1973 and August 2003 were retrieved. Pubmed and CAB direct scientific search engines were used to explore articles linked to the following keywords: probiotic, probiotics, *Lactobacillus*, *Bifidobacterium*, diarrhea and calves, regardless of language and source. The URL for google, <http://www.google.com>, was used to look for fugitive literature escaping the two scientific search engines. Secondary keywords and the reference lists from the primary publications were used to widen the search. The description of a clinical trial assessing a probiotic product and its effect on diarrhea in calves within the abstract was the main criterion for inclusion.

Over 250 abstracts were reviewed to finally select 44 clinical trials. Most of the studies were designed to investigate nutritional effects. Although positive health effects were reported in 55% of the clinical trials, there were some papers that drew conclusion from trends and not from statistically significant results. Randomized well-controlled blinded clinical trials were not found with this method of search. Likewise, colonization and meta-analyses studies had not been published. Interestingly, four recent publications dealt with shedding of *Escherichia coli* O157, however contradictory results were reported. Only one study was targeted against *E. coli* F5 (formerly K99). Regarding the probiotic microorganisms, the most relevant problems in interpreting the results were associated to the type of microorganisms studied, the inappropriate description of the inocula (14/44 studies) and the use of commercial products in 8 studies. In total, one yeast and 21 different bacterium species were reported, being *Lactobacillus acidophilus* the bacterium most commonly studied (24/44). However, *L. acidophilus* was used in mixed preparations in 13 out of the 24 clinical trials. The remaining bacteria have been only studied in a very few number of studies.

Meta-analysis studies are an important tool to compare well-controlled studies in order to draw statistical conclusions. However, the lack of repeatability associated to great variability of the microbial composition of the studies included in the present study precluded from conducting the analysis and making general conclusions.



#### ABSTRACT #44

SELECTION OF SPECIES-SPECIFIC BACTERIA FOR POTENTIAL PROBIOTIC USE IN CATTLE. A Rodriguez<sup>1</sup>; JS Weese<sup>1</sup>; T Duffield<sup>2</sup> & H Staempfli<sup>1</sup>. Departments of Clinical Studies<sup>1</sup>, and Population Medicine<sup>2</sup>, Ontario Veterinary College, University of Guelph, Canada.

Probiotics are selected living microorganisms that exert beneficial health effects when administered at proper doses. Probiotics are available commercially for prevention of *Escherichia coli* diarrhea in calves, however scientific evidence is lacking for most products. This study investigated 104 bovine-derived lactic acid bacteria (LAB). Acid tolerance (pH 2.0 and 4.0), bile tolerance (0.15 and 0.3% bile), inhibition of enterotoxigenic and enterohemorrhagic strains of *Escherichia coli* and aerotolerance were evaluated *in vitro*. Gastrointestinal tract survival and safety were evaluated for one selected isolate on neonate calves. 16S rRNA gene-PCR analysis was used to confirm results from preliminary biochemical identification.

Most LAB isolates did not grow when incubated at pH 2.0, whereas 12% of the isolates grew well at pH 4.0 (>80% of the growth rate obtained in control cultures). Bile salts were less restrictive for LAB growth. 42% of the isolates had good growth rates (>80% vs. control culture) on 0.3% bile-culture media. The growth of *E. coli* was significantly inhibited (at least 50% the growth rate of the control cultures) by 13 LAB isolates, whereas strong stimulation of the growth was seen with 7 LAB isolates. Among the best-ranked LAB isolates, *Lactobacillus plantarum* was the species most commonly identified (5/10). One isolate of *L. plantarum* (LPB80) was subjectively assessed to be superior and was chosen for the *in vivo* study. Animals received daily either placebo (n=3), 10<sup>7</sup>-10<sup>8</sup> colony-forming units (cfu) of LPB80 (n=5), or 10<sup>10</sup>-10<sup>11</sup> cfu of LPB80 (n=4) for five days. Fecal samples were collected and physical examinations were performed daily for 15 days. Intestinal samples were collected from euthanized calves at the conclusion of the study. No adverse clinical signs were observed in any of the calves that received LPB80. Administration of LPB80 did not affect the counts of fecal coliforms (F value, *P*>0.05). LPB80-like colonies were recovered from samples of both treated and placebo animals. Alignment of the 16S rRNA gene sequences showed 99.8-100% similarity with the administered strain.

Systematic screening studies are useful in the search of animal-species potential probiotic organisms. The results of this study suggested that *L. plantarum* strain LPB80 might have the potential to be used as a probiotic product to prevent/treat *E. coli*-associated bovine gastrointestinal disorders. Studies on the effect of oral administration of LPB80 on prevention of *E. coli* F5 diarrhea in calves, and carriage of *E. coli* O157 in calves and cattle are warranted. Further microbiologic studies on the other *L. plantarum* species are also required.

#### ABSTRACT #45

WEST NILE VIRUS MENINGOENCEPHALOMYELITIS IN ALPACAS. Jennifer L. O'Rourke, Robert J. Callan, David C. Van Metre. Colorado State University James L Voss Veterinary Teaching Hospital, Ft. Collins, CO.

First reported in New York in 1999, West Nile Virus (WNV) has now been reported in all states except Oregon and is considered endemic in the United States. West Nile Virus causes clinical disease primarily in horses, birds, and humans. However, WNV infection has also been reported in cattle, domestic sheep, big horn sheep, camels, mule deer, pigs, dogs, cats, frogs, and some reptiles. In this report we describe neurological disease associated with WNV infection in 4 alpacas in Colorado.

In August and September 2003, four alpacas (*Lama pacos*) were presented to the Colorado State University Veterinary Teaching Hospital with clinical signs suggestive of West Nile Virus encephalomyelitis. A wide variety of clinical signs were observed

including lethargy, inappetence, ataxia, weakness, head and neck tremors, muscle fasciculations, and recumbency. Two alpacas presented in lateral recumbency with opisthotonus and periodic, involuntary paddling; one animal died and the other was euthanized within 36 hours of hospitalization. Two animals survived; one was clinically normal within 96 hours of presentation and the other developed WNV-associated paralytic syndrome with a prolonged (4 month) convalescence.

Three of 4 patients were lymphopenic at presentation. Serum chemistry analyses were essentially within normal limits. Cerebrospinal fluid was collected from the 2 animals that died and the one animal that developed the paralytic syndrome. Two of the CSF samples showed mononuclear pleocytosis, and all three samples had markedly elevated protein concentration.

WNV antibody titers were determined on serum and/or CSF samples from the patients by plaque reduction neutralization test (PRNT) and microtiter virus neutralization (VN). Serum antibody titers were elevated at the time of presentation for the 3 animals where serum was available. Convalescent serum antibody titers remained elevated for the two surviving animals. Acute CSF antibody titers from the two fatal cases were negative on PRNT and had low positive titers on microtiter VN. The convalescent CSF antibody titer increased 20-fold in the alpaca demonstrating the paralytic syndrome. West Nile Virus infection was confirmed with reverse transcriptase PCR on fresh brain tissue from both fatal cases. Histopathological examination of brain and cervical spinal cord tissue from both animals that died showed lymphocytic meningoencephalomyelitis.

#### ABSTRACT #46

DETERMINATION OF *MYCOPLASMA BOVIS* SUSCEPTIBILITIES AGAINST 6 ANTIMICROBIAL AGENTS USING THE ETEST METHOD. David Francoz, Mado Fortin, Gilles Fecteau, Serge Messier. Faculté de Médecine Vétérinaire, Université de Montréal, Saint Hyacinthe, Québec.

The objective of this study was to determine susceptibilities of *M. bovis* against 6 different antibiotics using the Etest methodology.

Fifty nine isolates of *M. bovis* originating from 55 affected cattle were evaluated. Specimen sources were lungs tissues (n=18), synovial fluids (n=14), tracheo-bronchial wash (n=14), milk (n=9), and external or inner ear discharge (n=3). Antimicrobial agents tested were azythromycin (AZT), clindamycin (CDM), erythromycin (ETM), enrofloxacin (ENR), spectinomycin (STM) and tetracycline (TE). Antimicrobial agent concentrations tested ranged from 0.016 to 256 µg/ml for AZT, ETM, CDM and TE, 0.002 to 32 µg/ml for ENR and 0.064 to 1021 µg/ml for STM. One hundred and 50 µl of organism suspensions were placed on 150 mm and 90 mm SP 4 agar plate with arginine, respectively. Five Etest strips were placed on the surface of the 150 mm plate and 1 on the 90 mm plate according to the manufacturer's instructions. Plates were incubated at 35 °C in an atmosphere of 5% of CO<sub>2</sub> in air for 72 hours. At this time, minimal inhibitory concentrations (MIC) were read by determining where the zone of growth inhibition intersected the MIC scale on the strip. *M. bovis* Donetta isolate was used as a control. Susceptibilities to the antibiotics tested were based on the National Committee for Clinical Laboratory Standard recommendations.

Since there was no growth inhibition, MICs were not determined for ETM. MIC<sub>50</sub> and MIC<sub>90</sub> obtained for AZT were 3 and >256 µg/ml, respectively (range: 0.5 to >256 µg/ml). Thirty three percent of isolates were considered sensitive. MIC<sub>50</sub> and MIC<sub>90</sub> obtained for TE were 4 and 8 µg/ml, respectively (range: 0.094 to >256µg/ml). Seventy one percent of isolates were considered sensitive, but 18 isolates had MICs of 4 µg/ml. MIC<sub>50</sub> and MIC<sub>90</sub> obtained for STM were 2 and >1021 µg/ml, respectively (range: 0.38 to >1021 µg/ml). Sixty three percent of isolates were considered sensitive. MIC<sub>50</sub> and MIC<sub>90</sub> obtained for CDM were 0.19 and >256 µg/ml, respectively

(range: 0.094 to >256 µg/ml). Seventy six percent of isolates were considered sensitive. MIC<sub>50</sub> and MIC<sub>90</sub> obtained for ENR were 0.19 and 0.25 µg/ml, respectively (range: 0.047 to 0.5 µg/ml). Ninety three percent of isolates were considered sensitive. For each antibiotics, resistance was not associated with the specimen source.

*M. bovis* susceptibilities were easily determined by the Etest demonstrating acquired resistance to TE, STM, AZT and CDM and a good efficacy of ENR.

#### ABSTRACT #47

EFFECT OF INTRANASAL VACCINATION AGAINST BOVINE ENTERIC CORONAVIRUS ON THE OCCURRENCE OF RESPIRATORY DISEASE IN A COMMERCIAL BACKGROUNDING FEEDLOT. Paul Plummer, College of Veterinary Medicine, University of Tennessee, Knoxville, TN.

A randomized single-blind clinical trial was conducted to evaluate the effect of intranasal instillation of a modified live oral vaccine against bovine enteric coronavirus on the number of calves treated for bovine respiratory disease (BRD) in a commercial backgrounding feedlot. Four-hundred fourteen heifer calves weighing 350-750 pounds were purchased from various auction barns in the southeastern United States over a period from September 2001 to November 2002. On entering the feedlot, a swab of each naris was taken and a blood sample was obtained on entry and at the end of the observation period. Routine processing included vaccinations, deworming and growth implants. Calves were randomized to receive intranasal administration of 3.0 mL of a commercially available modified live oral vaccine against bovine enteric coronavirus and rotavirus or 3.0 mL of saline. After processing vaccinated and control calves were assigned to separate pens and observed for periods of 17-99 days. Pen assignments were reversed after each group of 150 calves was enrolled in the study. Diagnosis of respiratory disease and severity of disease scores were made by one of two authors blind to the treatment status of calves. Treatment for BRD was standardized. Nasal swabs were examined by ELISA test for the presence of BCV antigen and blood samples were tested by IFA test for antibody titer against BCV. BCV antigen was identified in (125/407) 31% and serum antibody titers  $\geq 20$  against BCV in (246/396) 62% of calves entering the feedlot. Seroconversion from  $< 20$  to  $\geq 40$  against BCV antigen occurred in 99% and 95% of vaccinated and control calves, respectively during the period of observation. In a multivariable analysis, vaccination was associated with a decrease ( $P < 0.01$ ) and the presence of intranasal BCV on entry to the feedlot was associated with an increased risk ( $P < 0.01$ ) of treatment for BRD. Among control calves, those with intranasal BCV on entry to the feedlot, RR = 1.6, and those with antibody titer  $< 20$ , RR = 1.6, were significantly more likely to be treated for BRD. These data provide further evidence of an association between BCV and respiratory disease in feedlot calves. An intranasal vaccine appears to reduce risk of treatment for BRD. The cost effectiveness of vaccination, and effect of vaccine to reduce the number of calves treated for BRD, is likely to be influenced by the prevalence of intranasal BCV and antibody titers  $< 20$  against BCV among calves entering the feedlot.

#### ABSTRACT #48

THE USE OF DIRECT IMMUNOFLOURESCENCE TO DETECT *CRYPTOSPORIDIUM PARVUM* OOCYSTS IN BOVINE COLOSTRUM CONTAMINATED EXPERIMENTALLY. Baillargeon Julie<sup>1</sup>, Fecteau Gilles<sup>2</sup>, Villeneuve Alain<sup>1</sup>, Faubert Gaétan M.<sup>3</sup>, and Baillargeon Paul<sup>4</sup>. <sup>1</sup> Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, Québec <sup>2</sup> Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, St-Hyacinthe, Québec <sup>3</sup> Institute of Parasitology, Macdonald Campus,

McGill University, Ste-Anne de Bellevue, Québec <sup>4</sup> Pfizer Animal Health Canada, Kirkland, Québec.

The purpose of this study was to determine the threshold of detection of *Cryptosporidium parvum* oocysts inoculated into bovine colostrum using direct immunofluorescence preceded by a concentration in water and ethyl acetate.

Using a sucrose flotation method, oocysts were isolated from feces of diarrheic calves submitted to the parasitology laboratory of the diagnostic services of the Faculty of Veterinary Medicine of the University of Montreal. Oocysts were suspended in sterile water and numbers were estimated using a hemacytometer.

Colostrum samples from five cows were harvested aseptically. The five colostrum samples were inoculated with the following numbers of *C. parvum* oocysts: 100, 1 000 or 10 000 oocysts/ml. Colostrum samples were then divided into 3 replicates of 10 ml each for a total of fifteen samples for each oocyst concentration. Fifteen negative controls were also analysed. A sedimentation technique using water and ethyl-acetate was first used to concentrate oocysts and to reduce lipids in colostrum samples. Sediments containing the oocysts were suspended in 500 µL of sterile water. Direct immunofluorescence with monoclonal antibodies specific to *C. parvum* (Merifluor, Meridian Bioscience, Inc., Cincinnati, Ohio) was used to detect concentrated oocysts in the samples. *C. parvum* oocysts were detected at every concentration (100, 1 000 and 10 000 oocysts/ml). No oocysts were detected in negative controls. For the higher concentrations of 10 000 and 1 000 oocysts/ml, oocysts were detected in all the samples (100%). For samples inoculated with 100 oocysts/ml, oocysts were found in 13 samples out of 15 (86,7%). The threshold of detection of this protocol appears to be useful for use on the field colostrum samples to determine the potential presence of *C. parvum* oocyst contamination on the farm.

#### ABSTRACT #49

PENETRATION OF CEFTIOFUR CRYSTALLINE FREE ACID STERILE SUSPENSION INTO STERILE VERSUS *MANNHEIMIA HAEMOLYTICA*-INFECTED TISSUE CHAMBERS IN BEEF CALVES AFTER SUBCUTANEOUS ADMINISTRATION IN THE EAR PINNA. Washburn KE,<sup>\*</sup> Johnson RJ,<sup>‡</sup> Clarke CR,<sup>\*</sup> Anderson K,<sup>†</sup> Bryson WL,<sup>‡</sup> Robinson JA,<sup>‡</sup> Hubbard VL,<sup>‡</sup> Callahan JK,<sup>‡</sup> Lucas MJ,<sup>‡</sup> Dame KJ,<sup>‡</sup> Robb EJ<sup>‡</sup>. <sup>\*</sup>Departments of Veterinary Clinical Sciences (Washburn) and Physiological Sciences (Clarke), Oklahoma State University, Stillwater, OK. <sup>†</sup>3<sup>rd</sup> year veterinary student, PhD student, Oklahoma State University, Stillwater, OK. <sup>‡</sup>Pfizer Animal Health, VMRD, Kalamazoo, MI.

The purpose was to determine the effect of *Mannheimia haemolytica* infection on the penetration of ceftiofur and desfuroylceftiofur into tissue chambers implanted in cattle following subcutaneous administration of ceftiofur crystalline free acid sterile suspension (CCFA-SS).

Four sterile tissue chambers were implanted into the paralumbar fossa of each calf. Two chambers on one side of each calf were randomly inoculated with *Mannheimia haemolytica*, while the remaining two chambers on the opposite side were inoculated with sterile saline. Each calf was injected with CCFA-SS at 6.6 mg ceftiofur equivalents/kg subcutaneously in the posterior aspect of the ear. Chamber fluid and blood samples were collected at predetermined times for 10 days following dosing and analyzed for total protein, and ceftiofur and desfuroylceftiofur metabolites by high-performance liquid chromatography.

Concentrations of ceftiofur and desfuroylceftiofur metabolites in plasma and tissue chamber fluid remained above a pharmacokinetic threshold of 0.2 µg/mL for at least 8 days. Infected tissue chamber fluid concentrations of ceftiofur and desfuroylceftiofur metabolites were significantly higher than those in non-infected tissue chamber

fluid, which correlated with significantly higher total protein concentration in infected tissue chambers.

These results indicate that a single subcutaneous administration of CCFA-SS at 6.6 mg/kg can be expected to provide effective therapy of susceptible bacterial infections for a period of at least 1 week. In addition, interstitial fluid is a better indicator of target site ceftiofur concentrations than plasma, and protein bound ceftiofur and related metabolites at active sites of infection may serve as a reservoir for microbiologically active drug.

#### **ABSTRACT #50**

**HEPATIC CYTOKINE AND CYCLOOXYGENASE EXPRESSION IN EQUINE & BOVINE LAMINITIS.** AJ Stewart, EB Belknap, H Huggins, A Cochran, JK Belknap. Auburn Univ., AL.

Deterioration of the digital laminae may occur as a complication of numerous systemic disease processes. We have previously shown increased cytokine and cyclooxygenase-2 (COX-2) expression in digital laminae during developmental laminitis, and are interested in determining whether these local digital inflammatory changes are a result of a systemic inflammatory response. Hepatic Kupffer cells are an important source of inflammatory mediators in states of systemic inflammatory response in rodent sepsis models. Due to reported portal endotoxemia in a bovine model of grain overload, we hypothesized that digital inflammation and degeneration may be secondary to inflammatory mediator production originating from the portal reticuloendothelial system. Our objectives were to determine hepatic cytokine expression in the developmental stages of experimental laminitis in the bovine grain overload (GO) model and the equine black walnut extract (BWE) model. Liver samples were collected and frozen from 31 steers: 6 hrs [*developmental stage/onset of decreased central venous pressure*, n=12; 6hr GO] and 12 hrs [*onset of clinical signs of laminitis*, n=12; 12hr GO] after administering grain at 3.5% of BW, and 6 hrs after water [n=7, controls]. Samples were collected in horses 2-4 hrs after either BWE administration (*onset of leukopenia*, n=5, BWE), or 2-hrs after water administration (n=5, control). RNA extraction followed by mRNA purification was performed on individual tissue samples. Real time quantitative PCR (LightCycler™, Roche, Inc.) was used to assess hepatic expression of IL-1 $\beta$ , IL-6, TNF $\alpha$ , COX-1, COX-2, and 4 housekeeping genes (to produce a normalization factor). One-way ANOVA was used to detect differences in means between groups. (P < 0.05).

There were no differences between control and BWE horses for hepatic mRNA expression of IL-1, IL-6, TNF or COX-2. Bovine liver mRNA expression of IL-1 was reduced 8-fold (P<0.009) in the 6hr GO group and 10-fold (P<0.009) in the 12hr GO group (compared with controls). There was no change in IL-6 between groups. Compared with controls, COX-1 decreased 6-fold (P<0.001) in the 6hr GO group and 2.5 fold (P<0.01) in the 12hr GO group. COX-2 expression was unchanged at the 6hr GO time point, but increased 11.9 fold (P<0.009) in the 12hr GO group.

In summary, there was no evidence of increased hepatic cytokine or COX-2 (equine) expression in the developmental stage of experimentally induced equine or bovine laminitis. Lack of hepatic inflammatory mediator expression may be due to a unique property of hepatic sinusoidal elements, which have been proposed to clear enterally absorbed toxins with minimal inflammatory response. Pulmonary intravascular macrophages may replace Kupffer cell reticuloendothelial function in horses and ruminants, or circulating leukocytes may be the source of systemic inflammation. Further investigation of inflammatory mediator expression by liver, lung and systemic leukocytes at different time points in horses and cows is needed to more thoroughly investigate a possible systemic source for the digital inflammation that occurs during the development of laminitis.

#### **ABSTRACT #51**

**THROMBOXANE CONTRIBUTES TO ENDOTOXIN-INDUCED EQUINE DIGITAL HYPOPERFUSION.** Menzies-Gow N.J., Marr C.M. and Elliott J. The Royal Veterinary College, University of London, Hertfordshire, U.K.

Infusion of a low dose of endotoxin (LPS) reduces digital perfusion in the horse and there is a temporal relationship between the onset of this hypoperfusion and increases in plasma concentrations of the vasoconstrictors serotonin and thromboxane (Tx). The aim of the study was to further determine the role of Tx in LPS-induced digital hypoperfusion.

A randomised three-way treatment protocol study was performed using six adult Thoroughbred horses (3 males, 3 females; 4-10 years). Saline (control) or aspirin (4 mg/kg, i.v.) was administered either 2 hours or 4 days prior to the endotoxin infusion. Blood flow in the lateral digital artery and vein of the right forelimb of was measured for 300 min following endotoxin infusion (E. coli 055:B5; 30 ng/kg given over 30 min) using Doppler ultrasonography. Hoof wall (HWST) and coronary band (CBST) surface temperatures, systemic arterial blood pressure and heart rate were monitored concomitantly. Plasma TxB<sub>2</sub> concentration was measured by radioimmunoassay in serial blood samples collected over 300 min. Platelet and leucocyte cyclo-oxygenase (COX) activities 4 days post-aspirin or saline treatment were assessed *ex vivo* by measuring calcium ionophore A23187 stimulated TxB<sub>2</sub> production. Values were compared between treatment groups using two-way analysis of variance and Bonferoni's *post hoc* test.

Following saline pre-treatment, LPS infusion caused significant reduction in digital blood flow from 60 min, reaching maximum at 90-120 min (arterial by 84%, venous by 87%), recovering by 300 min. These changes were corroborated by a similar pattern of changes in HWST and CBST. Plasma TxB<sub>2</sub> concentration had a biphasic increase following LPS infusion. The first peak (10 fold increase over baseline) occurred at 60 min and correlated with the onset of the digital hypoperfusion. The second peak (7 fold increase) occurred at 240 min and coincided with return of blood flow towards initial values. Two-hour aspirin pre-treatment abolished the LPS-induced increases in plasma TxB<sub>2</sub> concentration and significantly attenuated the LPS-induced decreases in digital arterial (by 8%) and venous (by 12%) flow, and HWST (by 63%) and CBST (by 42%). Four-day aspirin pre-treatment significantly reduced the first peak and abolished the second peak in plasma TxB<sub>2</sub>, delayed the LPS-induced reduction in digital blood flow without affecting the magnitude of the hypoperfusion and significantly attenuated the LPS-induced decreases in HWST (by 57%) and CBST (by 41%). *Ex vivo* studies showed that the 4-day aspirin treatment protocol had no significant effect on leucocyte COX but inhibited platelet COX activity by 75%.

Hypoperfusion of the equine digit induced by mild transient endotoxemia is significantly attenuated by inhibition of platelet TxB<sub>2</sub> production. Thus, platelet derived TxB<sub>2</sub> is partially responsible for the LPS-induced digital hypoperfusion. If LPS does play a role in the pathogenesis of acute laminitis, TxB<sub>2</sub> may contribute to the ischaemia preceding the onset of this disease.

#### **ABSTRACT #52**

**DEXAMETHASONE INDUCES INSULIN RESISTANCE IN QUARTER HORSES WITH POLYSACCHARIDE STORAGE MYOPATHY.** AM Firshman, SJ Valberg, T Karges, LE Benedict, EJ Annandale, ER Seaquist. College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

Polysaccharide storage myopathy (PSSM) is a glycogen storage disorder in Quarter Horses (QHs) associated with abnormal polysaccharide accumulation in skeletal muscle. Horses with PSSM have increased insulin-stimulated glucose excursion from the blood compared to controls indicating enhanced insulin sensitivity. The mechanism by which enhanced glucose uptake and glycogen

synthesis induces muscle necrosis with exercise in PSSM horses is unknown. Feeding a low starch/fat supplemented diet decreases postprandial glucose and insulin concentrations and reduces rhabdomyolysis in regularly exercised PSSM horses. We hypothesized that insulin-stimulated glucose uptake could be reduced and rhabdomyolysis minimized by administering dexamethasone (DEX) to PSSM horses since DEX inhibits GLUT4 translocation to the plasma membrane. The specific aims were 1) to determine if DEX decreases insulin sensitivity in PSSM horses compared to placebo and 2) to determine if DEX decreases 4-hour post exercise serum creatine kinase (CK) activity compared to placebo.

Four fit adult QHs with PSSM, fed grass hay and sweet feed/rice bran, were used in a 2 by 2 switch back design using 40 mg DEX IV every 48 hrs or saline placebo. Horses were exercised on a treadmill 5 days/week for 3 weeks per treatment with a 2-week washout period. Serum CK activity was measured 4 hr post-exercise. At the end of each treatment period, serum cortisol was measured, a 3 hr hyperinsulinemic euglycemic clamp was performed and [glycogen] were determined in gluteal muscle biopsies.

Serum cortisol was significantly lower after 48 hrs on DEX ( $0.38 \pm 0.08\mu\text{g/dl}$ ) than placebo ( $4.15 \pm 0.40\mu\text{g/dl}$ ). DEX treatment significantly decreased (by 2.5 X) the rate of glucose infusion necessary to maintain euglycemia during the clamp compared to placebo treatment indicating a reduction in insulin sensitivity with DEX treatment. Muscle glycogen concentrations of PSSM horses were 1.4 X greater than normal values and were not altered by DEX treatment. Mean serum CK activity ( $3,920 \pm 1,110\text{U/L}$ ) on placebo was not significantly different from DEX treatment ( $3,900 \pm 743\text{U/L}$ ). The results of this study indicate that although DEX treatment can significantly reduce insulin sensitivity in PSSM horses over a 3-week period it has no impact on reducing exercise induced rhabdomyolysis or decreasing muscle glycogen concentrations in PSSM horses. Thus, for the duration of this study, enhanced insulin stimulated glucose uptake did not appear to be the primary factor responsible for increased glycogen synthesis and exertional rhabdomyolysis in PSSM horses.

#### **ABSTRACT #53**

IDENTIFICATION OF GLYCOGEN BRANCHING ENZYME (GBE1) MUTATION IN AMERICAN QUARTER HORSES. TL Ward, SJ Valberg, DL Adelson, CA Abby, M Binns and JR Mickelson. Colleges of Veterinary Medicine, University of Minnesota, St. Paul, MN, Texas A&M University and The Animal Health Trust Newmarket UK.

Glycogen branching enzyme deficiency (GBED) has recently been reported in the American Quarter Horse. Varied clinical signs included late term abortion, weakness at birth, ventilatory failure, hypoglycemic seizures, contracted tendons, inability to rise, and/or sudden death by 18 weeks of age. Abnormal globular and crystalline polysaccharide is found in multiple tissues and isolated polysaccharide from liver and muscle has an unbranched structure in GBED. GBE1 enzyme assays from blood, muscle or liver tissue show virtually no activity in affected foals and GBE1 protein in liver determined by Western immunoblot is markedly absent in affected foals. Dams of affected foals have approximately half of the control levels of GBE1 activity and approximately half the amount of GBE1 protein. Biochemical and pedigree analyses are consistent with an autosomal recessive trait, however the mutation causing GBED in foals is not known. The purpose of this study was to determine the sequence of the GBE1 cDNA in horses and to determine if a mutation is present in foals with clinical and biochemical evidence of GBED.

Over 90% of the coding sequence of the equine GBE1 gene was obtained by RT-PCR. The remaining 5' and 3' sequence was obtained from BAC clones screened to ensure they contained GBE1 exon 2 and 15. PCR primers were designed to amplify a 266 bp segment of the equine GBE1 gene containing the 5' UTR and almost

all of exon 1. These primers were used to screen 16 controls, 11 GBED foals and at least one of their parents for a GBE1 mutation. A single base pair substitution resulting in conversion of tyrosine (Y) to a premature stop codon was identified in exon 1. All affected foals were homozygous for the mutation, all available dams and/or sires were heterozygous, and all control horses were homozygous for the Y allele. The previous findings of poorly branched glycogen, abnormal polysaccharide accumulation, lack of measurable GBE1 enzyme activity and lack of immuno-detectable GBE1 protein in foals with GBED can all be explained by this premature stop codon in the GBE1 mRNA. The pedigree contains prolific stallions with many thousands of offspring that are possible carriers of the recessive mutation. A DNA based test will allow easy identification of GBED foals and the ability to identify carriers will help prevent occurrence of this devastating disease in Quarter Horse related breeds.

#### **ABSTRACT #54**

ANALYSIS OF RECURRENT EXERTIONAL RHABDOMYOLYSIS IN THOROUGHBRED HORSE PEDIGREES AND EXCLUSION OF LINKAGE TO THE RYR1 GENE. PK Dranchak, SJ Valberg, GW Onan, EM Gallant, JM MacLeay and JR Mickelson. College of Veterinary Medicine, University of Minnesota, St. Paul MN; University of WI-River Falls, River Falls WI; Colorado State University, Fort Collins CO.

Exertional rhabdomyolysis (ER) occurs in 5-10% of Thoroughbred racehorses. Pedigree evaluation as well as biochemical and physiological analysis suggest that a form of ER known as recurrent exertional rhabdomyolysis (RER) is due to an inherited disorder of intracellular calcium regulation. Horses with RER have a lower contracture threshold to halothane and caffeine in intact intercostal muscle biopsies compared to controls. Thus, RER bears clinical and physiological resemblance to a heritable disorder in other species known as malignant hyperthermia caused primarily by mutations in the RYR1 gene. Specific Aim 1 in the present study was to determine the pattern of inheritance of RER. Specific Aim 2 was to test the RYR1 gene for linkage to RER. A well-defined family of horses was constructed by breeding Thoroughbred and crossbred horses with known clinical RER status and known phenotypes according to muscle contracture testing. The 22 resultant foals were tested for RER susceptibility with an *in vitro* muscle contracture test. Additional multigenerational families were provided through a collaborative effort with stables around the country. Thoroughbreds were diagnosed with ER based on history, clinical signs and whenever possible serum CK activity. Visual inspection and Chi Square analysis of the combined families indicated that the RER trait appeared to segregate in an autosomal dominant fashion (p-value for exclusion of dominance > 0.25). A recessive mode of inheritance was excluded due to the presence of an unaffected offspring from two affected parents. In the resource herd 5 foals tested positive for RER (3 males, 2 females); 12 foals tested negative (6 females, 6 males). In the additional families 13 offspring were assigned positive phenotypes (3 male, 10 female) and 10 offspring were described as normal (7 male, 3 female). Simulated linkage analysis was used to identify 96 individuals (36 affected) from the resource family and 3 additional families for a molecular genetic linkage analysis of RER to the RYR1. RYR1 was excluded as causative for RER in combined pedigrees by genetic linkage analysis with eight microsatellite markers from ECA10p, which all showed LOD scores below -1.00 for linkage to RER. Thus, RER appears to be a novel heritable disorder affecting muscle contractility and a future whole genome scan on these pedigrees should enable the mapping of the chromosomal locus of the RER gene.

#### ABSTRACT #55

GLUCOSE TRANSPORTER TYPE 4 GENE EXPRESSION IN EQUINE SKELETAL MUSCLE INCREASES AFTER GLYCOGEN DEPLETING EXERCISE INDEPENDENTLY OF MEAL TYPE AFTER EXERCISE. Eduard Jose-Cunilleras, Kathleen A Hayes, Ramiro E Toribio, Lawrence E Mathes, Kenneth W Hinchcliff. The Ohio State University, Dept Veterinary Clinical Sciences and Dept Veterinary Biosciences, Columbus, OH.

Exercise depletes muscle glycogen content. Replenishment of muscle glycogen is critical for optimal performance during subsequent high-intensity exercise. Availability of the substrate, plasma glucose, and translocation of the main glucose transporter from storage vesicles inside the muscle cell to the cellular membranes, are key factors for muscle glycogen replenishment in other species. The purpose of the present study was to clone and sequence the glucose transporter type 4 (GLUT4) in horses, and determine the effect of glycogen depleting exercise and of meal type after exercise on GLUT4 gene expression in equine skeletal muscle by quantification of GLUT4 mRNA by real-time RT-PCR normalized to expression of equine beta Actin.

Horses were exercised for 3 consecutive days in order to decrease muscle glycogen concentration by ~66%. Biopsies were obtained by biopsy needle from the gluteus medius muscle before 3 consecutive days of exercise and immediately after, 4 h, 8 h, and 24 h after the 3rd exercise bout. In the 3<sup>rd</sup> day of exercise, during the 8 hours following exercise in a randomized cross-over design, each of 6 horses were either not fed (NF), fed half of the daily energy requirements as mixed alfalfa and grass hay (H) or fed an isocaloric amount of corn (C) immediately and 4 hours after exercise. Total RNA was isolated from muscle biopsies and initially used to amplify various specific cDNA products of ~700-800 bp by conventional RT-PCR that overlapped and covered the entire coding region of the equine GLUT4 gene. Primers were designed by comparing published sequences of GLUT4 cDNA in human, rat, mouse and cow, and choosing oligonucleotides in areas with the highest homology between species. After cloning and sequencing the coding region of equine GLUT4 mRNA, species specific primers were designed to quantify GLUT4 mRNA in muscle biopsies using real-time RT-PCR and SYBR Green I dye.

With 4 overlapping RT-PCR products a total of 1629 bp were sequenced, of which 1527 bp corresponded to the coding region, encoding a protein of 509 aminoacids (GenBank # AF531753). Equine GLUT4 cDNA is >88% homologous to that of humans, cows, rats and mice.

GLUT 4 gene expression in muscle increased by ~2.3, ~4.3, ~3.3, and ~2.6 fold immediately, 4 h, 8 h and 24 h after exercise when compared to that prior to exercise (246±80, 556±194, 1048±337, 825±207 and 638±215 GLUT4 transcripts/ng total RNA in muscle). No differences were observed in the level of GLUT4 gene expression in muscle when comparing NF, C or H feeding during the 8 h following exercise. Lack of activation of GLUT4 gene expression after grain feeding and exercise may explain the apparently slower rate of glycogen synthesis after exercise in horses relative to that of other species.

#### ABSTRACT #56

ACID-BASE HOMEOSTASIS ACROSS THE LUNG AT REST AND DURING EXERCISE IN HORSES. M. Vengust<sup>1</sup>, H. Stämpfli<sup>2</sup>, L. Viel<sup>2</sup>, F. Teixeira-Neto<sup>2</sup>, A. Nunez de Moraes<sup>2</sup>, G. Heigenhauser<sup>3</sup>. <sup>1</sup>University of Ljubljana, Veterinary Faculty, Slovenia; <sup>2</sup>University of Guelph, Ontario Veterinary College, Guelph, Ontario, Canada; <sup>3</sup>McMaster University Medical Centre Hamilton, Ontario, Canada.

The control of acid-base homeostasis is a recognized function of lungs, but the interactive role of ions and CO<sub>2</sub> across the alveolo-capillary barrier remains controversial. The purpose of this study was

to investigate physiological situation in pulmonary capillaries to provide better insight into the electrolyte and volume homeostasis across the alveolo-capillary barrier.

Six horses (5-6 years) were exercised on a high-speed treadmill (Säto Sweden) at 80% VO<sub>2</sub> peak until fatigue. Resting arterial and mixed venous blood, as well as CO<sub>2</sub> elimination and O<sub>2</sub> uptake, were sampled simultaneously 5 minutes apart. During exercise, the sampling was performed in 60 sec intervals, and during the recovery period starting after the treadmill was stopped due to fatigue (0 min) and then at 1, 2, 3, 5, 10, and 15 min. Changes in blood dependent variables ([H<sup>+</sup>], [HCO<sub>3</sub><sup>-</sup>]) were quantified through independent physicochemical variables (strong ion difference [SID], weak electrolyte concentrations [A<sub>tot</sub>], and PCO<sub>2</sub>). Changes of volume in the pulmonary capillaries were calculated during and after exercise from changes in hemoglobin and hematocrit values in venous and arterial blood. Variables were analyzed using two-way repeated-measures ANOVA (P<0.05).

VCO<sub>2</sub>, VO<sub>2</sub>, PO<sub>2</sub>, and PCO<sub>2</sub> changed significantly during the exercise (P<0.05). When compared to resting values whole blood SID increased, intra-erythrocyte SID increased, and plasma SID decreased across the lung in blood passing pulmonary capillaries. SID changes, however, were not significantly different from resting values. Plasma [A<sub>tot</sub>] did not change across the lung. Plasma [H<sup>+</sup>] decreased across the lung by 17.7±1.4 nEq/L at fatigue compared to 2.4±0.5 nEq/L at rest (P<0.0001). Plasma [HCO<sub>3</sub><sup>-</sup>] decreased by 10.1±0.5 mEq/L across the lung at fatigue compared to 1.8±0.6 mEq/L at rest (P<0.0001). Blood volume, erythrocyte volume, and plasma volume did not change across the lung at rest. Plasma volume was unchanged throughout the experiment. Blood volume and erythrocyte volume decreased across the lung during exercise, which was not statistically significant from resting values.

CO<sub>2</sub> elimination and hemoglobin O<sub>2</sub> saturation in pulmonary capillaries coexisted with ion shifts and volume changes between different compartments, which provided means for H<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> reduction across the lung. Blood volume decrease was likely associated with an exercise-induced increase in pulmonary interstitial fluid. SID is a reliable indicator of electrolyte activity across the lung but A<sub>tot</sub> does not seem to play an important interactive role in lung regulated acid-base changes.

#### ABSTRACT #57

ACID-BASE HOMEOSTASIS ACROSS THE LUNG IN EXERCISING HORSES AFTER ACUTE AND CHRONIC INHIBITION OF CARBONIC ANHYDRASE. M. Vengust<sup>1</sup>, H. Staempfli<sup>2</sup>, G. Heigenhauser<sup>3</sup>, F. Teixeira-Neto<sup>2</sup>, A. Nunez de Moraes<sup>2</sup>, L. Viel<sup>2</sup>. <sup>1</sup>University of Ljubljana, Veterinary Faculty, Slovenia; <sup>2</sup>University of Guelph, Ontario Veterinary College, Guelph, Ontario, Canada; <sup>3</sup>McMaster University Medical Centre Hamilton, Ontario, Canada.

Specific airway diseases in horses, especially non-septic inflammatory airway disease and EIPH as it pertains to pulmonary interstitial fluid equilibrium and acid-base control during exercise, are poorly understood. This study was designed to determine the role of the lung in the control of acid-base responses, volume changes between the plasma and RBC, and gas exchange across the lung in blood passing through the pulmonary capillary during variable degrees of CO<sub>2</sub> retention.

Six horses (5-6 years) were exercised until fatigue on a high speed treadmill (Säto Sweden) at 80% VO<sub>2</sub> peak with the following treatment protocol: a) without carbonic anhydrase (CA) inhibition (control), b) acute CA inhibition without the associated metabolic acidosis, and c) chronic CA inhibition and associated metabolic acidosis. CO<sub>2</sub> retention was achieved with oral administration of CA inhibitor acetazolamide (Apo-Acetazolamide, Apotex Inc.) at a dose of 30 mg/kg of BW before the exercise for acute CA inhibition (AcIn), and 10 mg/kg of BW TID for 3 days and 30 mg/kg of BW

before the exercise for chronic CA inhibition (ChIn). Resting arterial and mixed venous blood, as well as CO<sub>2</sub> elimination and O<sub>2</sub> uptake were sampled simultaneously 5 minutes apart. During exercise, the sampling was performed in 60 sec intervals, and during the recovery period starting after the treadmill was stopped due to fatigue (0 min) and then at 1, 2, 3, 5, 10, and 15 min. [H<sup>+</sup>] and [HCO<sub>3</sub><sup>-</sup>] were quantified through [SID], [A<sub>tot</sub>], and PCO<sub>2</sub>. Changes in volumes were calculated from changes in hemoglobin and hematocrit values in venous and arterial blood. Variables were analyzed using two-way repeated-measures ANOVA (P<0.05). A significant F ratio was further analyzed using Tukey post-hoc analysis.

Treatment had a significant effect on performance (P<0.0001) as well as on VCO<sub>2</sub> and VO<sub>2</sub> (P<0.05). PCO<sub>2</sub> was higher in AcIn and ChIn (P<0.0001). Treatment affected [SID] across the lung in whole blood, RBC, and plasma (P≤0.01). Plasma [A<sub>tot</sub>] across the lung was not affected by CO<sub>2</sub> retention. AcIn and ChIn affected plasma [H<sup>+</sup>] across the lung and, less so, plasma [HCO<sub>3</sub><sup>-</sup>] across the lung (both P<0.05). Blood volume changes across the lung were affected by ChIn (P=0.004). RBC volume changes across the lung was affected by ChIn (P=0.009) and AcIn (P=0.002). Plasma volume across the lung was affected by AcIn (P=0.009).

CA inhibition was associated with changes in CO<sub>2</sub> homeostasis, which affected acid-base homeostasis across the lung in blood passing pulmonary capillaries. This was reflected by disturbances in strong ions and consequently [SID] in whole blood, RBC, and plasma. Volume regulation in whole blood, RBC, and plasma were disturbed by CO<sub>2</sub> retention, which probably affected the kinetics of the pulmonary interstitial fluid.

#### **ABSTRACT #58**

EVALUATION OF TRANSDERMAL GLIPIZIDE IN A PLURONIC LECITHIN GEL IN HEALTHY CATS. Nicole Bennett,<sup>1</sup> Mark Papich,<sup>2</sup> Martin Fettman,<sup>1</sup> Michael R. Lappin.<sup>1</sup> From Colorado State University, Fort Collins CO<sup>1</sup> and North Carolina State University, Raleigh NC.<sup>2</sup>

Although insulin and glucose concentrations have been evaluated in cats given oral glipizide, serum drug concentrations have not been measured. In addition, pharmacokinetics and pharmacodynamics of this drug in the cat are unknown. Transdermal glipizide has the potential to provide a safe, effective means of controlling diabetes mellitus in some cats without requiring oral administration. This study was performed to evaluate serum glipizide concentrations in healthy cats after the administration of a single 5 mg dose of either oral or transdermal glipizide. This study also evaluated the relationship between serum glipizide and plasma glucose levels.

Sixteen healthy, SPF, laboratory raised cats were randomly divided into three groups. Six control cats were assigned to receive either an oral or a transdermal placebo. Ten cats were assigned to receive either 5 mg of encapsulated glipizide powder orally or 0.1 ml (50mg/ml) of glipizide, compounded into a pluronic lecithin organogel, topically on the inner pinna. All cats were fasted twelve hours prior to drug administration. Blood was sampled at 0, 10, 20, 30, 45, 60, 90, 120, 240, 360 minutes and then every four hours thereafter for a total of 24 hours.

Serum glipizide was measured by HPLC in all cats receiving either the oral or transdermal formulation. Glipizide concentrations were significantly higher in those cats receiving oral drug compared to the cats receiving the transdermal preparation (p<0.0001). The maximum concentration (C<sub>MAX</sub>) achieved with a single 5 mg oral dose of glipizide was 5.6 ±3.2 µg/ml versus a C<sub>MAX</sub> of 0.99 ± 0.56 µg/ml after a single 5mg transdermal dose. Transdermal absorption was variable among cats and averaged 20% (+/-14) relative to the mean absorption of the oral dose. Glipizide was detected earlier in cats receiving oral glipizide than in cats receiving the transdermal gel, indicating a delay in transdermal absorption. The time to reach maximum concentration was only 5 ± 3.5 hr after oral dosing versus

16 ± 4.5 hr after transdermal administration. The elimination half lives were not statistically different between groups with a 16.8 ± 12 hr half life with oral administration and a 15.5 ± 15.3 hr half life with transdermal administration. Plasma glucose concentrations were significantly lower in cats receiving glipizide, either orally or topically, than in control cats (p<0.0001). Cats given oral glipizide had significantly lower plasma glucose levels compared to the transdermal cats (p<0.05) until 6 hours post dosing. After this time point, there was no significant difference in glucose concentrations between the treatment groups despite a significant difference in serum glipizide concentrations (p=0.01).

In this study, glipizide was detected in all cats receiving the drug either orally or transdermally. Plasma glucose concentrations declined in both treatment groups. Despite apparent delay in transdermal absorption, transdermal glipizide may provide an alternate route of administration. Further studies are required to assess the bioavailability and efficacy of this glipizide formulation in diabetic cats over longer periods of time.

#### **ABSTRACT #59**

FASTING AND POST PRANDIAL SERUM GASTRIN CONCENTRATIONS IN DOGS WITH PITUITARY DEPENDENT HYPERADRENOCORTICISM. RE Goldstein, J Pintar, JM Scarlett, Cornell University, Ithaca, NY.

The purpose of this study was to compare fasting and post prandial serum gastrin concentrations between dogs with pituitary dependent hyperadrenocorticism (PDH) and normal dogs and in dogs with PDH before and after initial treatment with mitotane. An increase in serum gastrin concentrations has been documented in humans with PDH, as well as in humans, dogs and rodents with chronic glucocorticoid administration.

Serum gastrin concentrations were measured by a validated radioimmunoassay in 16 client owned dogs with PDH and in 11 clinically normal control dogs of similar ages. Dogs with concurrent diseases or that had received medications that could alter serum gastrin concentrations were excluded. Samples were collected after a 12 hour fast (time 0) and 30 and 60 minutes following the ingestion of a standardized amino acid rich meal. In affected dogs, sampling was performed before and after a 3-10 day mitotane loading period (25-50mg/kg/day) for treatment of PDH. An ACTH stimulation test was performed in all dogs with PDH just following acquisition of samples for serum gastrin determination. The Wilcoxon rank sum test was used to compare serum gastrin concentrations in dogs with PDH to those of normal dogs and before and after mitotane loading, at each of the three time points. The Spearman rank correlation was used to determine if post ACTH stimulation serum cortisol concentrations correlated with serum gastrin concentrations in dogs with PDH. Statistical significance was set at p≤0.05.

Serum gastrin concentrations (median, ng/ml) were significantly higher at times 0, 30, and 60 minutes in untreated PDH dogs compared to control dogs (84.5, 154, 151 vs. 28, 80, 80 respectively, p<0.05). Serum gastrin concentrations following mitotane loading in dogs with PDH (73, 123, 112) were not different than pre mitotane loading concentrations at any time point and were different than serum gastrin concentrations of normal dogs at all time points. Post ACTH stimulation serum cortisol concentrations (median, pg/ml) differed before and after mitotane loading in dogs with PDH (22.75 vs. 3.67 respectively). Serum gastrin concentrations did not correlate with serum post ACTH cortisol concentration at any time point.

These results demonstrate that fasting and post prandial serum gastrin concentrations are increased in dogs with PDH compared to normal dogs. Loading treatment with mitotane did not reduce serum gastrin concentrations in affected dogs, and serum cortisol concentration did not correlate with the severity of hypergastrinemia. Therefore, hypergastrinemia in dogs with PDH may not be mediated by hypercortisolemia. However, since gastrin was measured just after

mitotane loading, it is possible that hypergastrinemia would resolve following chronic treatment. The cause of this hypergastrinemia and possible clinical affects in canine PDH including increased gastric acid secretion or contribution to hepatomegaly as a trophic factor require additional studies.

**ABSTRACT #60**

BLOOD PRESSURE, PROTEINURIA, AND MICROALBUMINURIA IN DOGS WITH HYPERADRENOCORTICISM AND RESPONSE TO THERAPY WITH AN ANGIOTENSIN CONVERTING ENZYME INHIBITOR. P. Boutillier, A. Carr, C. Waldner. Western College of Veterinary Medicine, Saskatoon, SK.

Hyperadrenocorticism (HAC) is a common endocrinopathy of older dogs. A variety of complications have been noted with this disease including hypertension, proteinuria and a hypercoagulable state. Proteinuria in HAC may lead to urinary antithrombin III (AT III) loss, decreased levels of which have been suspected to contribute to the hypercoagulable state seen with HAC. Work in a variety of species has shown that angiotensin converting enzyme inhibitors (ACEi) can decrease systemic blood pressure and reduce proteinuria with glomerulonephritis. Recently a microalbuminuria assay has become available which may be able to document lower levels of glomerular protein loss than is possible with the standard urine protein to urine creatinine ratio (UP:UC).

This prospective study was designed to investigate blood pressure, proteinuria and microalbuminuria in dogs with naturally occurring (pituitary dependent) HAC and their response to ACEi therapy. A total of 11 client-owned dogs were enrolled in the study. None of the dogs had been treated for their HAC. Baseline values were collected from all dogs and compared to an age-matched control group (8 dogs). An elevation of the UP:UC (>1.0) was found in 36.4% of the HAC dogs. An ELISA for microalbuminuria (>1mg/dL, Heska Corporation, Ft. Collins, CO) was positive in 63.6% of HAC dogs. All dogs with elevated UP:UC ratios were positive for microalbuminuria, 3 dogs had microalbuminuria without elevations in UP:UC. A Chi-square test for independence was used to assess microalbuminuria and a Wilcoxon signed rank test was used to assess UP:UC between HAC dogs and controls. HAC dogs were significantly higher for both microalbuminuria (p = 0.013) and UP:UC (p = 0.0004) than control dogs. Blood pressure was determined with an oscillometric device (Memoprint, S+B MedVet, Babenhausen, Germany). Using the criteria established by the Veterinary Blood Pressure Society 72.7% of the HAC dogs were moderately hypertensive (Systolic >160mmHg or diastolic >100mmHg). None of the control dogs were hypertensive by these criteria. Blood pressure was significantly higher in the HAC dogs than control dogs (systolic p = 0.03, diastolic p = 0.0056) using the Wilcoxon signed rank test.

The HAC dogs were placed on a 3-week course of enalapril (Enacard®, Merial, Baie d'Urfe, QC) at a dose of 0.5mg/kg/day. At the end of the 3-week period all measurements were repeated. Using the Wilcoxon signed rank test there were no statistically significant differences between pre- and post-ACEi values for blood pressure, proteinuria or microalbuminuria (p>0.05). The results of this study suggest that the proteinuria, microalbuminuria, and hypertension that occur in association with HAC are not responsive to ACEi therapy at standard dosages.

**ABSTRACT #61**

EVALUATION OF POST-ACTH SERUM 17-HYDROXY-PROGESTERONE CONCENTRATIONS IN CANINE HYPERADRENOCORTICISM. N. Benitah, EC Feldman, RW Nelson. University of California, Davis, CA.

The aim of this prospective study was to evaluate use of serum 17-hydroxyprogesterone (17-OHP) concentrations, a cortisol precursor,

after ACTH administration, as a screening test for the diagnosis of hyperadrenocorticism (HAC) in dogs. Fifty dogs with confirmed HAC (38 pituitary-dependent [PDH], 12 adrenocortical tumor [ATH]) and 55 healthy gender and neuter-matched control dogs were evaluated. A third group of 5 dogs with clinical signs and routine laboratory abnormalities suggestive of HAC that had ACTH stimulation and low-dose dexamethasone suppression (LDDS) test results within reference limits were also evaluated. All 38 dogs with PDH had abnormal ACTH stimulation or LDDS test results. All 12 dogs with ATH (3 adenomas, 9 carcinomas) had abnormal LDDS test results and 4/12 had abnormal ACTH stimulation test results. Serum concentrations of 17-OHP were assayed before and after ACTH stimulation in all 55 dogs with HAC and compared to the following reference ranges established using the controls:

Gender-Neuter status	N	Pre 17-OHP (ng/ml) Median (5-95%) <sup>b</sup>	Post 17-OHP (ng/ml) Median (5-95%) <sup>a,b,c</sup>
Female intact (FI)	12	0.51 (0.07-2.10) <sup>b</sup>	3.59 (0.72-8.36) <sup>a,b,c</sup>
Female spayed (FS)	15	0.10 (0.04-1.06)	0.98 (0.67-2.04) <sup>c</sup>
Male intact (MI)	12	0.20 (0.03-1.61) <sup>a</sup>	2.05 (1.12-3.24) <sup>b</sup>
Male neutered (MN)	16	0.07 (0.02-0.28) <sup>a,b</sup>	1.21 (0.62-3.37) <sup>a</sup>

<sup>a,b,c</sup> Values with same letters are significantly different from each other (P<0.05).

Results in dogs with HAC were as follows:

Gender-neuter status	N	PDH (n=38)		N	ATH (n=12)	
		Post 17-OHP (ng/ml) Median (range)	Abnormal (%)		Post 17-OHP (ng/ml) Median (range)	Abnormal (%)
FS	25	3.12 (0.72-19.10)	45	8	4.89 (1.11-25.2)	42
MN	11	6.21 (0.78-10.10)	21	4	2.88 (0.49-27.4)	17
MI	2	6.00, 17.36	5	0		
Total	38		71	12		59
ACTH st. test	38		68	12		33
LDDS test	34		79	12		100

Of dogs with ATH, median (range) post-ACTH 17-OHP concentration was 4.52 (3.79-27.4) and 1.96 (0.49-25.2) for adenomas and carcinomas, respectively. Of the 5 dogs with suspected HAC, 2 had abnormal post-ACTH 17-OHP concentration (median [range]: 2.02 [1.35-6.38]). Results of this study suggest that serum 17-OHP concentration measured after ACTH administration could be useful as a screening test for diagnosis of canine HAC if other screening tests are equivocal.

**ABSTRACT #62**

PULMONARY HISTOPATHOLOGY ASSOCIATED WITH DIABETES MELLITUS IN DOGS AND CATS. Angela M. Mexas, Eleanor C. Hawkins, and Linda D. Martin. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Diabetes mellitus is a common endocrinopathy of dogs, cats and people. Human diabetics have a high incidence of pulmonary pathology, often dying as a result of pulmonary infections. Changes in pulmonary function and histopathology in human diabetics and rodent models have been documented. However, the association between diabetes mellitus and pulmonary pathology in dogs and cats has never been studied, even though the respiratory tract is a common site of occult infection associated with insulin resistance and poor control of glucose levels in these species. Therefore, the objective of this study was to determine whether dogs and cats with diabetes mellitus have an increased incidence of pulmonary pathology compared with non-diabetic dogs and cats.

Cases presented to the North Carolina State University College of Veterinary Medicine from 1999-2003 were identified in the hospital's database using key word 'diabetes mellitus'. Patient ID numbers were cross-referenced to necropsy files in order to identify diabetic dogs and cats with necropsy reports during the same time period. All cases with complete necropsy reports and a clinical diagnosis of diabetes mellitus were included. The necropsy reports were analyzed for presence and description of pulmonary pathology and categorized as having histopathologically normal or abnormal lungs. Hospital records were reviewed for the presence of clinical signs referable to the respiratory tract before death. Necropsy records of age-matched dogs and cats without diabetes or clinical evidence of pulmonary disease from the same time period were used as a control population.

Of 218 dogs and cats with diabetes mellitus, 15 had complete necropsies performed. None of the 15 cases had clinical signs referable to the respiratory tract before death. Abnormal histopathologic findings in the lungs were found in 12 out of the 15 cases (80%). Only one of five diabetic dogs and two of 10 diabetic cats had normal lungs. Pulmonary pathology included non-specific inflammatory changes (7/15), pulmonary edema (3/15), bacterial pneumonia (4/15), lipid pneumonia (1/15), and neoplasia (3/15). Although the incidence of pulmonary histopathology in diabetic dogs and cats was higher than that in the control population (0.8 compared to 0.53), the difference was not statistically significant ( $p$ -value = 0.12; Fischer Exact Test).

Although this data did not show a statistically significant difference between diabetic and non-diabetic dogs and cats, the study is currently limited by the small number of cases. However, the findings remain consistent with our hypothesis that there is an increased incidence of pulmonary pathology associated with diabetes mellitus. Continued studies will incorporate greater numbers of cases and further characterization of the histopathologic changes seen in the lungs of diabetic dogs and cats in an effort to determine whether health monitoring of the respiratory system could lead to decreased morbidity from occult pulmonary diseases in these animals.

#### **ABSTRACT #63**

**INSULIN GLARGINE AND A HIGH PROTEIN-LOW CARBOHYDRATE DIET ARE ASSOCIATED WITH HIGH REMISSION RATES IN NEWLY DIAGNOSED DIABETIC CATS.** RD Marshall<sup>1,2</sup>, JS Rand<sup>1</sup> <sup>1</sup>Centre for Companion Animal Health, University of Queensland, <sup>2</sup>Creek Rd Cat Clinic, Brisbane, Australia.

Insulin glargine is a new human synthetic insulin analogue that is very long-acting. Its pharmacokinetics and pharmacodynamics were determined in healthy cats, and this is the first report of its use in diabetic cats.

Six newly-diagnosed diabetic cats (2 male, 4 female) were treated with glargine and fed a high protein-low carbohydrate diet (Purina DM canned). Cats were allowed to eat ad lib for the first 2 weeks, and then calories fed were adjusted to achieve optimal body weight. Initial dose of glargine was 0.5U/kg BID S/C, rounded to the nearest unit. Insulin dose was then adjusted based on serial blood glucose curves and water intake.

At diagnosis, mean age was 10.7 $\pm$ sem0.8yrs, body weight was 6 $\pm$ 0.7kg and body condition score was 6.8 $\pm$ 0.7 (scale 1-9). Three cats were Burmese and 3 were Domestic Shorthair. No diabetogenic drugs were known to have been administered to any cat in the preceding 12 months. Four cats presented with a plantigrade stance, and 1 cat presented with signs associated with an infected tooth root. Four cats were initially ketotic and 3 of these were also acidotic. Mean glucose concentration at diagnosis was 478 $\pm$ 45mg/dl (range=351-648mg/dl), and mean fructosamine concentration was 552  $\pm$  27 $\mu$ mol/L. All cats were still eating at presentation, and were treated with subcutaneous injections of glargine from the time of diagnosis.

Mean 12hr glucose concentration 10 days after beginning glargine was 265 $\pm$ 63mg/dl and at day 17 was 178 $\pm$ 65mg/dl. All 6 cats treated with glargine went into diabetic remission within 4 months of beginning treatment, and 5 cats went into remission within 4 weeks (mean=4.5 $\pm$ 1.9 weeks, median=2.5 weeks). Five cats remained in remission at the time of publication (mean remission time=7.6 $\pm$ 2.1 months, range=3-15 months). Body weight at time of remission was 5.9 $\pm$ 0.6kg, which was not significantly different to initial weight. All cats had fructosamine concentrations within the reference range once in remission (246 $\pm$ 14 $\mu$ mol/L). Only 1 cat required an increase in insulin dose above 0.5U/kg BID, and was given 0.7U/kg BID. Mean insulin dose at day 10 of treatment was 0.26U/kg (range=0.2-0.6U/kg) BID, and range at day 17 was =0.25-0.73U/kg BID (n=2).

Total insulin dose per cat during the trial ranged from 1U SID to 4U BID. No cat experienced clinical hypoglycemia with glargine.

In conclusion, glargine was safe and very effective for the treatment of newly diagnosed diabetic cats. When combined with a high protein-low carbohydrate diet, it resulted in a very high remission rate in newly diagnosed diabetic cats. The relative euglycemia achieved with glargine was assumed responsible for rapid reversal of beta cell glucose toxicity and early diabetic remission.

#### **ABSTRACT #64**

**POST-PRANDIAL AND LIPOLYTIC RESPONSES OF DOGS TO DIACYLGLYCEROL PROVIDES A METABOLIC ENVIRONMENT FOR OBESITY PREVENTION AND WEIGHT MANAGEMENT.** B. Porterpan<sup>1</sup>, K. Bigley<sup>1</sup>, D. Nagaoka<sup>1</sup>, T. Umeda<sup>2</sup>, K. Otsuji<sup>2</sup>, J.E. Bauer<sup>1</sup>. <sup>2</sup>Companion Animal Nutrition Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA. <sup>2</sup>Kao Corporation, Tokyo, Japan.

Obesity of companion animals contributes to respiratory, orthopedic and other disorders. Diets to support healthy weight loss utilize caloric restriction at the sacrifice of palatability. Metabolic approaches to increase adipose tissue mobilization or prevent adipose accumulation have the potential to become important alternatives to weight management. To address this concept, a new vegetable oil has been developed containing 80% diacylglycerol (DAG) which is a naturally occurring yet minor component of traditional dietary oils containing triacylglycerol (TAG). DAG has been studied in rodents and human trials and is generally regarded as safe. Primary digestion products of DAG are 1- (or 3-) monoacylglycerol and fatty acids the latter of which appear to be favorably beta-oxidized in the intestinal mucosa and liver rather than re-esterified as TAG in the adipocyte. Under this condition, less secretion of TAG- rich chylomicra and less adipose tissue accumulation would occur. This study was designed to confirm the observed post-prandial effects of DAG in normal dogs and extend the work to include the determination of lipoprotein lipase and hepatic lipase activities. Normal adult Beagles were fed meals enriched in dietary DAG compared with TAG. Four different meals were fed containing cooked chicken breast combined with either DAG or TAG in the presence of either high or low glycemic index carbohydrate sources. High amylose corn -starch (low glycemic index) and waxy corn- starch (high glycemic index) were used as carbohydrate sources. The meals were randomly fed to each of 12 dogs and post-prandial blood samples were collected at 0, 0.5, 1, 2, 3, 4 and 6 hours. Post-heparin plasma was also obtained following the 6hour blood sample. Serum triglyceride, non-esterified (NEFA) fatty acids, and glucose were evaluated using standard methods. Lipoprotein lipase (LPL) and hepatic lipase (HL) of post-heparin plasma were assayed by differential substrate techniques. Significant differences were found for both the peak and extent of post-prandial hypertriglyceridemia when DAG was consumed independent of high or low glycemic-index starch. When low glycemic carbohydrate was fed a peak serum NEFA response was seen after 2-3 hours. High glycemic carbohydrate plus TAG showed a more precipitous decrease of NEFA during the first 30 minutes after feeding. Serum NEFA also varied more widely in both TAG groups compared to DAG. Greater post-prandial peak elevations of glucose were seen with high glycemic carbohydrate. Preliminary analysis of the lipase data found higher LPL activity when DAG was fed but similar HL activities among the groups. We conclude that meals containing DAG decreased post-prandial hypertriglyceridemia compared to TAG while maintaining a more consistent NEFA and serum glucose response. DAG may also be involved in modulating LPL activity, and assisting fatty acid uptake and its utilization.



## ABSTRACT #65

MEASUREMENT OF 17-HYDROXYPROGESTERONE (17OHP) IN TUMOR-BEARING DOGS AND DOGS WITH SUSPECTED HYPERADRENOCORTICISM. EN Behrend, AL Boozer, EM Whitley, KA Busch, RJ Kempainen. Auburn University College of Veterinary Medicine, Auburn, AL.

Recently, measurement of 17OHP has been advocated as a means for diagnosis of occult canine hyperadrenocorticism. However, the sensitivity and specificity of this test has not been defined. Our objectives were to assess serum 17OHP concentration pre- and post-injection of ACTH in tumor-bearing dogs and in dogs being screened for hyperadrenocorticism (HAC).

In Phase I, ACTH stimulation tests were performed on 16 normal and 33 tumor-bearing dogs. Plasma endogenous ACTH (eACTH) was measured in the baseline plasma sample of the tumor-bearing dogs. Trasyolol was added to all blood samples used for measurement of eACTH. For the ACTH stimulation test, Cortrosyn was injected (5 mcg/kg IV) and blood samples taken before and 1-hr after injection. For statistical comparison of serum 17OHP concentrations between normal and tumor-bearing dogs, the Mann-Whitney Rank Sum Test was used. The relationships between serum cortisol, 17OHP and plasma eACTH concentrations were assessed using linear regression analysis. Statistical significance was set at  $p < 0.05$ . In Phase II, samples from 127 ACTH stimulation tests submitted to the Auburn Endocrine Diagnostic Service for screening for canine HAC were used.

The reference range for post-ACTH serum 17OHP concentration was  $< 0.1$ -2.8 ng/ml. Serum 17OHP concentration post-ACTH was significantly higher in tumor-bearing dogs than in normal dogs ( $p = 0.0130$ ). Ten tumor-bearing dogs had an elevated post-ACTH serum 17OHP concentration yielding a 70% specificity for diagnosis of HAC. Although the pre-ACTH serum cortisol and 17OHP concentrations were significantly correlated ( $R = 0.67$ ,  $p < 0.001$ ), neither concentration correlated with endogenous ACTH concentration ( $R = 0.044$  and  $0.13$ , respectively).

Of the 127 dogs suspected to have HAC, 68 had a normal post-ACTH serum cortisol concentration. Of these 68 dogs, 10 (15%) had an elevated post-ACTH serum 17OHP concentration. Of the 59 dogs with an elevated post-ACTH serum cortisol concentration, 17 (29%) had a normal post-ACTH serum 17OHP concentration (the rest had values above normal).

Our results suggest: 1. Measurement of post-ACTH serum 17OHP concentration has a relatively low specificity for diagnosis of HAC. 2. In tumor-bearing dogs, secretion of neither 17OHP nor cortisol is related to eACTH concentration. 3. In dogs suspected to have HAC, if post-ACTH serum cortisol concentration is normal, post-ACTH serum 17OHP concentration also is normal in the majority of cases. 4. Approximately 30% of dogs suspected to have HAC with an elevated post-ACTH serum cortisol concentration have a normal post-ACTH serum 17OHP concentration. While it is unclear whether the 10 dogs suspected of HAC with normal post-ACTH serum cortisol but high post-ACTH serum 17OHP would have benefited from mitotane therapy, the data suggest that post-ACTH serum 17OHP values may not necessarily correlate with adrenal hyperfunction.

## ABSTRACT #66

USE OF SERUM PROGESTERONE CONCENTRATIONS TO PREDICT WHELPING DATES. CA Johnson<sup>1</sup>, ML Yoerg<sup>2</sup>, NB Olivier<sup>1</sup>, S Petersen-Jones<sup>1</sup>. <sup>1</sup>College of Veterinary Medicine, Michigan State University, East Lansing, MI. <sup>2</sup>United States Army Medical Department.

Accurate prediction of parturition is important for optimizing neonatal survival, especially in high risk pregnancy and those for which a Cesarean section is planned. As determined by breeding dates, average canine gestation length is 63 days from the first

breeding (range 57-70 days). This 14-day range is too broad for accurate monitoring of high risk pregnancies. Because initial changes in progesterone correlate with the luteinizing hormone (LH) surge, and thus with ovulation, serum concentrations of progesterone are related to gestation length. Parturition normally occurs  $65 \pm 1$  day from the LH surge. Quantitative progesterone assays are readily available and do not require daily sampling as does LH. Previous reports compared gestation lengths with progesterone concentrations of 1.0-10.0 ng/ml (3.18-31.8 nmol/L) at breeding. In our colony, pregnancy rates are highest when breeding occurs at progesterone concentrations of 30-64 nmol/L. Therefore, further study was needed.

Serum samples were obtained between 9-11 AM during 41 estrus cycles. Breeds included Corgi, Briard, Beagle, Schnauzer, Jack Russell terrier and mixes thereof. Progesterone was determined using Coat-a-Count® RIA without ether extraction. Two inseminations were performed when serum progesterone concentrations were between 30-64 nmol/L. Inseminations were 48 hours apart unless the first insemination occurred when progesterone was nearly 64. Then the second was done in 24 hours. Pregnancy rate was 96%. The day of parturition was defined by the first observation of clinical signs of Stage I labor. Length of gestation was recorded in whole days.

The average length of gestation from first breeding to parturition was 60.4 days (range: 56-69 days; SD 2.1), whereas from the second breeding the average was 58.8 days (range: 55-62 days; SD 1.4). A prediction error was calculated as an absolute value of actual gestation length minus predicted gestation length based on the group average. There was no statistically significant difference in the prediction error between the 2 breeding dates. There was a statistically significant relationship ( $p = .01$ ) between progesterone concentration at the time of first breeding and subsequent gestation length. For every 10 nmol/L increase in progesterone, gestation length declined by a half day, such that average gestation length was 61 days when breeding occurred at progesterone concentrations of 20 nmol/L, 60 days when progesterone was 40 nmol/L, and 59.2 days when progesterone was 60 nmol/L. The average error predicting whelp date from breeding date was 1.3 days. The average error predicting whelp date from all progesterone concentrations at the time of breeding was 1.9 days. However, when only those progesterone concentrations above 40 nmol/L were considered, the prediction error was 1.2 days. The prediction errors were not statistically different from each other. Under these breeding conditions, progesterone concentrations at the time of breeding were useful for predicting whelping date.

## ABSTRACT #67

CHRONIC, SUBCLINICAL, EXOCRINE PANCREATIC DISEASE IS COMMON IN DIABETIC DOGS. LM Fleeman<sup>1</sup>, JS Rand<sup>1</sup>, JM Steiner<sup>2</sup>, and DA Williams<sup>2</sup>. <sup>1</sup>Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Australia; <sup>2</sup>GI Laboratory, Texas A&M University, College Station, TX.

The pathogenesis of canine diabetes mellitus is not well characterized. There is evidence for beta cell autoimmunity in a proportion of affected dogs, and also for an association between pancreatitis and diabetes mellitus. The association between diabetes mellitus and pancreatitis in dogs warrants particular attention, because pancreatic inflammation may play a role in initiation of beta cell autoimmunity in susceptible dogs, and lead to destruction of beta cells. Concurrent pancreatitis also has important implications for the clinical and nutritional management of diabetic dogs. The aim of this study was to evaluate the incidence of exocrine pancreatic disease in 12 diabetic dogs over 6 months.

All dogs had spontaneous diabetes mellitus and received subcutaneous porcine lente insulin every 12 hours. Stable glycemic control was established prior to entry into the 6-month trial, and insulin dose was adjusted throughout the study to maintain glycemic

control. Dogs were excluded from entry into the trial if there was a history or clinical signs compatible with pancreatitis. None of the dogs showed clinical signs consistent with either pancreatitis or exocrine pancreatic insufficiency during the trial. At the start of the study and then every 2 months, complete physical examination and ultrasonographic examination of the pancreas was performed on each dog, and serum concentrations of pancreatic lipase immunoreactivity (cPLI), trypsin-like immunoreactivity (cTLI), and C-reactive protein (CRP) were assayed.

Increased serum cPLI concentrations above the cut-off value for pancreatitis (200 $\mu$ g/L) were present in 8 of the 12 dogs on at least one occasion during the 6-month study. No increases in CRP were found, suggesting that these animals had chronic pancreatic disease with negligible acute inflammation. From the beginning of the study, exocrine pancreatic insufficiency (cTLI <2.5 $\mu$ g/L) was present in 2 dogs, and in 1 of these dogs findings consistent with end-stage pancreatitis were present at post-mortem examination 18 months later. Another 2 dogs had serum cTLI concentrations in the questionable range (3.5-5.0 $\mu$ g/L) on a single occasion during the study. Both of these dogs had increased cPLI, however the 2 dogs with exocrine pancreatic insufficiency did not have increased cPLI, possibly because of insufficient pancreatic parenchyma. Only 2 of the 12 dogs had no evidence of either pancreatitis or exocrine pancreatic insufficiency during the 6 months. Ultrasonographic examination revealed no pancreatic structural abnormalities in any dog.

We conclude that: 1) Subclinical exocrine pancreatic disease is common in diabetic dogs. 2) Ten of 12 (83%) diabetic dogs had laboratory evidence of either chronic pancreatitis or exocrine pancreatic insufficiency during a 6-month period. 3) Further investigation of the role of chronic pancreatitis in the pathogenesis of canine diabetes is warranted.

#### **ABSTRACT #68**

**IMPROVED GLYCEMIC CONTROL IN DIABETIC DOGS IS ASSOCIATED WITH IMPROVED SENSITIVITY TO INSULIN.** Linda M Fleeman and Jacquie S Rand. Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Australia.

Chronic hyperglycemia causes insulin resistance in human beings and dogs. This has clinical relevance in the treatment of diabetic patients. Because sensitivity to insulin is improved once chronic hyperglycemia is reduced with insulin therapy, the insulin dose may need to be decreased to avoid life-threatening hypoglycemia. Insulin resistance caused by hyperglycemia occurs in dogs with experimental diabetes mellitus, but has not been studied in dogs with spontaneous diabetes that are receiving insulin therapy. The purpose of this study was to evaluate the relationship between degree of glycemic control and insulin sensitivity in dogs with spontaneous diabetes. It was hypothesized that improved glycemic control in diabetic dogs may be associated with increased sensitivity to insulin. If an association were demonstrated, this may have important clinical implications for management of insulin therapy in diabetic dogs.

Sixteen, insulin-modified, frequently-sampled, intravenous, glucose tolerance tests were performed in 10 dogs with spontaneous diabetes mellitus. Bergman's Minimal Model analysis of serum glucose and insulin concentrations was used to calculate insulin sensitivity ( $S_I$ ). Each diabetic dog had received insulin therapy for a variable duration and no attempt was made to standardize glycemic control before testing. Glycemic control was based on the mean of serial blood glucose measurements obtained every 2 hours over 48-hours immediately prior to the glucose tolerance test. The dogs' usual insulin dosing and feeding regimen was followed except on the morning of the glucose tolerance test, when food and insulin were withheld. The relationship between mean blood glucose concentration and insulin sensitivity was evaluated using Pearson

Product Moment Correlation analysis and significance was set at  $P < 0.05$ .

Glycemic control varied among the dogs, with mean blood glucose concentration ranging from 170 to 386 mg/dL (9.4 to 21.5 mmol/L). Insulin sensitivity ( $\times 10^{-4} \text{ min}^{-1} / (\text{uU/mL})$ ) in all dogs was less than that reported for non-diabetic dogs (mean  $\pm$  SEM, 6.06  $\pm$  0.66), and ranged from 0.27 to 3.24 (mean  $\pm$  SEM, 1.42  $\pm$  0.19). Significant negative correlation was found between insulin sensitivity and mean blood glucose concentrations ( $P = 0.03$ ). This indicates that lower mean blood glucose concentration in diabetic dogs is significantly associated with greater sensitivity to insulin.

We conclude that: 1) Insulin sensitivity in spontaneously diabetic dogs is approximately four times lower than in non-diabetic dogs. 2) Improved glycemic control in diabetic dogs is associated with greater insulin sensitivity. 3) Once therapy has resulted in improved glycemic control, insulin dosage may need to be reduced in diabetic dogs to decrease the risk of insulin-induced hypoglycemia.

#### **ABSTRACT #69**

**DETERMINATION OF ADIPONECTIN, A NOVEL ADIPOCYTE HORMONE, IN HEALTHY AND DIABETIC NORMAL WEIGHT AND OBESE CATS.** C Brömel, RW Nelson, AJ Fascetti, JL Graham, KL Stanhope, PH Kass, RD Berghaus, PJ Havel. University of California, Davis, CA.

Adiponectin is a large (30 kD) protein produced and secreted by adipocytes. It increases tyrosine phosphorylation of the insulin receptor in humans and rodents (insulin sensitizer). Low circulating adiponectin is associated with obesity, decreased insulin sensitivity, insulin resistance and type 2 diabetes mellitus (DM) in humans and monkeys. The aim of this study was to validate an assay for adiponectin in feline serum and compare serum adiponectin in healthy and diabetic normal weight and overweight/obese cats.

Seventy-nine cats were studied and divided in five groups: healthy normal weight (HN,  $n = 15$ ), healthy overweight/obese (HO, 16), diabetic normal weight (DN, 14), diabetic overweight/obese (DO, 19) (all over 2 years old), and healthy normal weight cats younger than 2 years (HN<2, 15). Cats were clinically healthy or had been diagnosed with DM based on clinical signs and persistent hyperglycemia and glycosuria. Diabetics were treated with insulin. Glycemic control was evaluated using clinical signs, serum fructosamine and serial blood glucose (BG). Body condition score (BCS) was determined on a scale of 1-9 (BCS of 4-5=normal,  $\geq 6$ =overweight/obese). Serum adiponectin was measured using an ELISA with recombinant mouse adiponectin standards (B-Bridge, Inc, San Jose, CA). Data were analyzed with ANOVA, Tukey's comparisons, t-test, correlation and regression.

The ELISA was validated for sensitivity, dilutional parallelism, recovery, precision and analyte stability. The assay was sensitive for measuring feline adiponectin to 0.15 ng/ml. Ratios of observed to expected values for dilutional parallelism ranged from 108.3 to 145.5%. Spiking recovery ranged from 115.2 to 125.0%. Coefficients of variation for intra- and interassay precision ranged from 2.3 to 11.9% and 9.1 to 16.8%, respectively. Samples were stable for  $\geq 22$  months.

In normal weight groups, BCS (mean  $\pm$  SD) was 4.7  $\pm$  0.5 (HN) and 4.5  $\pm$  1.1 (DN). In obese groups, BCS was 7.8  $\pm$  1.2 (HO) and 6.8  $\pm$  1.1 (DO). Adiponectin (mean  $\pm$  SD, ng/ml) was lower in all obese (3.1  $\pm$  2.3) than in all normal weight cats (4.9  $\pm$  2.7,  $P < 0.01$ ) and lower in all diabetic (3.1  $\pm$  2.4) than in all healthy cats (4.9  $\pm$  2.7,  $P < 0.01$ ). DO cats also had a lower adiponectin (2.5  $\pm$  2.1) than all healthy and all normal weight cats ( $P < 0.001$ ). Adiponectin (mean  $\pm$  SD, ng/ml) was higher in intact (4.8  $\pm$  2.7) than neutered (3.1  $\pm$  2.4,  $P < 0.01$ ) and in male intact (6.6  $\pm$  3.1) than female intact (4.0  $\pm$  2.0,  $P < 0.001$ ) cats. Adiponectin was negatively correlated with BCS ( $r = -0.37$ ), body weight ( $r = -0.34$ ), age ( $r = -0.36$ ), and mean BG ( $r = -0.48$ ), all  $P < 0.01$ . It was not correlated with glycemic control and

fructosamine. Using multiple linear regression, BCS ( $P < 0.01$ ) and diabetic status ( $P < 0.05$ ), but not age, were significant predictors of adiponectinemia.

The results indicate that obesity and DM are associated with low serum adiponectin concentrations in cats. Low adiponectin concentrations could contribute to insulin resistance in feline obesity and diabetes mellitus.

#### ABSTRACT #70

CHANGES IN CANINE IONISED CALCIUM UNDER THREE STORAGE CONDITIONS. S.F. Brennan\*, J. O'Donovan\*\*, C.T. Mooney\*. \*Department of Small Animal Clinical Studies and \*\*Department of Veterinary Pathology, University College Dublin, Ireland.

Measurement of circulating ionised calcium (iCa) concentration is a more accurate indicator of calcium status than either total calcium or albumin adjusted calcium values. Ideally iCa should be measured immediately after anaerobic blood collection to minimise alterations induced by changes in sample pH. Unfortunately this is not always practical in the clinical setting. In humans when sample analysis is delayed the iCa concentration is adjusted to pH 7.4 using a specific regression equation appropriate to the analyser being used. The purpose of this study was to investigate iCa and pH in canine blood under three storage conditions to determine the change in both over time and whether the iCa measurement adjusted for pH accurately reflects the actual iCa value. The storage conditions were chosen to mimic sample handling in the practice situation

Blood was collected from 18 dogs referred to the University Veterinary Hospital. A commercial pre-heparinised syringe was used as recommended by the manufacturer, and iCa measured within 10 minutes (WB10) on the Bayer Rapidpoint 400 blood gas analyser. A sample of blood was also placed in a commercially available heparinised tube and analysed within 10 minutes (HT). This sample was then set aside for storage at room temperature for 48 hours (WB48) before repeating the ionised calcium measurement. A further aliquot was immediately centrifuged at 4°C and the plasma subsequently divided for storage at room temperature (PR48) and 4°C (PF48) for 48 hours each, after which time iCa was measured again. Statistical analysis was carried out using a Friedman test with post hoc pairwise comparison (Minitab™).

The ionised calcium concentration was significantly decreased ( $P < 0.001$ , respectively) in PR48 (median, 1.20 mmol/l) and in PF48 (1.21 mmol/l) compared with WB10 (median, 1.31 mmol/l) and HT (median, 1.27 mmol/l). Ionised calcium was also significantly lower ( $p < 0.001$ ) in WB48 (median, 1.24 mmol/l) compared with WB10. The median pH decreased from 7.4 in WB10 and HT to 7.10 in WB48 and increased to 7.74 in PR48 and 7.68 in PF48. Regression equations using ionised calcium alone or ionised calcium with pH as predictors were calculated for each group.

Ionised calcium concentrations decreased under all storage conditions irrespective of the direction of pH change. The median decrease in ionised calcium concentration was 7.2 % and adjustment of the concentration for this change resulted in an ionised calcium concentration similar to that obtained using regression equations. The analyser used provided reasonable ionised calcium concentrations even when pH was outside its working range.

#### ABSTRACT #71

EUGLYCEMIC HYPERINSULINEMIC CLAMP AND [<sup>3</sup>H]-GLUCOSE KINETICS IN LEAN AND OBESE CATS. J. Brandao<sup>a</sup>, K. Thomaseth<sup>b</sup>, D.C. Ferguson<sup>a</sup>, M. Hoenig<sup>a</sup>, <sup>a</sup>University of Georgia College of Veterinary Medicine, Athens, GA and <sup>b</sup>Institute of Biomedical Engineering of the Italian National Research Council, Padova, Italy.

The “gold standard” of insulin sensitivity testing is the euglycemic hyperinsulinemic clamp (EHC). The addition of <sup>3</sup>H-glucose to the infusion solutions allows simultaneous determination of glucose metabolism through the glycolytic pathway to <sup>3</sup>H-H<sub>2</sub>O. In these studies, we sought to establish the tracer-supplemented EHC technique in lean and obese cats.

Regardless of the protocol, plasma samples were collected at various time intervals for determination of total radioactivity, radioactive glucose after evaporation of radioactive water, and glucose and insulin concentrations. In 4 cats, a <sup>3</sup>H-glucose bolus (0.5 uCi/kg) was administered in to estimate the rate and extent of glucose distribution. For the EHC, in 9 lean (3.7±0.4 (SD) kg BW and 14.0 ± 4.7% body fat) and 12 obese cats (6.8 ± 0.8 kg BW and 39.4 ± 5.8 % BF), <sup>3</sup>H-glucose was infused at 6 nCi/kg/min for the duration of the 4 hour study. At 120 minutes, an infusion of insulin (8 pmol/min/kg) was begun while glucose was maintained near baseline values. Considerable improvement in modeling fit was observed by nonlinear transformation of the data followed by fitting the entire dataset with a nonlinear mixed-effects model to establish the most parsimonious compartmental model. A 2 compartmental model fit the data in both protocols with the following calculated parameters: fractional clearance rate of glucose (k<sub>01</sub>), the total volume of distribution for glucose (V<sub>tot</sub>), the ratio between the central and peripheral compartments (fKc). In the EHC studies, tracer specific activity rose slightly, and therefore a non-steady state analysis of cold and radioactive glucose was performed. Insulin sensitivity (S<sub>I</sub>, liters/(pmol\*min) x 10<sup>4</sup>) was calculated as the ratio of cold glucose infused (mg/min) divided by the steady-state insulin concentration. k<sub>01</sub> (1/min x 10<sup>3</sup>) was estimated from the pre-insulin clamp tracer data.

In the bolus studies, V<sub>tot</sub> varied with lean, not total body weight. fKc averaged 7.8%/min and fell 14.7% per kg increase in body weight with a 47% reduction in the obese animals. Total radioactivity cleared at 50% of the rate of <sup>3</sup>H-glucose reflecting that essentially all cleared glucose is eventually metabolized to <sup>3</sup>H-H<sub>2</sub>O. The central compartment for the average cat was 31% of the total glucose distribution volume. k<sub>01</sub>, the fractional glucose clearance, fell with body weight. In the clamp studies, S<sub>I</sub> was 0.61±0.10 for lean and 0.27±0.02 for obese cats, and k<sub>01</sub> was 12.81±0.13 for lean and 9.80±0.2 for obese cats (all  $p < 0.0001$ ).

In conclusion, data from bolus tracer glucose studies aided predictions of “true” steady-state values and calculation of S<sub>I</sub> in EHC studies. These results support the dosing of glucose for intravenous glucose tolerance tests based upon lean body weight. Glucose dosages based upon total body weight may result in higher initial glucose concentrations in obese animals, resulting in a greater relative stimulation of beta cell secretion. Insulin sensitivity (S<sub>I</sub>) was 56% lower and fractional glucose clearance (k<sub>01</sub>) was 24% lower in obese cats. While technically more complicated than static glucose/insulin ratios or glucose tolerance tests, very small differences in insulin sensitivity are detectable with the clamp technique.

#### ABSTRACT #72

PLASMA ENDOTHELIN-1 CONCENTRATIONS IN HEALTHY DOGS AND DOGS WITH ACQUIRED HEART DISEASE. Robert Prošek<sup>1</sup>, David Sisson<sup>1</sup>, Mark Oyama<sup>1</sup>, Alexander Biondo<sup>2</sup>, and Philip Solter<sup>1</sup>. <sup>1</sup>College of Veterinary Medicine, University of Illinois, Urbana, IL and <sup>2</sup>Universidade Federal do Parana, Curitiba - Parana, Brazil.

Plasma levels of endothelin-1 (ET-1), the most potent pressor substance discovered to date, are elevated in humans with congestive heart failure (CHF). We sought to measure plasma ET-1 concentrations in healthy dogs, and in dogs with acquired heart disease with or without radiographic evidence of pulmonary edema.

A commercially available sandwich ELISA kit, designed to measure endothelin-1 (ET-1) levels in human subjects, was first validated for use in dogs and then used to measure the plasma ET-1 immunoreactivity in plasma samples obtained from 32 healthy dogs and 46 dogs with either dilated cardiomyopathy (DCM, n = 27) or degenerative valvular disease (CDVD, n = 19), with (n = 30) and without (n = 16) congestive heart failure (CHF). Dogs were categorized based on clinical history, physical examination findings, thoracic radiographs and echocardiography. Statistical analysis was performed using one-way ANOVA with Bonferroni's post test on both raw and log-transformed data.

Plasma ET-1 concentrations (average  $\pm$  SD) were  $1.231 \pm 0.363$  fmol/ml in the 32 healthy control dogs,  $1.423 \pm 0.735$  fmol/ml in 16 dogs with DCM (n = 9) or CDVD (n = 7) without CHF, and  $2.792 \pm 1.344$  fmol/ml in 30 dogs with DCM (n=18) and CDVD (n=12) with CHF. Plasma immunoreactivity of ET-1 was significantly higher in dogs with CHF in comparison to healthy dogs ( $P < 0.001$ ) and dogs with heart disease without CHF ( $P < 0.001$ ). No significant difference was found between healthy dogs and dogs with heart disease without CHF ( $P > 0.05$ ). There was no significant difference in ET-1 levels when comparing dogs with DCMs and CDVD in either the CHF or non-CHF groups. There was a significant correlation between plasma ET-1 levels and the following variables: left atrial/aortic ratio ( $P < 0.0001$ ,  $r = 0.6016$ ), LVIDd indexed to aortic diameter ( $P < 0.0001$ ,  $r = 0.4952$ ), LVIDs indexed to aortic diameter ( $P = 0.0058$ ,  $r = 0.3220$ ), LVIDd indexed to body surface area (BSA) ( $P = 0.0096$ ,  $r = 0.3034$ ) and LVIDs indexed with BSA ( $P = 0.0015$ ,  $r = 0.3667$ ).

ET-1 levels are significantly elevated in dogs with CHF due to DCM or CDVD compared to healthy dogs and dogs with DCM or CDVD without CHF. Furthermore, ET-1 correlated with some clinical variables of disease progression. Measurement of plasma endothelin may have diagnostic, therapeutic, and prognostic utility in dogs with acquired heart disease.

#### **ABSTRACT #73**

**PROSPECTIVE EVALUATION OF PLASMA CARDIAC TROPONIN I CONCENTRATION IN CATS WITH MILD HYPERTROPHIC CARDIOMYOPATHY.** William E Herndon<sup>o</sup>, Carl D Sammarco<sup>\*\*</sup>, Donald Schrope<sup>+</sup>, SouthPaws Veterinary Referral Center, Springfield, VA<sup>o</sup>; Redbank Animal Hospital, Redbank, NJ<sup>\*\*</sup>. Oradell Animal Hospital, Oradell, NJ<sup>+</sup>.

Measurement of plasma cardiac troponin I concentration ([cTnI]) is a sensitive and specific circulating biochemical marker of myocardial cell damage. Previous studies have shown that cats with moderate to severe hypertrophic cardiomyopathy (HCM) have significantly increased plasma [cTnI] compared to normal cats. The purpose of this study was to determine if [cTnI] is increased in cats with mild HCM compared to normal cats.

Plasma [cTnI] was quantified using a two-site sandwich assay with analytical sensitivity of 0.03ng/ml. Plasma [cTnI] greater than or equal to 0.16 ng/ml was considered abnormal. We attempted to prospectively enroll cats with a familial history of HCM. An echocardiogram and [cTnI] (observation) was obtained every six months until the study concluded, or the patient was lost to follow up. The study included 23 cats and 49 total observations (number of observations/number of cats = 4/3, 3/6, 2/5, 1/9). The median age upon enrollment was 5.2 years (range 1-7).

Throughout the study, 6 cats (totalling 13 observations) developed echocardiographic evidence of mild HCM. Following diagnosis of HCM, only one cat had subsequent observations. This cat six months after initial echocardiographic diagnosis had increased [cTnI] at 0.51ng/ml. Seventeen cats (totalling 35 observations) remained echocardiographically normal throughout the study. One of these cats initially had increased [cTnI] at 0.2ng/ml, which normalized on the subsequent observation. Although analysis of [cTnI] is an easily

obtainable and noninvasive diagnostic test, it appears to remain normal in many cats with mild HCM.

#### **ABSTRACT #74**

**PLASMA CARDIAC TROPONIN I CONCENTRATION IN CATS WITH CARDIAC AND NONCARDIAC CAUSES OF RESPIRATORY DISTRESS.** William E Herndon<sup>o</sup>, Donald Schrope<sup>+</sup>, Kenneth J Drobatz<sup>\*</sup>, Carl D Sammarco<sup>\*\*</sup>, Kirstin N Boddy<sup>\*</sup>, Meg M Sleeper<sup>\*</sup>. SouthPaws Veterinary Referral Center, Springfield, VA<sup>o</sup>; Oradell Animal Hospital, Oradell, NJ<sup>+</sup>; Redbank Animal Hospital, Redbank, NJ<sup>\*\*</sup>; Ryan Veterinary Hospital of the University of Pennsylvania<sup>\*</sup>, Philadelphia, PA.

Measurement of plasma cardiac troponin I concentration ([cTnI]) is a sensitive and specific circulating biochemical marker of myocardial cell damage. The molecular structure of cTnI is highly conserved across species, and current assays developed for its detection in humans have been validated in many species, including cats. The purpose of this study was to determine if [cTnI] could discriminate cardiac (n=29) from noncardiac (n=12) causes of respiratory distress in the cat. [cTnI] was significantly higher in cats with a cardiac cause of respiratory distress (i.e. congestive heart failure) (median, 1.49 ng/mL; range, 0.20 – 30.24 ng/mL) as compared with noncardiac causes of respiratory distress (median, 0.28 ng/mL; range, <0.03-1.42 ng/mL) ( $P < 0.0002$ , Wilcoxon Ranksum test). A receiver operator characteristic curve was analyzed to determine the accuracy of [cTnI] in diagnosing cardiac disease as a cause of respiratory distress. The area under the curve was 0.87. A [cTnI] greater than or equal to 0.2 ng/ml had 100% sensitivity, 58% specificity, and 88% diagnostic accuracy. Plasma [cTnI] greater than or equal to 1.49 ng/ml had 52% sensitivity, 100% specificity, and 66% diagnostic accuracy. Based on this preliminary cTnI study, most all cats with cardiac disease sufficient to cause congestive heart failure have evidence of cardiomyocyte damage. Plasma [cTnI] appears to be clinically helpful in differentiating cardiac from noncardiac causes of respiratory distress in cats.

#### **ABSTRACT #75**

**USE OF PLASMA ANP, BNP, ENDOTHELIN-1 AND TROPONIN-I LEVELS IN DISTINGUISHING BETWEEN CARDIAC AND NON-CARDIAC CAUSES OF ACUTE DYSPNEA IN DOGS.** Robert Prošek, David Sisson, Mark Oyama, Philip Solter, and Robyn Ostapkowicz. College of Veterinary Medicine, University of Illinois, Urbana, IL.

The aim of this study was to determine whether circulating levels of NT-proANP, BNP, ET-1 and cTn-I can be used to help distinguish between cardiac and non-cardiac causes of dyspnea in dogs. Forty-eight dogs were included in the study. Inclusion criteria were coughing, tachypnea, or increased respiratory effort severe enough to limit exercise capacity and adversely impact quality of life. Dogs with obvious trauma or metabolic disease, such as renal failure, were excluded from study. NT-proANP and BNP were measured by RIA, and ET-1 and cTn-I were measured by a sandwich ELISA. Final diagnosis was based on information derived from a minimum of physical exam, thoracic radiographs and echocardiography. Congestive heart failure (CHF) was diagnosed in 21 dogs (CHF Group = 10 dilated cardiomyopathies, 10 acquired degenerative valvular diseases, patent ductus arteriosus, subaortic stenosis) and dyspnea of non-cardiac origin was diagnosed in 27 dogs (noHD Group = 12 with pneumonia, 8 with pulmonary neoplasia, 3 with pleural neoplasia/effusion, 2 with laryngeal paralysis, 2 with chronic bronchitis).

Plasma NT-proANP concentrations (mean  $\pm$  SD, median, range) were  $0.3381 \pm 0.2137$  nmol/ml, 0.2870 nmol/ml, 0.0080-0.9320 nmol/ml for noHD dogs; and  $1.723 \pm 0.9426$  nmol/ml, 1.430 nmol/ml, 0.5990-4.409 nmol/ml for dogs with CHF. Plasma BNP

concentrations (mean  $\pm$  SD, median, range) were 12.77  $\pm$  6.473 pg/ml, 11.63 pg/ml, 3.520–28.09 pg/ml for noHD dogs; and 53.50  $\pm$  64.46 pg/ml, 30.98 pg/ml, 4.449–245.9 pg/ml for dogs with CHF. Plasma ET-1 concentrations (mean  $\pm$  SD, median, range) were 0.4254  $\pm$  0.4141 fmol/ml, 0.3510 fmol/ml, 0.0270–2.057 fmol/ml for noHD dogs; and 1.844  $\pm$  1.557 fmol/ml, 1.691 fmol/ml, 0.1770–7.073 fmol/ml for dogs with CHF. Plasma cTn-I concentrations (mean  $\pm$  SD, median, range) were 2.344  $\pm$  8.028 ng/ml, 0.160 ng/ml, 0.020–38.090 ng/ml for noHD dogs; and 1.454  $\pm$  2.526 ng/ml, 0.455 ng/ml, 0.020–10.85 ng/ml for dogs with CHF. Data was analyzed using an unpaired t-test on raw or log-transformed data, depending on distribution of data points. Differences were highly significant between groups for NT-proANP (P<0.0001), BNP (P<0.0001), and ET-1 (P<0.0001) while cTn-I did not show a significant difference between groups (P= 0.3474). A cut-off value of 0.55 nmol/ml for NT-proANP, 19.24 pg/ml for BNP, and 0.84 fmol/ml for ET-1 was associated with a sensitivity of 100% and a specificity of 82.6% for NT-proANP, a sensitivity of 85.0% and a specificity of 84.6% for BNP, and a sensitivity of 76.2% and a specificity of 92.0% for ET-1 in predicting CHF as the cause of dyspnea. These preliminary results suggest that plasma NT-proANP, BNP and ET-1, but not cTn-I, are useful for distinguishing between dogs with CHF and non-cardiac causes of dyspnea.

#### ABSTRACT #76

CARDIAC MAGNETIC RESONANCE IMAGING MORE ACCURATELY QUANTIFIES LEFT VENTRICULAR MASS THAN ECHOCARDIOGRAPHY IN DOMESTIC CATS. KA MacDonald<sup>1</sup>, MD Kittleson<sup>1</sup>, RF Larson<sup>2</sup>, ER Wisner<sup>2</sup>. <sup>1</sup>Dept. of Medicine and Epidemiology;

<sup>2</sup> Dept. of Surgical and Radiological Sciences; University of California, Davis; Davis, CA.

Noninvasive quantification of left ventricular (LV) mass is one variable that can be used to assess severity of LV diseases and response to interventions aimed at reducing LV hypertrophy. Echocardiography (ECHO) has been traditionally used to quantify LV mass, but requires precise alignment, and clear delineation of endocardial and epicardial borders. Also, one must assume that the LV is a uniform, ellipsoid shape unless Simpson's rule is utilized.

The hypotheses of this study were that cardiac MRI would accurately determine LV mass in normal cats and would be more accurate than echocardiography. Seven domestic cats weighing 4.4 to 5.8 kg were sedated with acepromazine and hydromorphone, and ECHO was performed. LV mass was calculated using the truncated ellipse formula from a right parasternal long-axis view (RPV) at end-diastole rather than using a left apical 4-chamber view or Simpson's rule because of better visualization of the LV apex and inconsistent visualization of the right endocardial border of the septum from the RPV. MRI was performed (GE Signa 1.5 Tesla) on cats anesthetized with propofol. Heart rate ranged from 83 to 108 beats/minute (mean = 98 bpm; SD = 10.8 bpm) T1 weighted MR images were acquired during multiple phases of the cardiac cycle. The short-axis images were obtained by acquiring 3 mm thick contiguous slices perpendicular to the long-axis of the heart. Images were acquired using the gradient echo sequence with and without hyperventilation-induced apnea and cine pulse sequence without apnea. LV mass was determined using Simpson's rule. Endocardial and epicardial borders were traced on each slice at end-systole, end-diastole, and mid-cycle. LV myocardial volumes were summated, and multiplied by myocardial density (1.05) to obtain LV mass at each measured phase of the cardiac cycle. Cats were euthanized and the LV was dissected and weighed to determine the true mass.

The best MRI edge detection was obtained with the gradient echo sequence during apnea. The mean number of slices was 10 (range = 9 to 11 slices). MRI at end-systole was the most accurate method of determining LV mass based on use of Lin's concordance coefficient

(MRI end-systole  $\rho_c$  = 0.94; MRI mid-cycle  $\rho_c$  = 0.76, MRI end-diastole  $\rho_c$  = 0.81; ECHO end-diastole  $\rho_c$  = 0.6). Actual LV mass ranged from 6.5 to 10.5 g (mean 8.5 g; SD 1.6 g) compared to MRI LV mass at end-systole, which ranged from 6.7 to 11.1 g (mean 8.7 g; SD 1.7 g) and echocardiographic LV mass at end-diastole, which ranged from 5.2 to 9.1 g (mean 7.1 g; SD 1.8 g).

Cardiac MRI obtained at end-systole is a very accurate method to noninvasively quantify LV mass in domestic cats and is more accurate than the echocardiographic method used in this study, which consistently underestimated LV mass. Cardiac MRI may be useful in the future to monitor progression of disease and serially assess LV mass in response to therapy.

#### ABSTRACT #77

PREVALENCE AND EFFECT ON LIFE EXPECTANCY OF DCM IN A POPULATION OF 1,019 IRISH WOLFHOUNDS EVALUATED BETWEEN 1990 – 2003. A. Vollmar<sup>1</sup>, P.R. Fox<sup>2</sup>, and B.W. Keene<sup>3</sup>. <sup>1</sup>Wissen, Germany; <sup>2</sup>Animal Medical Center NY, NY; <sup>3</sup>North Carolina State University CVM, Raleigh, NC.

Background: Dilated cardiomyopathy (DCM) constitutes the predominant form of heart disease in Irish Wolfhound dogs (IW). While atrial fibrillation and heart failure are well recognized sequelae of DCM, the life expectancy of IW and overall effect of DCM on that expectation has not been well characterized.

Methods: From May 1990 to December 2003, IW in the Netherlands, Belgium and Germany were examined by one individual (AV) as part of a prospective, longitudinal study to evaluate the life expectancy, prevalence of DCM and effect of DCM and other diseases on survival. Screening criteria for DCM included the following echocardiographic parameters: LVIDs > 41mm, LVIDd > 60mm, and FS < 25%; additional changes were variably present and could include EPSS > 10mm, LAd > 56 mm, RVIDd 35 mm, and the presence of atrial fibrillation or other arrhythmias. Following initial examination, dogs were echoed annually or when possible, and their owners were instructed to report the date and circumstances of death when it occurred. Because DCM can develop at any time during a dog's life, we restricted this interim survival analysis to dogs that have died, and whose definitive DCM status was thus known. Kaplan-Meier survival curves were constructed from the date of birth until the date of death for dogs with and without DCM, and compared by the Log Rank test.

Results: 1,019 Irish Wolfhound dogs (442 males and 577 females) with known dates of birth were enrolled in the study during this 12.5 year period. The median age of dogs at initial screening was 2.4 years. The age of dogs at the time they were diagnosed with DCM was 4.4  $\pm$  2.0 years (mean  $\pm$  SD; range 0.68–9.54 yrs). DCM was diagnosed in 301 IW (170 males, 131 females) which represented 29.5% of the 1,019 enrolled IW dogs. The mean age of unaffected dogs at the time of the latest examination was 3.36  $\pm$  1.71 yrs (range 0.84 to 9.86 yrs). Of the 1,019 dogs entered into the study, 402 had died at the time of this writing; 201 of the 402 had DCM- of these 201, 145 died of causes attributable to DCM, and 56 died of non cardiac conditions. The remaining 201 IW dogs had no evidence of DCM and died of non cardiac diseases. Survival curves for those with and those without DCM were significantly different (P = 0.036). The median survival of dogs that developed DCM was 2,208 days, compared to 2,351 days for those that had no evidence of DCM.

Conclusions: The prevalence of DCM detected in a large population of Irish Wolfhound dogs was 29% (male:female ratio = 1.3:1). While DCM has a clear and statistically significant adverse effect on survival, the magnitude of this effect may not be as great in IW dogs as it is generally considered to be in other breeds. Median duration of life for IW dogs that have died to date in this longitudinal study was about 6 years.

## ABSTRACT #78

COIL EMBOLIZATION OF PATENT DUCTUS ARTERIOSUS VIA THE CAROTID ARTERY IN FIVE DOGS. S.J. Miller, DVM, Veterinary Medical Teaching Hospital; WP Thomas, DVM, Department of Medicine and Epidemiology, University of California, Davis.

The traditional approach to the correction of congenital patent ducts arteriosus (PDA) in dogs has been ligation via left thoracotomy. During the past 7 years, an increasing percentage of cases have been corrected using catheter delivered occlusion devices, primarily Gianturco coils delivered via the femoral artery. The femoral approach provides the most direct access to the PDA with the least amount of catheter maneuvering. Because of the caliber of currently available catheter and coil systems and the small size of many affected dogs, delivery via the femoral artery may be technically difficult. The purpose of this study was to evaluate the feasibility and limitations of transcatheter coil occlusion of PDA using a carotid artery approach in dogs.

Five dogs (4 related dachshunds, 1 Newfoundland retriever) presented to the University of California, Davis Veterinary Medical Teaching Hospital (VMTH) in the fall of 2002 for evaluation of congenital heart disease had congenital PDA diagnosed by characteristic physical, electrocardiographic, radiographic, and anatomic and Doppler echocardiographic findings. Dogs were anesthetized for transesophageal echocardiography, followed by transcatheter coil embolization of the PDA via surgical access of the right external carotid artery. A 5Fr-7Fr catheter was easily inserted into the artery. Because of the angles between the brachiocephalic trunk, aorta and ductus, catheters were pre-formed with an S-shaped curve to allow the catheter tip to enter the aortic side of the ductus. Using detachable coils, 1-3 coils were placed in the PDA of each dog. Placement of the first coil was relatively easy. Placement of additional coils was more difficult, as the curved course of the catheter, ductus angle and reduction in ductal flow made controlled manipulation of coil position more difficult compared to the femoral approach.

Coil embolization was achieved in all five dogs. A single coil was delivered in 4 dogs and 2 coils in 1 dog. There were no major complications. There were two minor complications (two additional coils were delivered using a femoral arterial approach in dog 2 and one small coil embolized into a left femoral artery branch in dog 5). All dogs recovered uneventfully. One year later all dogs were reported to be normal. No murmur was heard in 3 dogs, and a very soft, localized grade 1/6 continuous murmur was heard in 2 dogs. Re-evaluation by echocardiography at one year showed complete PDA closure in one dog and trivial to mild residual ductal flow in the other 4 dogs. Although technically more demanding than the femoral artery approach, the carotid artery approach may be a viable alternative, especially in very small dogs.

## ABSTRACT #79

PLASMA CARVEDILOL LEVELS IN DOGS WITH SPONTANEOUS CARDIOVASCULAR DISEASE RECEIVING CHRONIC ORAL CARVEDILOL: A PILOT STUDY. S.G. Gordon\*, D.M. Boothe\*, L. Braz-Ruivo\*\*, I. Petrikovics\* M.W. Miller\*, and K. Glaze\*. \* Department of Small Animal Medicine and Surgery and Michael E. DeBakey Institute, College of Veterinary Medicine, Texas A&M University, College Station, Texas, \*\*Dog & Cat Veterinary Referral, Glenn Dale, Maryland.

The purpose of this study was to determine the correlation between plasma carvedilol (Coreg®), a 3<sup>rd</sup> generation non-selective beta-blocker with ancillary alpha<sub>1</sub>-blocking and antioxidant properties, and chronic oral carvedilol in canine patients with acquired spontaneous cardiovascular disease. The pharmacokinetics (PK) and pharmacodynamics (PD) of carvedilol in normal conscious dogs has been previously reported. Carvedilol has a low and variable oral

bioavailability. Based on an isoproterenol challenge study in normal dogs receiving chronic oral carvedilol (1.5 mg/kg twice per day), plasma carvedilol levels were found to correlate well with the magnitude of beta-blockade. Additionally, maximum plasma carvedilol concentrations are reportedly higher in human patients with spontaneous cardiac disease relative to control subjects. In light of the aforementioned data and the potential for adverse effects with rapid over zealous beta blockade in canine patients with spontaneous cardiac disease, evaluation of PK data in this population of dogs is warranted prior to initiation of clinical trials to assess its efficacy.

Six canine patients with spontaneous cardiovascular disease were evaluated. The dogs (4.4-27.3kg BW) reported herein had various forms and degrees of severity of acquired spontaneous cardiovascular disease, were receiving other clinically appropriate medications, and had been on a stable oral dose of carvedilol for at least 30 days (0.25-0.9 mg/kg orally every 12 hours). Four samples were collected at 1-hour intervals starting 1 hour after oral administration. Carvedilol concentrations were measured in plasma using HPLC analysis. Lower limit of quantification was 1 ng/ml.

Peak plasma carvedilol level was most commonly observed at the 4-hour time point (50% of dogs) ranging from 0-100 ng/ml. Peak carvedilol plasma levels correlated with the oral dose in mg/kg when a simple logarithmic regression analysis was performed. The equation for the regression analysis is as follows:  $y = 0.4638 + 0.774 * \ln(x)$ , where  $y$  = oral carvedilol dose in mg/kg and  $x$  = plasma carvedilol concentration in ng/ml. The adjusted R<sup>2</sup> was 0.74 with a p-value of 0.02. The 2 dogs receiving the lowest dose of carvedilol (0.25 and 0.3 mg/kg) had no measurable plasma carvedilol at any of the 4 time points.

Based on prior reported work in normal conscious dogs, a reasonable target plasma carvedilol concentration is 50-100 ng/ml with clinically significant beta blockade occurring at plasma levels > 10 ng/ml. In this small series, dogs dosed above 0.5 mg/kg twice per day achieved plasma levels that should result in clinically significant beta blockade while those dosed at 0.3 mg/kg or below failed to achieve this level. However, to achieve close to maximum beta blockade doses > 0.7-0.9 mg/kg may be required. Given this data and the reported variations in oral bioavailability, up-titration protocols and clinical trials may benefit from plasma monitoring.

## ABSTRACT #80

DOPPLER ECHOCARDIOGRAPHIC PREDICTION OF PULMONARY HYPERTENSION IN DOGS WITH INTERSTITIAL LUNG DISEASE USING SYSTOLIC TIME INTERVALS OF PULMONARY ARTERY FLOW. <sup>1</sup>Schober KE, Baade H. University of Leipzig, Germany and <sup>1</sup>The Ohio State University, Columbus, OH.

Systolic, diastolic, and mean pulmonary artery pressures (PAP) may be determined non-invasively by use of Doppler peak tricuspid (TR) or pulmonic (PR) regurgitation velocities in the absence of pulmonic stenosis or dynamic right ventricular (RV) outflow tract obstruction. However, assessment of PAP may be difficult in patients with a lack of detectable TR or PR. Additionally, poor Doppler beam alignment may be observed in dogs with eccentric regurgitant jets and result in an underestimation of PAP. The evaluation of PAP may be important in a wide variety of clinical settings with regard to therapeutic decisions and prognostic implications in patients with cardiovascular and systemic disease. The aim of the study was to assess the ability of Doppler derived systolic time intervals of pulmonary artery flow to diagnose pulmonary hypertension (PH) in dogs.

Normal West Highland white terriers (WHWT; n=40), normal boxer dogs (n=32), and WHWT with interstitial pulmonary fibrosis (IPF) and without (n=24) or with (n=18) evidence of PH based on Doppler peak TR and PR velocities were evaluated. Among others, heart rate (HR), RV segmental shortening fraction (RV SF),

pulmonary artery flow variables (peak velocity [ $V_{\max}$  PA], acceleration time [AT], ejection time [ET], and AT to ET ratio [AT:ET ratio]), and peak velocities of TR and PR were obtained by transthoracic 2D and Doppler echocardiography. Reference values of AT:ET were established from the normal WHWT and boxers and compared to dogs with PH using a Mann Whitney rank sum test. Linear regression analysis to detect a relationship between the AT:ET ratio and the estimated PAP was also performed.

The AT:ET ratio in normal WHWT and boxers (median, 5 to 95 percentiles) was 0.40 (0.28 to 0.55) and 0.41 (0.33 to 0.50), respectively, in dogs with IPF and no PH based on TR and PR 0.36 (0.21 to 0.43), and in WHWT with IPF and PH 0.30 (0.20-0.42). In the latter, peak velocities of TR were 3.90 m/s (3.23 to 5.44). There was a linear negative correlation between the AT:ET ratio and systolic PAP in dogs with PH ( $r=-0.62$ ,  $P=0.016$ ). The sensitivity, specificity, and positive and negative predictive values of an AT:ET ratio  $< 0.32$  to predict PH (systolic PAP  $> 45$  mmHg) were 83%, 90%, 63%, and 97%, respectively. There were no differences between groups of WHWT with regard to HR, RV SF, and  $V_{\max}$  PA.

Pulmonary hypertension is a common finding in dogs with IPF. The Doppler derived AT:ET ratio may be used to help diagnose PH in those dogs, in particular in the absence of TR or PR. Dogs with an AT:ET ratio  $\geq 0.32$  have a high likelihood of normal systolic PAP.

#### ABSTRACT #81

COMPARISON OF ELECTROCARDIOGRAPHY, THORACIC RADIOGRAPHY, AND ECHOCARDIOGRAPHY TO DIAGNOSE LEFT ATRIAL ENLARGEMENT IN CATS. <sup>1</sup>Schober KE, Maerz I, Ludewig E. University of Leipzig, Germany and <sup>1</sup>The Ohio State University, Columbus, OH.

Left atrial (LA) enlargement is an important finding in cats with cardiac disease that has diagnostic, therapeutic, and prognostic implications. The gold standard to diagnose LA size non invasively is two dimensional (2D) echocardiography in small animals. However, availability and expense may be limiting factors for this technique in routine practice. The objective of this study was to evaluate the ability of electrocardiographic, radiographic, and echocardiographic variables to assess left atrial size in cats.

Normal cats ( $n=19$ ) and 25 cats with different forms of cardiomyopathy were evaluated without sedation. Standard six lead electrocardiograms (ECG) were recorded in right lateral recumbency. The P-wave duration and amplitude were measured. Thoracic radiographs were taken in dorsal and lateral recumbency. Left atrial size was assessed subjectively by three independent observers and quantified by using a novel index of LA size ("LA vertebral heart score" [LA-VHS]). A 2D, M-mode, and Doppler echocardiographic examination was performed. The LA dimensions were assessed by measurement of maximum LA diameter (LAD) and LA area (LAA) from the right parasternal 4-chamber view and used as gold standard of LA size. Values obtained in normal cats were used as reference values. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) for ECG and radiographic methods were calculated and compared with each other. Linear regression analysis to detect relationships between different indices of LA size was performed.

A P-wave duration of  $> 0.035$  sec, a P-wave amplitude of  $> 0.20$  mV, a LA-VHS of  $> 1.30$ , a LAD of  $> 1.57$  cm, and a LAA of  $> 2.80$  cm<sup>2</sup> were used as cut-offs to diagnose LA enlargement. 25 cats had LA enlargement and 19 cats had normal LA size. Test characteristics to diagnose LA enlargement are listed below:

	Se (%)	Sp (%)	PPV (%)	NPV (%)
EKG	28	90	78	49
LA-VHS (radiography)	36	95	90	53
Subjective assessment (radiography)	76	74	80	70

Interobserver variability (IOV) for LA-VHS was 7.2 %. IOV for the subjective assessment of LA size based on radiography was 3.5% for

Se, 25.9% for Sp, 12.4% for PPV, and 10.1% for NPV. There was a positive correlation between LAD and LA-VHS ( $r=0.61$ ,  $P < 0.001$ ).

Variables of LA size based on electrocardiographic and radiographic measures yield a high specificity and PPV, however, a very low sensitivity and NPV for the detection of LA enlargement. Subjective assessment of thoracic radiographs and 2D echocardiography should be used to diagnose and quantify LA enlargement in cats.

#### ABSTRACT # 82

PATENT DUCTUS ARTERIOSUS OCCLUSION WITH A SELF-EXPANDING DEVICE AND PER-CATHETER DEPLOYMENT PROCEDURE DEVELOPED SPECIFICALLY FOR USE IN DOGS. Anthony H. Tobias, and Dewey H. Carpenter. University of Minnesota, St Paul, MN.

The purpose of the study was to develop a device and per-catheter deployment procedure specifically for patent ductus arteriosus (PDA) occlusion in dogs. The device is composed of 2 to 3 layers of nitinol (nickel and titanium alloy) mesh, and it is highly compressible and self-expanding. The device takes the form of 2 disks connected by a short (2 mm) waist, and devices with a range of waist diameters have been manufactured. Waist diameter is selected to be  $1\frac{1}{2}$  to 2 times the diameter of the PDA ostium at its junction with the pulmonary artery (PA), as measured by echocardiography and angiography.

The device is attached to a delivery cable and deployed via a 6 French guiding catheter. The guiding catheter is passed through the PDA and into the main PA from the arterial side. The distal disk is then deployed by advancing the device through the guiding catheter with the delivery cable. Next, the guiding catheter, device, and delivery cable are withdrawn simultaneously until the deployed disk engages the PDA ostium. Correct positioning of the device at the PDA ostium is confirmed by noting, first, a tugging sensation and straightening of the guiding catheter and delivery cable, and second, disappearance of the continuous murmur during transthoracic or transesophageal auscultation. The proximal disk is then deployed within the ampulla of the PDA by stabilizing the device with the delivery cable, and partially withdrawing the guiding catheter. This step fully deploys the device across the PDA ostium. Stability of the device is checked by gently pushing and pulling the fully deployed device with the delivery cable. Once the device is assessed to be correctly positioned and stable, the delivery cable is detached and removed together with the guiding catheter. An aortic angiogram is then performed to confirm PDA occlusion.

The procedure has been performed in 6 dogs ranging in weight from 4.2 to 30.0 kg, with PDA ostium diameters of 2.5 to 5.0 mm. Aortic angiograms performed at the end of each procedure confirmed complete PDA occlusion in all cases. On the day following each procedure, physical examination, thoracic radiography, and echocardiography disclosed that the device was correctly located with no residual ductal flow in 5 dogs. The device had migrated in 1 dog with the largest PDA ostium, prompting modification of the device (increasing its stiffness) for cases with large PDA ostia.

Whereas further research and development are necessary and ongoing, we conclude that this device and per-catheter deployment procedure have considerable potential for PDA occlusion in dogs.

#### ABSTRACT # 83

COMPARISON OF ECHOCARDIOGRAPHIC AND ANGIOGRAPHIC ESTIMATES OF MINIMAL DUCTAL DIAMETER IN DOGS WITH PATENT DUCTUS ARTERIOSUS. A.B. Saunders,<sup>1</sup> M.W. Miller,<sup>1</sup> S.G. Gordon,<sup>1</sup> A. Bahr,<sup>2</sup><sup>1</sup>Department of Small Animal Medicine and Surgery and Michael E. DeBaKey Institute, and <sup>2</sup>Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA.

Transcatheter coil embolization is a minimally invasive treatment option for patent ductus arteriosus (PDA) in dogs. Complications including residual ductal flow and embolization of coils to the pulmonary or systemic vasculature may be minimized with appropriate estimates of minimum ductal diameter. Measurements of minimal ductal dimensions in dogs are traditionally based on a right lateral, monoplane angiogram.

15 dogs referred for definitive treatment of PDA were evaluated; coil embolization was accomplished in 14. There were 13 purebred dogs and 2 mixed breed dogs; 13 females and 2 males. Age ranged from 2.8 to 71.2 months (median 5.3). Weight ranged from 2.3 to 24 kg (median 5.4). Minimum ductal diameter was measured with two dimensional (2D) and color Doppler (CD) modalities via transthoracic echocardiography (TTE) from the right (R) and left (L) parasternal views and transesophageal echocardiography (TEE). Measurements were compared to angiographically derived minimal diameter. TTE was performed with intravenous buprenorphine (0.007 mg/kg). Angiography and TEE were performed under general anesthesia. CD TTE and first pass nuclear angiography (FPNA) were utilized to assess initial and residual ductal flow postoperatively. 14/15 dogs were determined to be completely occluded at 1 month, documented by a FPNA ratio of pulmonary to systemic blood flow (QP:QS) <1.2 and no CD TTE flow.

Minimal ductal diameter determined by monoplane angiography was compared to minimum ductal diameter obtained with TTE from both the right and left hemithorax and TEE using both 2D and CD as well as FPNA and results are reported in table 1. Measurements are reported as the mean +/- 2 standard error.

Angiogram	2.32+/-0.31mm	R <sup>2</sup> value	P-value
TTE-R	3.78+/-0.25mm	0.09	0.3
TTE-R CD	3.76+/-0.24mm	0.24	0.07
TTE-L	3.50+/-0.21mm	0.01	0.68
TTE-L CD	3.96+/-0.49mm	0.29	0.05
TEE	1.97+/-0.24mm	0.68	0.0003
TEE CD	2.36+/-0.28mm	0.42	0.01
FPNA initial	2.16+/-0.16	0.02	0.65

Minimal ductal diameter as measured by 2D and CD TEE correlated best with angiographically determined minimal diameter. Additionally, TEE provided anatomic information regarding PDA morphology, coil deployment and confirmation of intraoperative ductal closure. In one dog prior to angiography, TEE accurately identified a Type III ductal morphology that was not amenable to coil embolization thus ductal ligation was performed.

#### ABSTRACT #84

PRESENCE OF INTRACELLULAR MYOCARDIAL UBIQUITIN COMPLEXES IN CANINE DILATED CARDIOMYOPATHY.

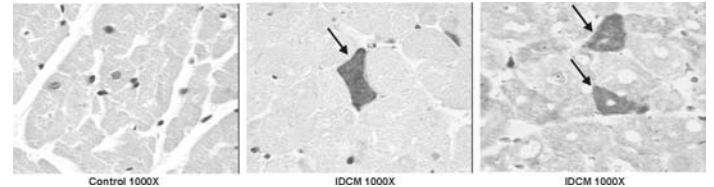
Mark A. Oyama, Phil F. Solter, University of Illinois, Urbana, IL.

Ubiquitin is a highly conserved 72-aa chain involved in intracellular protein degradation by the 26S proteasome system. Ubiquitin recognizes and tags damaged intracellular proteins prior to delivering them to the proteasome system for degradation. The ubiquitin-proteasome system (UPS) is critical to the normal maintenance and turnover of cell constituents. Defects of the UPS may affect the balance of cytoplasmic proteins, cause accumulation of non-functional proteins, and lead to loss of normal cellular function and/or cell death. Explanted human hearts with idiopathic dilated cardiomyopathy (IDCM) contain over 200 distinct ubiquitin-labeled proteins, possess a 5-fold increase in overall ubiquitin labeling, and reveal occasional myocytes with massive intracellular deposits of ubiquitin complexes. Canine IDCM possesses many morphologic and biomolecular similarities to the human disease. We hypothesized that intracellular ubiquitin accumulation occurs in dogs with IDCM.

Immunohistochemical staining using a rabbit-derived anti-ubiquitin polyclonal antibody (Stressgen Biotechnologies, Victoria, BC, Canada) was performed on LV and septal tissues from a dog with severe IDCM (EF=17%) and compared to control tissues from a healthy animal. Thirty randomly selected areas of 1000X magnification were examined and the prevalence of myocardiocytes with large ubiquitin inclusions was calculated.

Cardiac tissues from the affected dog demonstrated occasional myocardiocytes with massive accumulation of ubiquitin-stained complexes. No such cells were found in the control tissues. In the affected tissue, the prevalence of myocardiocytes with large ubiquitin accumulations was 0.11%.

Large deposits of ubiquitin complexes are found in myocardiocytes of dogs with IDCM, a finding which is similar to that reported in humans. Abnormalities of the UPS may play an important role in cardiac cell death and cardiac dysfunction in canine IDCM.



#### ABSTRACT #85

CORRELATION BETWEEN SERUM POTASSIUM CONCENTRATION, SERUM IONIZED CALCIUM CONCENTRATION, SERUM SODIUM: POTASSIUM RATIO AND ELECTROCARDIOGRAPHIC FINDINGS IN CATS WITH URETHRAL OBSTRUCTION. Evason M., Carr A., and Waldner C. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK., Canada.

The veterinary literature supports a close relationship between hyperkalemia and ECG changes such as flattening of the P-wave, spiking of the T-wave and prolongation of the QRS duration. Cats with urinary obstruction commonly develop hyperkalemia and may have abnormal electrocardiograms. Concurrent metabolic changes occur in obstructed cats, such as hypocalcemia, that could effect the ECG. The aim of this prospective study was to obtain blood gas parameters concurrently with an ECG in cats with urethral obstruction.

Eleven male neutered feline patients that presented to the emergency service of the Veterinary Teaching Hospital with complete urethral obstruction were evaluated. Diagnosis was made on the basis of a large, firm urinary bladder from which urine could not be expressed. This was later confirmed via the passage of a urinary catheter. A standard 6 lead ECG and venous blood sample for blood gas analysis were obtained on all patients immediately prior to instituting therapy. ECG analysis was performed by the primary investigators who were blinded to blood test results. All measurements were performed on lead II, and the relationship of serum potassium, serum ionized calcium, and sodium: potassium ratio to the following characterized; amplitude of P, R and T waves, duration of P, QRS, Q-T, and P-R intervals, P to T ratio, ST segment depression or elevation, and heart rate. Data was analyzed using a generalized estimating equation with a normal distribution to account for multiple observations per animal (SAS version 8.2). In all statistical analyses, differences were considered significant at P < 0.05.

Significant correlation was found between ionized calcium values and the following; P wave amplitude and duration, T wave amplitude, heart rate, and duration of QT, P-R, and QRS intervals. Significant correlation was also found between serum potassium concentration and amplitude of T, heart rate, and duration of PR and QRS intervals. A significant association was found between serum sodium: potassium ratio and heart rate, and duration of PR and QRS interval.



Preliminary data suggests that serum ionized calcium levels may have profound effects on cardiac impulse generation and conduction in cats with urethral obstruction. Further analysis on a larger patient population is pending, and may support altering current therapeutic recommendations for this common veterinary emergency.

#### **ABSTRACT #86**

NEURONAL CEROID LIPOFUSCINOSIS IN THE AMERICAN BULLDOG. Jason Evans<sup>1</sup>, Martin Katz<sup>2</sup>, Donald Levesque<sup>1</sup>, Alexander de Lahunta<sup>3</sup>, Diane Shelton<sup>4</sup>, Dennis O'Brien<sup>5</sup>; <sup>1</sup>Veterinary Neurological Center (Las Vegas, NV), <sup>2</sup>Mason Eye Institute (Columbia, MO), <sup>3</sup>Cornell University (Ithaca, NY), <sup>4</sup>Comparative Neuromuscular Laboratory (La Jolla, CA), <sup>5</sup>College of Veterinary Medicine, University of Missouri (Columbia, MO).

Neuronal ceroid lipofuscinoses (NCLs) are inherited neurodegenerative diseases characterized by accumulations of autofluorescent lipopigments (ceroid and lipofuscin) within the cells of the nervous system. NCL typically causes retina degeneration resulting in blindness and cerebral dysfunction resulting in mental deterioration and seizures. Tremors, dysmetria and proprioceptive deficits may be seen if the cerebellum or brainstem is affected. NCLs cause highly variable clinical manifestations and are histopathologically diverse. Diagnosis is based on ultrastructural identification of lipopigment inclusions. The precise enzymatic deficiencies leading to most NCLs are not well understood. The purpose of this investigation was to characterize the clinical and histopathological manifestations as well as the mode of inheritance of a previously undiagnosed neurodegenerative disease in a population of related American Bulldogs.

Nine related American Bulldogs were examined for signs of a slowly progressive dysmetria and UMN paraparesis. General physical and cranial nerve examinations were normal. The average age at the initial examination was 3.2 +/- 1.06 years (1.4 to 4.25 years) and the average age of the onset of signs was 1.53 +/- 0.65 years (0.9 to 3 years). Results of urine organic acid and plasma amino acid assays were not contributory to a diagnosis. Specific enzymatic activities for 10 different lysosomal storage diseases evaluated from leukocytes from groups of both affected and related, but unaffected dogs were normal. Four dogs were necropsied. Histopathological examinations identified diffuse accumulation of PAS-positive inclusions in the cytoplasm of neurons and prominent axonal spheroids along the entire neuraxis particularly in the brainstem and spinal cord. The stored material was autofluorescent and immunohistochemical staining on the substrate was positive for products of lipid peroxidation. Similar autofluorescent inclusions were present in retinal neurons. Ultrastructural analysis was consistent with NCL.

In conclusion, NCL has not been previously reported in the American Bulldog and these findings suggest a variant form of the canine disease. The most severe pathology in these dogs was found in the proprioceptive nuclei of the brainstem and spinal cord resulting in the characteristic clinical signs. The pedigree evaluation supports an autosomal recessive mode of inheritance. The accessibility to multiple related affected and unaffected dogs provides an opportunity to study this disease as a natural model.

#### **ABSTRACT #87**

FECAL INCONTINENCE AS A PRIMARY PRESENTING COMPLAINT IN 5 DOGS WITH CYSTIC ABNORMALITIES OF THE SPINAL CORD. AV Chen, RS Bagley, CL West, PR Gavin, RL Tucker. Washington State University, Pullman, WA.

Five dogs with suspected primary neurogenic fecal incontinence were identified. Breeds represented include Shih Tzu (2), Pug (1), Jack Russell Terrier (1), and Great Dane (1). Age of dogs ranged from 2 to 10 years. Three dogs were spayed females and two were castrated males. Incontinence was characterized by defecation

without posturing and normal stools. Duration of clinical signs prior to presentation ranged from 5 months to 3 years. Neurologic examination revealed upper motor neuron (UMN) paraparesis in four dogs and UMN tetraparesis in one dog. Two of five dogs also had notable pelvic limb ataxia. Physical exams, including rectal exams, were normal in all dogs. Diagnostic workup included a complete blood count, serum biochemistry panel, and magnetic resonance (MR) imaging. Four of five dogs had lumbar cerebrospinal fluid (CSF) collection.

The complete blood count and serum biochemistry panel were non-contributory. CSF analysis was abnormal in two of four dogs. One had mildly elevated protein and the other had elevated protein and lymphocytic pleocytosis. This dog had an IgG antibody titer for Toxoplasmosis in both serum and CSF. All dogs had dural or intradural cystic lesions identified on MR imaging. All lesions were hypointense on T1-weighted images and hyperintense on T2-weighted images. Signal intensity of the abnormalities was equivalent to CSF. Three lesions were focal and two were more diffuse in the spinal cord. Of the focal lesions, two were extramedullary in location, consistent with arachnoid cysts (at T12-13 and C2-3). One dog had an intramedullary abnormality at T11-12 associated with a thickened dura. This was the dog with the positive antibody titer for Toxoplasmosis. Of the diffuse spinal lesions, one was consistent with syringomyelia extending from T1-L3. The other appeared extramedullary in location extending from T8-L2. All cystic abnormalities predominately involved the dorsal aspect of the spinal cord. Exploratory spinal surgery was performed on the three dogs with focal lesions. Arachnoid cysts were confirmed in 2 dogs. The third dog had a mononuclear infiltrated dural scar causing focal cerebrospinal fluid accumulation. Post-operatively, the two dogs with the arachnoid cysts had their fecal incontinence resolved (follow up at 6 months and 5 ½ years). The dog with the dural scar was treated for Toxoplasmosis. Fecal incontinence persisted and neurologic status deteriorated after two months. Additional workup was not pursued to determine the cause for the neurologic deterioration.

In conclusion, neurogenic fecal incontinence may be associated with UMN spinal lesions, specifically dorsal cystic abnormalities. Successful resection of focal cystic abnormalities (arachnoid cysts) can improve fecal incontinence.

#### **ABSTRACT #88**

FELINE MYOKYMIA AND NEUROMYOTONIA. Heather R. Galano<sup>1</sup>, Natasha Olby<sup>1</sup>, James F. Howard<sup>2</sup>, Diane Shelton<sup>3</sup>. 1. North Carolina State University Veterinary Teaching Hospital, Raleigh, NC. 2. University of North Carolina Medical School, Chapel Hill, NC. 3. Comparative Neuromuscular Laboratory, San Diego, CA.

Myokymia and neuromyotonia are two rare clinical phenomena that represent a continuum of motor axon or motor nerve terminal hyperexcitability. They are both high frequency discharges distinguished by their electrophysiological parameters. Myokymia is characterized by undulating, vermicular, rippling, and wave-like movements spreading across the muscle surface, which persist despite sleep or anesthesia. Myokymic discharges are short bursts of ectopically generated motor unit potentials, firing at rates of 5-62 Hz and appearing rhythmically as doublets, triplets, or multiplets. Neuromyotonia is characterized by muscle stiffness and persistent contraction related to underlying spontaneous repetitive firing of motor unit potentials. Neuromyotonic discharges are characterized by prolonged bursts of motor unit potentials, firing at rapid rates of 150-300 Hz, which begin and end abruptly, do not fire rhythmically, and have a characteristic waning amplitude. In humans, neuromyotonia is commonly associated with immune-mediated disorders, such as myasthenia gravis, thymomas, amyloidosis, and lymphoma. Myokymia is commonly associated with Guillain-Barré Syndrome, multiple sclerosis, radiation plexopathy, brainstem

tumors, and timber rattlesnake envenomation. Myokymia and neuromyotonia can also be primary autoimmune disorders, associated with antibody production against voltage-gated potassium channels. Myokymia has only been reported once in the veterinary literature involving a Yorkshire Terrier Dog. The authors present a case of myokymia and neuromyotonia in a cat.

A 6-year-old female spayed DSH presented to NCSU Neurology Service for a 2-week history of rhythmic muscle movements. Physical examination demonstrated forelimb rigidity, contracture of the carpi, generalized muscle atrophy, and rhythmic rippling of all limb muscles. Neurological examination was otherwise normal. The CK was elevated at 28,380 IU/L (normal 50-502). EMG demonstrated spontaneous muscle activity in all muscle groups tested, including fibrillation potentials, positive sharp waves, and spontaneous firing of motor unit potentials characteristic of myokymic and neuromyotonic discharges. Motor nerve conduction studies were normal. Muscle biopsies from the triceps and biceps femoris revealed a non-inflammatory myopathy with no underlying etiology identified.

Treatment with carbamazepine was unsuccessful. Immunosuppression with prednisone was also unsuccessful. Phenytoin, however, did result in resolution of muscle movements, increased range of motion of the limbs, improved ambulation, and resolution of the carpal contracture. The cat continues to do well on Phenytoin. Repeat electrodiagnostics revealed improvement but not complete resolution of the spontaneous activity. The CK level has normalized. Serology for detection of antibodies to potassium channels is pending, which would further confirm the diagnosis and identify the etiology of myokymia and neuromyotonia in this case.

#### **ABSTRACT #89**

SUSPECTED CEREBRAL VASCULAR ABNORMALITIES IN GREYHOUND DOGS. CL West, RS Bagley, KJ Wardrop, PR Gavin, AV Chen. College of Veterinary Medicine, Washington State University, Pullman, WA.

Cerebrovascular (CV) disease is increasingly recognized as a cause of intracranial dysfunction in dogs. Clinical features, breed-associations, predisposing factors, and imaging characteristics of vascular-associated abnormalities are just now beginning to be further clarified. Five greyhound dogs were evaluated beginning in 1999 for acute cerebral disease suspected to be associated with CV abnormalities. Dogs ranged in age from 5 to 13 years. All clinical signs began acutely. Three dogs' signs were consistent with a central vestibular dysfunction including ataxia (3/3), head tilt (3/3), paresis (3/3), and one dog had a horizontal nystagmus. Two dogs had signs of supratentorial abnormalities including ipsilateral head turn, decreased facial sensation and vision deficits contralateral to the lesion as well as limb paresis. Clinical signs in 1 dog reflected a right-sided lesion, while the other had signs of a left-sided lesion. No dog had generalized seizures. Systolic Doppler blood pressures were performed in two dogs; one dog had pressures consistently above 180 mm Hg, and the other was at 167 mm Hg. No significant abnormalities were found in the serum chemistry (4/4), complete blood count (5/5) or urinalysis (3/4). One dog had protein loss in the urine with an elevated urine protein: creatinine ratio. Coagulation evaluations in 2 dogs were normal. CSF was collected in 3 dogs and was normal in these dogs. Four dogs had magnetic resonance (MR) imaging of the intracranial region using standard sequences. Two of these dogs had vestibular signs, and 2 had supratentorial signs. All dogs had focal abnormalities within the brain. In one of the dogs with vestibular signs, MR imaging was normal. In the other dog with vestibular signs there was a focal lesion in the left cerebellum and brain stem. In the two dogs with supratentorial signs, 1 had a lesion in the internal capsule, and 1 had a lesion involving the caudate nucleus. MR imaging features included hyperintense abnormalities on T2-weighted and Flair sequences and isointense to hypointense

abnormalities on T1-weighted sequences. Enhancement following intravenous contrast administration (Gadolinium DTPA) was variable. Two dogs had echocardiograms, 1 being normal and 1 having a suspected myocardial infarction as well as increased left ventricular wall thickness. All dogs improved rapidly initially with supportive care and had continual improvement over the ensuing 2 to 4 weeks. All dogs were discharged to their owner's care at home. One dog with the lesion in the caudate nucleus was necropsied two years after initial presentation and had pathologic features consistent with that of a previous CVA within the caudate nucleus. Clinical course and MR imaging characteristics in these dogs support the possibility of CV disease.

#### **ABSTRACT #90**

MUCOPOLYSACCHARIDOSIS TYPE VI IN MINIATURE PINSCHERS: SCREENING FOR THE MUTATION. P.Fouremant<sup>1,2</sup>, L. Berman<sup>1</sup>, K. Stieger<sup>1</sup>, M. Van Hoeven<sup>1</sup>, N. M. Ellinwood<sup>1</sup>, M. E. Haskins<sup>1</sup>, E. Kirkness<sup>3</sup>, U. Giger<sup>1</sup>. <sup>1</sup>Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. <sup>2</sup>Veterinary Neurological Center, Phoenix, AZ. <sup>3</sup>The Institute for Genomic Research, Rockville, MD.

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders in which glycosaminoglycans accumulate. MPS VI is caused by a deficiency of the enzyme N-acetylgalactosamine-4-sulfatase, also known as arylsulfatase B (ASB). MPS VI is inherited as an autosomal recessive trait, and is clinically characterized by short stature, facial dysmorphism, corneal dystrophy, secondary neurological disturbances, and such orthopedic abnormalities as loss of the femoral head and remodeling of acetabular bone. MPS VI has been diagnosed in Siamese and domestic short hair cats, as well as in several breeds of dogs, including miniature pinschers, miniature schnauzers, corgis and Chesapeake Bay retrievers. Miniature pinschers affected with MPS VI have come from Louisiana, California and South America. They were diagnosed by a positive urinary MPS spot test, white blood cell inclusions, and deficient ASB enzyme activity.

The goals of this study were to sequence the normal canine ASB gene, determine the mutation responsible for MPS VI in miniature pinschers, and develop a rapid DNA-based test for distinguishing normal, affected and carrier individuals. We then screened miniature pinschers in order to identify carrier and affected dogs, estimate the gene frequency in the population, and to test the hypothesis of an association between MPS VI and Legg-Calve-Perthes (LCP) disease, another disease affecting the hip joint. The normal canine ASB cDNA sequence was determined using primers developed based on conserved sequences of the human and feline ASB, and by screening clones from a canine cDNA library. When the DNA coding sequence from miniature pinschers affected with MPS VI was compared to the normal canine sequence, a single missense mutation (G to A) was identified. This mutation, occurring in exon V, replaces the tiny amino acid glycine with a bulky arginine in a highly conserved region of the protein and is found only in affected dogs. A DNA test based on restriction enzyme analysis of PCR products was developed. Samples were solicited for testing from AKC registered miniature pinschers, including nine dogs diagnosed with LCP disease. DNA obtained from cheek swab or blood samples from 89 miniature pinschers has been analyzed. One affected and ten carrier dogs were identified. None of these had LCP. The affected dog and all identified carrier dogs were related to each other. In this biased population, the frequency of the mutant allele was approximately 0.07 (7%).

Because the MPS VI mutation appears to be widespread in the miniature pinscher population, we recommend genetic screening of miniature pinschers in order to guide informed restriction of carrier matings and to thereby ultimately eradicate this disease from the miniature pinscher population. Future studies will focus on the

frequency of this mutation in the general population and identification of the MPS VI causing mutations in other dog breeds.

#### ABSTRACT #91

MAGNETIC RESONANCE IMAGING OF INTRACRANIAL CRYPTOCOCCOSIS IN DOGS AND CATS. <sup>1</sup>T.L. Stevenson, <sup>1</sup>P.J. Dickinson, <sup>1</sup>B.K. Sturges, <sup>1</sup>K.M. Vernau, <sup>1</sup>G.D. Kortz, <sup>2</sup>RE Levitski, <sup>2</sup>D. Lipsitz, <sup>1</sup>R.A. LeCouteur. <sup>1</sup>School of Veterinary Medicine, University of California Davis CA. <sup>2</sup>Veterinary Specialty Hospital, Rancho Santa Fe, CA.

Cryptococcosis is an uncommon but important infectious disease of cats and dogs caused by infection with the saprophytic fungal organism *Cryptococcus neoformans*. Involvement of the central nervous system is common, particularly in dogs, however information relating to the value of advanced imaging as a potential diagnostic tool is limited. This retrospective study was done to evaluate the magnetic resonance imaging (MRI) characteristics, clinical presentation and diagnostic findings of intracranial cryptococcosis in 7 dogs and 5 cats. MR images were evaluated for the following characteristics: lesion location, signal intensity of mass lesions, edema, mass effect and contrast enhancement.

All cats were over 5 years of age. All dogs were medium to large breed with most being less than 6 years of age. Onset of clinical signs was less than 5 weeks in 5/7 dogs and 3/5 cats. Cryptococcal organisms were found in CSF of 3/6 dogs and 4/5 cats. Cryptococcal antigen testing was positive in 6/8 serum samples and 6/6 CSF samples. Two dogs had positive CSF titers with negative serum titers. CSF total nucleated cell counts were elevated in 10/11 animals. CSF total protein was elevated in 10/11 animals, and greater than 100mg/dl in 6/11 animals.

MRI characteristics were variable in both cats and dogs. Location of MRI lesions included diffuse or patchy meningeal enhancement alone, meningeal, ependymal and choroid plexus enhancement, solitary mass lesions, and multifocal mass lesions with and without meningeal enhancement. MR imaging was unremarkable in 1 cat. Mass lesions were present in olfactory/frontal regions in 6/12 cases. Some degree of meningeal enhancement (beyond the apparent margins of mass lesions) was typical in most canine (6/7) and feline (3/5) cases. Most mass lesions were iso to hypo intense on T1 weighted images and hyperintense on T2 weighted images. Edema was usually perilesional in nature. The majority of mass lesions were contrast enhancing, however degree of enhancement was variable, as was definition of the margins of the lesions. There was no apparent correlation between presence of larger mass lesions and length of duration of clinical signs.

It is concluded from this study that considerable variation can occur in the MRI characteristics of intracranial cryptococcosis, although meningeal enhancement is a common finding. MRI appearance may be suggestive of a wide variety of differential diseases, including primary and secondary neoplasia, inflammatory and other infectious diseases.

#### ABSTRACT #92

NEONATAL ENCEPHALOPATHY IN STANDARD POODLES. <sup>1</sup>O'Brien DP, <sup>1</sup>Shelton GD, <sup>2</sup>Johnson GC, <sup>3</sup>Johnson GS, <sup>4</sup>Patterson E. <sup>1</sup>Dept of Vet Medicine & Surgery, <sup>2</sup>Dept of Pathobiology, <sup>3</sup>and Vet Med Diagnostic Lab, University of Missouri, Columbia MO; <sup>4</sup>Dept of Pathology, University of California, San Diego, La Jolla CA; <sup>5</sup>Dept Small Animal Clinical Sciences, University of Minnesota, Minneapolis MN.

We report the genetics, clinical signs, and organic acid profiles in a familial, neonatal encephalopathy in Standard Poodles. Twenty-five affected pups were identified in thirteen litters (95 total pups) born to normal parents. Puppies that died within the first few days of life could not be accurately phenotyped and were not included in the

analysis. Males and females were equally affected (13:12 respectively). The observed segregation frequency was 26.3%. Correction for ascertainment bias (Davies method) yielded a frequency of 23.9%. Chi square analysis did not rule out autosomal recessive mode of inheritance (chi-square = 0.056, p = 0.81). All affected dogs could be traced to a single common ancestor 4 generations prior to the first identified affected pup.

Affected pups nursed poorly at first and often required extra care to survive. They subsequently nursed normally but were developmentally delayed and smaller than their littermates. At three weeks of age, some affected pups were able to walk though they were weak, ataxic, and mentally dull. In addition to limb weakness, axial weakness was apparent in some pups as a ventroflexion of the neck. They showed a wide-based stance, tremors, increased extensor tone, and frequent falls. In the puppies that survived to 4-5 weeks of age, weakness progressed to lateral recumbency with extensor rigidity and opisthotonus. Generalized, tonic-clonic seizures were also observed. CBC, serum chemistries, and liver function tests were normal in the puppies tested.

Urine organic acids were quantified by gas chromatography-mass spectrometry in four affected pups, two litter-mate controls, and two age-matched controls of another breed. Affected pups showed elevated levels of citric acid (mean 1217, range 450-2593 mmol/mol creatinine) compared to age-matched controls (mean 82, range 5-174 mmol/mol creatinine) and mild elevations of isocitric, aconitic, and succinic acids, a pattern suggestive of a TCA cycle abnormality.

The neonatal onset of severe encephalopathic signs would be consistent with an inborn error of metabolism. The altered organic acid profiles could reflect such an error, or they could be secondary, such as to severe seizures in a neonate. The genetic analysis suggests an autosomal recessive trait, which may be wide-spread in the breed.

#### ABSTRACT #93

CLINICAL CHARACTERISTICS AND TOPOGRAPHICAL MAGNETIC RESONANCE OF SUSPECTED BRAIN INFARCTION IN DOGS. <sup>1</sup>LS Garosi, <sup>1</sup>JF McConnell, <sup>1</sup>SR Platt, <sup>1</sup>G Barone, <sup>2</sup>JC Baron, <sup>3</sup>A de Lahunta, <sup>4</sup>SJ Schatzberg, <sup>5</sup>- <sup>1</sup>Animal Health Trust, Newmarket, Suffolk, England; <sup>2</sup>Long Island Veterinary Specialists, New York, NY; <sup>3</sup>University of Cambridge, Dept of Neurology and Addenbrooke's NHS Trust, Cambridge, England; <sup>4</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY; <sup>5</sup>Animal Medical Centre, New York, NY.

The purpose of this retrospective study was to determine the location and associated neurological signs of brain infarcts in dogs. Medical records of 38 dogs that presented for evaluation of acute onset, non-progressive (after 24 hours), intracranial neurological signs were reviewed [1999-2003]. All dogs had a lesion on magnetic resonance imaging (MRI) compatible with brain infarction. Based on the MRI findings, infarcts were classified based upon: 1) location within the brain (telencephalic, thalamic/midbrain, cerebellar, pons/medulla or multifocal), 2) suspected arterial territory involved, 3) presence or absence of hemorrhage and 4) pattern of contrast-enhancement. Common neurological findings were recorded for each infarct location. The location distribution of the brain infarcts were as follows: 6/38 telencephalic, 12/38 thalamic/midbrain, 17/38 cerebellar and 3/38 multifocal (thalamic and medulla). Telencephalic infarcts occurred within the territory of the middle cerebral (4/6) and rostral cerebral arteries (2/6). Thalamic/midbrain infarcts occurred within the territory of the striate arteries (5/12) and perforating arteries of the rostral brainstem (7/12). Cerebellar infarcts were all within the territory of the rostral cerebellar artery. The appearance of the telencephalic and cerebellar infarcts resembled territorial infarcts seen in humans, while the thalamic/midbrain infarcts resembled lacunar infarcts. All infarcts appeared as non-hemorrhagic and contrast-enhancement was observed in only 3/38 dogs, all of which were imaged more than 7 days after the onset of

neurological signs. Common neurological findings of the brain infarctions were as follow: telencephalic infarct (abnormal mental status, contralateral postural reaction deficits, contralateral nasal hypalgesia, contralateral menace deficits and ipsilateral circling), thalamic/midbrain infarct (contralateral or ipsilateral postural reaction deficits, contralateral menace deficits, ipsilateral head tilt or turn, nystagmus, ventrolateral strabismus and anisocoria), cerebellar (asymmetric cerebellar quality ataxia, head tilt, intermittent opisthotonus, nystagmus, ipsilateral menace deficit with apparent normal vision). Three dogs were presented for paroxysmal events suggestive of a vestibular disorder. In conclusion, brain infarctions are a cause of acute, non-progressive neurological signs in dogs, cause clinical signs consistent with the neuroanatomic location of the lesion, occur in similar vascular territories to those seen in humans, and typically are non-hemorrhagic.

#### **ABSTRACT #94**

EVALUATION OF CEREBROSPINAL FLUID URIC ACID LEVELS IN DOGS WITH INTRACRANIAL MENINGIOMAS. S. R. Platt,<sup>1</sup> D. Marlin,<sup>2</sup> N. Smith,<sup>2</sup> V. Adams<sup>3</sup> L. Garosi<sup>1</sup> Centre for Small Animal Studies,<sup>1</sup> Centre for Equine Studies,<sup>2</sup> Centre for Preventative Medicine,<sup>3</sup> The Animal Health Trust, Newmarket, Suffolk, UK.

Cerebrospinal fluid (CSF) uric acid level is an index of turnover of nucleic acid and the degree of cellular destruction in the brain leading to increases in interstitial uric acid (UA) and may reflect glutamate-mediated excitotoxicity. Elevated CSF uric acid levels are associated with the degree of malignancy of human brain tumors serving as an indirect prognostic marker for patient outcome. The objectives of this pilot study were to evaluate a technique for the analysis of UA levels in canine CSF and compare UA levels in the CSF of dogs with intracranial meningiomas to those with a normal central nervous system based on clinical signs, in addition to MRI and CSF analysis.

Six dogs with histopathologically diagnosed intracranial meningiomas (group I) and five dogs with no evidence of intracranial disease (group II) as determined by magnetic resonance imaging and CSF analysis were included in the study. All dogs had a cisternal CSF tap performed under general anesthesia; 0.3ml of CSF from each dog was stored in fluoride oxalate containers at -80°C until analysis. Uric acid in dog CSF was measured using HPLC with ultraviolet detection. The method used a citrate/acetate buffer with 6% methanol run isocratically. The uric acid peak eluted after 3 minutes and was detected at a wavelength of 292nm. The limit of detection of this method is approximately 200nM. The intra- and interassay CVs were 5.1% and 8.3% respectively. Results are presented as arithmetic mean  $\pm$  SD. Data were analyzed with standard statistical methods, using a two sample T-tests for independent samples. Significance was set at  $P < 0.05$ .

The mean CSF uric acid level was 13.60 $\mu$ M ( $\pm$ 5.83) in group I and 6.90 $\mu$ M ( $\pm$ 2.35) in group II and these values were significantly different ( $P=0.04$ ) with a mean difference of 6.7 (95% CI:0.4, 13).

This small pilot study demonstrates that CSF uric acid concentrations can be reliably measured in dogs and that there is a significant elevation in CSF uric acid in dogs with intracranial meningiomas. This research is now being expanded to evaluate larger groups of dogs with other intracranial tumor types and grades in an attempt to associate CSF UA concentrations with tumor malignancy and ultimately survival of the patient.

#### **ABSTRACT #95**

USE OF MAGNETIC RESONANCE IMAGING IN EVALUATION OF STEROID RESPONSIVE MENINGOENCEPHALITIS SUBTYPES. AE Farabaugh, D Faissler, AS Tidwell, J McDonnell Tufts University, School of Veterinary Medicine, North Grafton, MA.

Steroid-responsive meningoencephalitis (SRME) is an idiopathic condition which can be divided into subtypes such as granulomatous meningoencephalomyelitis, necrotizing encephalitis, unclassified viral, allergic and eosinophilic meningoencephalitis. A diagnosis of SRME and its subtypes is presumptive, and it is based on a combination of different findings as well as ruling out infectious causes. Definitive diagnosis is by histopathology.

The goal of this retrospective study was to evaluate the usefulness of magnetic resonance imaging (MRI) in differentiating SRME lesions. Clinical data of dogs with suspected SRME were analyzed and compared to MRI results. MRI studies included T1 and T2 weighted spinecho, FLAIR, T2\*, and T1 weighted post Gadolinium contrast sequences. Parameters were pattern, location of lesion, and lesion intensity on different sequences, as well as the presence of mass effect, hemorrhage, ventriculomegaly and edema. The relationship between the MRI data and other diagnostic information including lesion location, neurological signs, and response to treatment and survival times was examined.

Twenty-one dogs were enrolled with Pug dogs and West Highland Terriers being the most frequently represented breeds. The median age of onset of the disorder was 4.8 years (0.9-11.6 years). Clinical signs such as dullness (n=9), behavioral changes (n=3), circling (n=7), head tilt (n=9), ataxia (n=15), pathological nystagmus (n=5), anisocoria (n=4), proprioceptive dysfunction (n=12), and seizures (n=10) indicated an intracranial lesion. CBC, chemistry profile, urine analysis (n=11), blood ammonia levels (n=4), Neospora (n=6), Toxoplasma (n=6), Ehrlichia (n=3), Rocky Mountain spotted fever (n=1) and canine distemper (n=1) titers were within normal limits.

The MRI findings revealed multifocal, poorly to moderately well-margined brain lesions. The dogs were divided into two groups based on the contrast enhancing pattern. Group A: These dogs presented with a post-contrast pattern, which we describe as a halo effect. This effect appeared as a thin ring of contrast enhancement around a hypointense core (n=6). Group B: The enhancement of the lesion appeared to be homogeneous (n=16).

Lesion location was compared between the two groups; there was no significant correlation between the enhancement pattern and lesion location ( $p=0.098$ ). There was a strong trend ( $p=0.051$ ) for the dogs in group A to exhibit a greater degree of mass effect than the patients in group B. CSF of the dogs of group A showed a mixed pleocytosis with increased protein levels similar to group B. Survival time of group A was significantly shorter than in group B (35 $\pm$ 61 versus 258 $\pm$ 209 days,  $p=0.02$ ). After a median follow up time of 399 days, 7 of 15 dogs in group B are still alive whereas all dogs of group A died regardless of the treatment type.

This study indicates that MRI can provide important prognostic information when evaluating SRME. Further investigation in distinguishing SRME subtypes appears to be warranted.

#### **ABSTRACT #96**

LEVETIRACETAM THERAPY FOR LONGTERM IDIOPATHIC EPILEPTIC DOGS. M. Steinberg, D Faissler, Tufts University, School of Veterinary Medicine, North Grafton, MA.

Multiple drugs are administered to idiopathic epileptic dogs refractory to monotherapy in order to reduce seizure frequency and duration while simultaneously reducing the risk of side effects. Phenobarbital (PB) and potassium bromide (KBr) are often used in conjunction to control the seizure activity. The addition of a third anticonvulsive medication was reported to further reduce seizure frequency.

Our hypothesis was that levetiracetam (LEV), a newer drug which is liver cytochrom P450 independent and excreted mostly unchanged by the kidneys, lowers the seizures frequency without producing significantly more side effects when added to the previous treatment. Our study population consisted of a group of dogs with longstanding, typical grand mal seizures that were difficult to manage. All dogs

were initially treated with PB and/or KBr resulting in insufficient seizure control or significant side effects that warranted another antiepileptic medication. Methods of data collection were telephone questionnaires, physical examinations, tabulation of patient records, blood tests, and medication serum levels. All parameters were evaluated before and after the addition of LEV.

Fifteen dogs were evaluated. Grand mal seizures were observed for a median of 38 months (13.8 to 95.5 months). The median age of onset of seizure activity was 31 months (9 to 64 months). Fourteen of 15 dogs were treated with PB and KBr whereas one dog received only KBr. The median time on therapy with PB and/or KBr was 17 months (3.3 to 58.3 months). Four of the 14 dogs treated with PB developed clinical signs of liver toxicity, increased liver enzymes, low albumin and glucose levels and abnormal bile acid stimulation tests. Liver biopsies confirmed moderate chronic active hepatitis in these dogs. These 4 dogs with confirmed liver disease had higher mean PB serum levels (32.1±14.4 versus 22.0±18.1 µg/ml) and were under treatment longer (31.1±18.5 versus 20±6.9 months) than dogs with no obvious liver dysfunction, but neither serum PB levels nor treatment time were significantly different.

Prior to the addition of LEV, the average seizure frequency was 12.8 seizures per 3 months. Oral LEV was added at doses ranging between 7.1 and 23.8 mg/kg three times a day. With the addition of LEV, the mean number of seizures was reduced from 12.8 to 5.9 seizures per 3 months. This investigation demonstrated a significant 54% decrease (p<0.05) in seizure frequency after LEV was added to PB and/or KBr. There was no significant difference between the mean PB and KBr serum levels before and after the addition of the LEV (PB 23.4±12 versus 19.4±5.5 µg/ml; KBr 2.0±0.9 versus 2.2±0.7 mg/dl). The addition of LEV was not associated with additional side effects that would cause reduction or cessation in medication. Based on the improved seizure control with LEV, the oral PB dose was reduced in the 4 dogs suffering from clinical apparent liver toxicity without increasing the seizure frequency.

#### **ABSTRACT #97**

TARGETING OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN CANINE PRIMARY BRAIN TUMORS. <sup>1</sup>P.J. Dickinson, <sup>1</sup>B. Roberts, <sup>1</sup>C.M. Leutenegger, <sup>2</sup>T. Harding, <sup>2</sup>A. Lalani, <sup>2</sup>K. Jooss, <sup>1</sup>R.A. LeCouteur. <sup>1</sup>School of Veterinary Medicine, University of California Davis CA. <sup>2</sup> Cell Genesys Inc., South San Francisco, CA.

Inhibition of tumor angiogenesis is a promising approach in the treatment of highly vascularized solid tumors such as primary brain tumors. Defining the presence of critical angiogenic pathway targets, such as vascular endothelial growth factor (VEGF) and its receptors VEGFR-1 and VEGFR-2, is essential in selecting appropriate clinical cases for inclusion in therapeutic trials. Antiangiogenic treatment strategies for canine primary brain tumors have been developed utilizing intratumoral delivery of adeno-associated viral (AAV) vectors targeting the VEGF pathway in mice. Before these novel therapies can be used in spontaneous canine tumors, the therapeutic targets and viral vectors must be validated in canine tissues.

This study was done to determine the expression of the 3 major VEGF isoforms (120,164,188) and their major receptors (VEGFR-1,2) in canine primary brain tumors in order to validate specific tumor types as potential targets for anti-VEGF therapy. A selection of AAV vectors was also assayed for their ability to transduce canine tumor cells. Expression of VEGF and VEGFR transcripts was determined using semiquantitative real time TaqMan® RT-PCR. Samples were obtained from snap frozen clinical specimens of meningioma, astrocytic and oligodendroglial tumors obtained at surgery or necropsy as well as paraffin embedded archival tissue. Transduction efficiency of 3 AAV serotypes in canine cells was assayed using AAV serotypes 2,5 and 6 expressing a GFP reporter

gene. *In vitro* assays were done using canine thymus (Cf2th), osteoblast (D17), and glioma (J3T, SDT-3) cell lines.

Transcripts from all three VEGF isoforms and their receptors were expressed in canine tissue with VEGF-164 being the predominant isoform in both normal and neoplastic samples. Over expression of VEGF (relative to normal canine brain) was seen in all tumor types, however significant overexpression (>10 fold and as high as 1000 fold) was seen only in high grade astrocytic and oligodendroglial tumors. VEGFR-1,2 expression generally paralleled VEGF expression, however VEGFR-1 expression was down-regulated in some tumors. *In vitro* transduction of canine cell lines was achieved with all 3 AAV serotypes, however transduction efficiency of AAV-2 was significantly (~30-60%) higher, in all canine cell lines.

Results of this study demonstrate that AAV vectors may be useful for intratumoral delivery of novel therapeutic agents in the dog, however additional *in vivo* studies are required. High grade canine gliomas are potentially good candidates for VEGF and VEGF receptor targeted therapies based on their over expression of both the ligand and its receptors.

#### **ABSTRACT #98**

USE OF CYCLOSPORINE TO TREAT GRANULOMATOUS MENINGOENCEPHALITIS IN DOGS. P. Filippo Adamo, DVM, DECVN, Robert T. O'Brien, DVM, MS, DACVR School of Veterinary Medicine, University of Wisconsin – Madison.

This present report describes four dogs with a presumptive diagnose of granulomatous meningoencephalomyelitis (GME) treated with Cyclosporine (CyA). In all cases presumptive GME diagnosis was based on history, clinical signs, CT (1 case), MRI (3 cases), cerebrospinal fluid abnormalities, and by ruling out infectious diseases. Two dogs had the focal form associated with the ocular form, one dog had the focal form and one dog had the disseminated form of GME.

In three dogs oral CyA therapy replaced corticosteroid therapy and in one dog CyA was the only therapy used. The dog with the disseminated form was euthanized due to progression of the clinical signs and GME was histopathologically confirmed on necropsy. In the three surviving dogs a follow-up at 8 month (1 case) and 1 year (2 cases), including serial CSF analysis and CT (1 case) and MRI (1 case) showed remission of the diseases. CyA was considered effective at an initial dose of 6mg/kg orally BID resulting in blood CyA concentrations between 235ng/ml and 370ng/ml. In this preliminary study oral CyA appeared effective to treat focal and ocular GME.

#### **ABSTRACT #99**

RESULTS OF DIAGNOSTIC INVESTIGATION AND OUTCOME OF SUSPECTED AND CONFIRMED BRAIN INFARCTION IN DOGS. LS Garosi<sup>1</sup>, JF McConnell<sup>1</sup>, SR Platt<sup>1</sup>, V Adams<sup>1</sup>, G Barone<sup>2</sup>, JC Baron<sup>3</sup>, A de Lahunta<sup>4</sup>, SJ Schatzberg<sup>5</sup> - <sup>1</sup>Animal Health Trust, Newmarket, Suffolk, England; <sup>2</sup>Long Island Veterinary Specialists, New York, NY; <sup>3</sup>University of Cambridge, Dept of Neurology and Addenbrooke's NHS Trust, Cambridge, England; <sup>4</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY; <sup>5</sup>Animal Medical Centre, New York, NY.

The purpose of this retrospective study was to determine the typical case signalment and medical conditions associated with brain infarctions, to identify associations between medical conditions and brain infarct types and locations, and to assess the outcome of dogs with brain infarcts. Medical records of 33 dogs that presented for acute onset, non-progressive (after 24 hours), intracranial neurological signs were reviewed. All dogs had magnetic resonance imaging findings compatible with brain infarction (30/33) or post-mortem confirmation (3/33). All dogs were evaluated with complete hematology, biochemistry, thyroid, coagulation and adrenal profile,

urinalysis, thoracic and abdominal imaging and cerebrospinal fluid (CSF) analysis. Arterial blood pressure was evaluated in 28/33 dogs. Based on the imaging findings, infarcts were classified depending on their type (large 'territorial' or small 'lacunar') and location within the brain (telencephalic 6/33, thalamic/midbrain 12/33, cerebellar 15/33). Associations between type or location of brain infarction and clinical outcome were assessed using chi-square or Fisher's exact tests. There were no significant associations between region or type of infarct on one hand and patient age and sex, occurrence of hypertension (systolic >160 mmHg and/or diastolic >120 mmHg), presence of CSF abnormalities or presence or absence of an associated medical condition on the other hand. Small breed dogs ( $\leq 15$  Kg) were significantly more likely to have large cerebellar infarcts while large breed dogs (>15 Kg) were significantly more likely to have a small/thalamic/midbrain infarct (OR=16.5, 95% CI=1.7-163,  $P=0.01$ ). The only individual breed effect was a significant breed predisposition in spaniels (all 7 had cerebellar infarcts). An associated medical condition was detected in 18/33 infarcts. Chronic kidney disease (8/33) and hyperadrenocorticism (6/33) were the two most commonly encountered conditions. Ten of the 33 dogs were euthanized due to the lack of improvement of their neurological status (3) or severity of their associated medical condition (7). There was no association between type or region of infarct and outcome, however, dogs with an associated medical condition had a significantly shorter lifespan (7 deaths and 9 still alive, OR=12, 95% CI=1.3-111,  $P=0.02$ ) than those dogs with no identifiable medical condition (14 still alive). Dogs with an associated medical condition also were significantly more likely to suffer from recurrent neurological signs due to subsequent infarcts (OR=8.9, 95% CI=1.0-84,  $P=0.05$ ).

#### ABSTRACT #100

DIFFERENCES IN SURFACTANT FUNCTION AND COMPOSITION BETWEEN NEONATAL FOALS AND ADULT HORSES. U. Christmann<sup>1</sup>, J. Taintor<sup>1</sup>, L. Livesey<sup>1</sup>, E. Welles<sup>2</sup>, J. Schumacher<sup>1</sup>, B. Waldrige<sup>1</sup>, B. Grier<sup>3</sup>, D. Hite<sup>3</sup>. <sup>1</sup> Department of Clinical Sciences and <sup>2</sup> Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL; <sup>3</sup> Section Pulmonary and Critical Care, Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC.

Pulmonary surfactant maturation occurs in the last trimester of gestation. It undergoes changes at birth, when the lung makes the transition from a fluid to an air-filled state. Even during postnatal development, surfactant function and composition are varied and adapted to the specific respiratory physiology of a mammalian species. The purpose of this study was to evaluate possible differences in surfactant isolated from neonatal foals and adult horses.

Samples of surfactant were obtained by bronchoalveolar lavage (BAL) from normal adult horses (n=6), normal foals at less than 24 hours (n=7), 2 days (n=2) and 5 days (n=2) of age, and premature foals (n=4) euthanized for reasons unrelated to this study. Samples of surfactant from premature foals were obtained at less than 2 hours postmortem. BAL fluid was obtained from horses using standard procedures and obtained from sedated (normal) or euthanized (premature) foals in sternal recumbency, using an endoscope or BAL tube and instilling 100-200 ml of sterile saline. The cell fraction of BAL fluid was removed using centrifugation, and the surfactant pellet was isolated from the cell-free BAL fluid supernatant using ultracentrifugation. The large surfactant aggregates (LA) from the surfactant pellet and the small surfactant aggregates (SA) from the BAL fluid supernatant were analyzed for phospholipid (PL) and protein content using the Bartlett and BCA method, respectively. Surface tension of LA (1mgPL/ml) was measured using a pulsating bubble surfactometer. PL composition was determined using high

performance liquid chromatography with an evaporative light scatter detector.

Surfactant surface tension was abnormally high ( $\gamma_{\min}$ : 20mN/m) in all foals until 2 days after birth. Normal surfactant function ( $\gamma_{\min}$ : 2mN/m) was measured in 5-day-old foals. Surfactant PL composition in all foals showed decreased levels of phosphatidylglycerol (PG) but increased levels of phosphatidylinositol (PI) compared to values in adults (PG: 3.5% versus 6.5% and PI: 2.4% versus 1.1%). The amount of surfactant PL recovered was not different amongst groups of animals. The amount of protein recovered in the surfactant pellet was lower in normal foals compared to premature foals and adult horses.

Our study demonstrated changes in surfactant function and composition in the neonatal period of foals. Normal surfactant function was not achieved in foals until several days after birth. The sum of anionic PL (PG and PI) was decreased in normal foals compared to adults.

#### ABSTRACT #101

DISPOSITION OF ORAL CEFPODOXIME PROXETIL IN FOALS AND ADULT HORSES, AND MINIMUM INHIBITORY CONCENTRATION OF THE DRUG AGAINST COMMON EQUINE BACTERIAL PATHOGENS. N. Carrillo, S. Giguère, R.R. Gronwall, M.P. Brown and K. Merritt. College of Veterinary Medicine, University of Florida, PO Box 100136, Gainesville, FL.

Availability of orally administered broad-spectrum antimicrobial agents would represent a major advantage in the treatment of many equine neonatal infections including sepsis. Cefpodoxime proxetil (Vantin<sup>®</sup>) is an orally administered third generation cephalosporin approved for use in humans. The disposition of this drug was investigated in six healthy 7- to 14-day-old foals, in the same foals when aged between 3-4 months, and in 6 adult horses. A single dose of cefpodoxime proxetil oral suspension (10 mg/kg of body weight) was administered to each animal by nasogastric tube. In 7- to 14-day-old foals, 5 additional doses were administered intragastrically at 12-h intervals. In addition to serum, CSF, peritoneal fluid, synovial fluid and urine were collected following administration of the last dose. A microbiological assay was used to measure cefpodoxime concentrations. Minimum inhibitory concentration (MIC) of cefpodoxime against 173 equine bacterial isolates was determined using the E-test<sup>®</sup>. Time to peak serum concentration ( $T_{\max}$ ) in 7- to 14-day-old foals (mean  $\pm$  SD) was 1.67  $\pm$  0.68 h, maximum serum concentration ( $C_{\max}$ ) was 0.81  $\pm$  0.22  $\mu$ g/ml, and elimination half-life was 7.17 h (harmonic mean). The disposition of cefpodoxime in 3 to 4 month-old foals was not significantly different from that of neonates. Adult horses had significantly higher  $C_{\max}$  and significantly lower  $T_{\max}$  when compared to foals. Concentrations of cefpodoxime in synovial and peritoneal fluids were similar to that of concurrent serum concentrations. Urine concentrations were 12 to 72 times higher than concurrent serum concentrations. The drug could not be detected in CSF. No adverse reactions were noted in foals whereas mild colic developed in 2 adult horses. MIC<sub>90</sub> of cefpodoxime against *Salmonella enterica*, *Escherichia coli*, *Pasteurella* spp., *Klebsiella* spp., and  $\beta$ -hemolytic streptococci was 0.38, 1.0, 0.16, 0.19, and 0.09  $\mu$ g/ml, respectively. Dosing schedules for  $\beta$ -lactam antimicrobials should maintain serum concentrations above the MIC of a given pathogen for at least 50% of the dosing interval to achieve therapeutic efficacy. Oral cefpodoxime administered to 7- to 14-day-old foals at a dose of 10 mg/kg every 12 h resulted in serum concentrations above the MIC<sub>90</sub> of *Klebsiella* spp., *Pasteurella* spp. and  $\beta$ -hemolytic streptococci for more than 50% of the dosing interval. The same dose given at 8-h intervals would be required for therapy of *S. enterica* infections. Administration at 8-h intervals would also result in serum concentrations above the MIC of 75% of *E. coli* isolates for approximately 50% of the dosing interval. Further studies are

required to determine the efficacy and safety of these dosages in a clinical setting.

#### **ABSTRACT #102**

COMPARISONS OF D-DIMER CONCENTRATION IN SEPTIC AND HEALTHY FOALS. MD Figueiredo, TJ Divers, MB Brooks, HO Mohammed, J Reetz, TD Byars. Cornell University, Ithaca, New York.

D-dimer is a degradation fragment produced by the action of plasmin on cross-linked fibrin. In human patients, high plasma D-dimer is associated with DIC, deep vein thrombosis, pulmonary embolism, and catheter-related venous thrombosis. We performed this study to determine whether measurement of D-dimer could aid in the diagnosis of DIC and other thrombotic disorders in foals. Specific objectives were: 1. Determine plasma D-dimer concentration of healthy foals (n=15) 2. Compare plasma D-dimer concentration of septic and age-matched healthy foals (n=10) 3. Monitor D-dimer concentration of septic foals (n=14) at admission and after 24 hr. treatment.

Citrate plasma samples were collected from 15 healthy foals at 3 time points: birth, 18-24hr., and 7-10 days. Five of these foals also had blood collected from the umbilical vein during delivery before rupture of the umbilical cord. The 2nd study group consisted of 14 foals, aged 24 to 48 hrs, hospitalized for clinicopathologic signs of sepsis. D-dimer, aPTT, PT, antithrombin and fibrinogen were measured on admission and 24hrs later. D-dimer and fibrinogen concentration were measured in the control group. D-dimer concentration was measured using a latex agglutination assay (Accuclot D-dimer). The expected equine plasma D-dimer concentration for this method is < 500ng/mL (based on assay of 30 healthy adult horses).

A scoring system for D-dimer results was implemented to facilitate statistical analyses as follows: 0 = < 250ng/mL, 1 = 250-500ng/mL, 2 = 500-1000ng/mL, 3 = 1000-2000ng/mL, 4 = >2000ng/mL. We found that mean ( $\pm$ SD) D-dimer of healthy foals at birth = 1.33 ( $\pm$ 1.63), 18 hr. = 2.00 ( $\pm$ 1.29) and 7-10 days = 2.30 ( $\pm$ 1.34). There was no significant difference between the D-dimer values at birth and 18-24hr. (paired t-test,  $p = 0.17$ ), 18-24hr. and 7-10 days (paired t-test  $p = 0.18$ ), birth and 7-10 days (paired t-test  $p = 0.06$ ). All foals that had blood drawn from the umbilical vein prior to rupture of the cord had D-dimer values < 250ng/mL. D-dimer values for septic foals on admission and 24hr. post admission were 1.69 ( $\pm$ 1.55) and 2.62 ( $\pm$ 1.39) respectively, with no significant difference (paired t-test,  $p = 0.08$ ). These values were not different from the age matched control group (two-sample t-test,  $p = 0.43$ , Wilcoxon rank sum test,  $p = 0.57$ ).

These results reveal that healthy foals, unlike adult horses, typically have plasma D-dimer concentration > 500ng/mL. D-dimer concentration increased over the first 7-10 days of life, however the magnitude of change was not significant in this study population. Blood taken from the umbilical vein had no detectable D-dimer, indicating that fibrin degradation occurs during the post-natal period. We found no significant difference between D-dimer concentration of septic foals and healthy foals less than 48hr old. We conclude that the finding of high (> 500ng/mL) D-dimer concentration in foals less than 10 days of age should not be interpreted as evidence of a pathologic thrombotic condition.

#### **ABSTRACT #103**

EFFECT OF ANTIMICROBIAL DRUG RESISTANCE ON SURVIVAL OF BACTEREMIC FOALS. Jamie E. Murphy, Cheyney Meadows, Kenneth W. Hinchcliff, Allison Stewart\*. Departments of Veterinary Clinical Sciences and Veterinary Preventive Medicine, College of Veterinary Medicine, The Ohio

State University, Columbus, OH. (\*Current address: College of Veterinary Medicine, Auburn University, Auburn, AL).

Bacterial resistance to antimicrobial drugs poses a challenge to the clinical management of infected animals. In foals suspected to be bacteremic, antimicrobial therapy is initiated before the results of blood culture and antimicrobial susceptibility testing are available. The objective of this study was to determine the impact of bacterial resistance to initial antimicrobial treatment on clinical outcome of bacteremic foals treated in a teaching hospital setting.

Case records of 101 foals with blood culture-confirmed bacteremia were examined to determine if the occurrence of antimicrobial resistance to initial naïve therapy influenced survival. Foals were examined because of clinical abnormalities consistent with increased risk of bacteremia. This population of foals has been described (Stewart et al. J. Vet. Int. Med. 2002;16:464-471). Blood for culture was collected at time of initial examination. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Initial antimicrobial therapy consisted of administration of penicillin or ampicillin and an aminoglycoside (amikacin or gentamicin). A Cox proportional hazards regression model was used in the evaluation. Type I error rate was 5%.

Among the 101 foals, there was complete information regarding therapy and antimicrobial susceptibility for 81 foals, which were the focus of the analysis. Bacteria isolated from 9/81 foals (11%) were classified as resistant to antimicrobials used in initial therapy. Thirty-six foals (44%) died or were euthanized in hospital. Three percent (1/36) of foals that did not survive and 18% (8/45) of foals alive at discharge had bacteria isolated that were resistant to initial antimicrobial therapy. Median days of hospitalization were 7 (range: 4, 21) and 10 (1, 37), respectively among surviving foals with or without isolates resistant to initial therapy. These medians were not significantly different (Fisher's Exact  $P = 0.24$ ). A univariable Cox proportional hazards regression did not find antimicrobial resistance to be a significant risk factor for death (RR = 0.20; 95%CI = 0.03, 1.50;  $P = 0.118$ ). Additional analyses controlling for sepsis score and presence of pneumonia did not find antimicrobial resistance to be a risk factor for death.

Results of these analyses suggest that resistance to initial antimicrobial therapy, as judged by the Kirby-Bauer method, does not increase the risk of death or the length of hospitalization of bacteremic foals. The small number of foals with bacteria resistant to initial therapy may have hindered identification of an important effect of antimicrobial resistance on survival. However, there was no evidence of such an effect in the foals in this study, indicating that any effect of resistance, if present, is small. Alternatively, and importantly, these results may be indicative of a poor correlation between susceptibility *in vivo* as defined by the Kirby Bauer method.

#### **ABSTRACT #104**

GLUCOSE KINETICS IN SEPTIC NEONATAL FOALS. Eduard Jose-Cunilleras, Kenneth W. Hinchcliff, Yvette S. Nout, Raymond J. Geor. The Ohio State University, Dept. Veterinary Clinical Sciences, Columbus, Ohio, USA and University of Guelph, Dept. Biomedical Sciences, Guelph, Ontario, Canada.

Sepsis and the consequent alteration in hormones and proinflammatory mediators can lead to a state of relative insulin resistance and hyperglycemia in humans and laboratory animals. Septic neonatal foals often present with abnormal blood glucose concentrations (both hypo and hyperglycemia). However, the underlying derangements in gluco-regulatory mechanisms are unknown in septic foals. This observational study was undertaken to quantitatively determine rates of glucose turnover (glucose production and utilization) in septic neonatal foals.

Five neonatal foals with evidence of failure of transfer of passive immunity and septicemia were enrolled in this study after obtaining client consent. All foals had a positive blood culture, 4 of 5 had

sepsis score >11, were foaled at term and presented at 36 h to 3 days of age. Three foals survived, one was euthanized and another died. Glucose kinetics studies were performed 12-24 h after presentation to allow fluid repletion and cardiovascular stabilization. Foals received a primed constant intravenous infusion of dideuterated glucose for 5 h (17.5  $\mu$ mol/kg prime and 0.5  $\mu$ mol/kg/min infusion) to allow estimation of rates of plasma glucose appearance (index of gluconeogenesis, hepatic glycolysis and gastrointestinal absorption of glucose) and rates of plasma glucose disappearance (index of glucose disposal by tissues). Plasma glucose and dideuterated glucose, serum immunoreactive insulin, immunoreactive glucagon and cortisol concentrations were determined hourly. Dextrose supplemented fluids were administered to 2 of 5 foals. The rate of plasma glucose appearance was corrected to account for glucose administered intravenously. Glucose kinetics studies were performed while foals were being fed milk via indwelling nasogastric tube at rates of ~3.5-6 ml/kg as hourly feedings (~42-72 kcal DE [digestible energy] per kg/day or ~40-70% of daily energy requirements if these are 100 kcal DE per kg/day).

Glucose turnover rates in these septic neonatal foals after initial stabilization were ~25-35  $\mu$ mol/kg/min (min 17, max 53  $\mu$ mol/kg/min), which is ~3 fold greater than that of healthy adult horses. Plasma glucose concentrations during the same period were ~7.3-8.7 mM [131-157 mg/dL] (min 2.4 mM [43 mg/dL], max 12.8 mM [230 mg/dL]). On presentation 3 of 5 foals were hypoglycemic (39, 39 and 27 mg/dL) and during hospitalization blood glucose concentrations fluctuated between 50 and 175 mg/dL. At the time of the glucose kinetic studies, concentrations of serum immunoreactive insulin were 5-25  $\mu$ IU/ml (36-180 pmol/L), serum immunoreactive glucagon were 67-415 pg/ml (19-120 pmol/L), and serum cortisol were 96-322 nmol/L (35-117 ng/ml). Septic foals tended to have lower serum insulin and glucagon, and higher plasma cortisol concentrations, when compared to hormone concentrations reported in healthy neonatal foals.

This study suggests that, when extrapolating from a 5 h measurement, a 45 kg septic neonatal foal utilizes ~350 g of glucose in 24 h.

#### **ABSTRACT #105**

SERUM AMYLOID A IN EQUINE COLOSTRUM AND EARLY MILK. Vivienne E Duggan, G Reed Holyoak, Charles G MacAllister, Anthony W Confer, Oklahoma State University College of Veterinary Medicine, Stillwater, Oklahoma.

This study aimed to define the concentration of serum amyloid A (SAA) in equine colostrum and early milk. It also aimed to determine whether this protein is absorbed in the neonatal intestine to interfere with the accuracy of measuring endogenous SAA concentration in neonatal serum as an aid in the differential diagnosis of sepsis.

19 mares and their foals were included in the study. A serum sample and a colostrum sample at parturition were obtained from each mare and milk samples at 12, 24 and 48 hours post-partum were obtained from 12 of the mares. Serum samples at 0 (pre-colostrum), 12, 24, 36, 48, 60 and 72 hours post-partum were obtained from all normal foals. The SAA concentration of each sample was measured using a commercially available ELISA.

This study demonstrated a mean SAA concentration in mare serum at parturition similar to previously published values. Equine colostrum and early milk demonstrated consistently higher concentrations of SAA than that observed in the serum. Correlation was demonstrated between mare serum SAA concentration at parturition and colostrum SAA concentration. There was no correlation between mare serum SAA concentration at parturition and milk SAA concentration at any measured time. Correlation was demonstrated between colostrum SAA concentration and foal serum SAA concentration at 48 and 60 hours in normal foals. There was no

correlation between 12-hour milk SAA concentration and foal serum SAA concentration at any measured time in normal foals.

Our study confirmed that SAA is elevated in the serum of periparturient mares. This study also demonstrated that SAA is present at high concentrations in equine colostrum and early milk. Correlation between SAA concentration in colostrum and normal neonatal foal serum at 48 and 60 hours suggests that this protein may be absorbed in the early post-partum period of increased macromolecular absorption in the neonatal intestine and could interfere with the accuracy of serum SAA concentration measurement as an aid in the diagnosis of sepsis in foals if colostrum concentrations were high. Absorption of this protein does not appear to persist past 12 hours in the normal equine neonatal intestine. It is unclear if colostrum/milk SAA represents maternal hepatic-derived SAA or if it is mammary gland-derived. Milk amyloid A-3 (MAA-3), a mammary gland derived isoform of SAA, has been previously demonstrated in bovine, ovine, equine and human colostrum. Unfortunately, currently available assays do not distinguish SAA and MAA-3. Human and bovine MAA-3 have been shown to have a protective function in the infant neonatal intestine through up-regulation of mucin production and prevention of pathogen adhesion. Further studies are required to define the role of MAA-3 in the protection of the equine neonatal intestine.

#### **ABSTRACT #106**

SERUM IMMUNOGLOBULIN G AND LACTOFERRIN CONCENTRATIONS IN HEALTHY AND SEPTICEMIC NEONATAL FOALS. M. Henry Barton,<sup>1</sup> D. Hurley,<sup>1</sup> N. Norton,<sup>1</sup> G. Heusner,<sup>1</sup> L. Costa,<sup>2</sup> S. Jones,<sup>3</sup> D. Byars,<sup>4</sup> and K. Watanabe.<sup>5</sup> University of Georgia, Athens, GA,<sup>1</sup> Louisiana State University, Baton Rouge, LA,<sup>2</sup> North Carolina State University, Raleigh, NC,<sup>3</sup> Hagyard-Davidson-McGee Associates, Lexington, KY,<sup>4</sup> and Kitasato University, Towada, Japan.<sup>5</sup>

The strong association between increased risk for septicemia and mortality in foals with inadequate serum IgG concentrations has focused the veterinary literature solely on the role of IgG in neonatal infection. However, evidence in other species suggests that additional proteins in colostrum may also be important in innate immunity. Lactoferrin is a glycoprotein in the transferrin family that is found in colostrum, milk, and neutrophils. It exerts antimicrobial and anti-inflammatory actions by several mechanisms. The purposes of this study were to establish the normal serum concentration of lactoferrin in healthy foals before and after ingestion of colostrum, to determine serum IgG and lactoferrin concentrations in sick neonatal foals, and to determine if serum lactoferrin concentration is associated with sepsis or mortality.

Foals (n=16) that were at least 330 gestational days and with a normal physical examination served as healthy controls. Blood was collected from the healthy foals within 30 minutes of birth and between 1 to 3 days of age and from foals  $\leq$  4 days of age (n=111) that were ill for any reason and referred for further evaluation. Serum was stored frozen at -70C until tested for IgG and lactoferrin concentrations by RID and ELISA, respectively. Equine lactoferrin, purified from seminal plasma, served as the standard for the lactoferrin assay and for production of antisera in rabbits. The limit of detection of the ELISA is 1 ng lactoferrin/ml with a 5% and 6% intra-assay and inter-assay coefficient of variation, respectively. Clinical records of the referred foals were reviewed and the sepsis score, blood culture results, and survival to discharge were categorically recorded. Data were evaluated by use of an ANOVA with significance set at  $P < 0.05$ .

Compared to values obtained at 30 minutes of age ( $18 \pm 2$  mg/dl, SEM), the mean serum value of IgG was significantly greater in healthy foals after ingestion of colostrum ( $2,921 \pm 245$ ). Likewise, compared to values obtained before ingestion of colostrum ( $249 \pm 39$  ng/ml), serum lactoferrin concentration significantly increased in



healthy foals 1 to 3 days of age ( $445 \pm 63$ ). In healthy foals, there was a significant correlation between IgG and lactoferrin concentration ( $P=0.002$ ,  $r^2=.28$ ). The serum IgG concentration was significantly less in referred foals, compared to healthy foals. For referred foals, serum IgG was significantly less in foals with a positive sepsis score, as well as those that did not survive. There was no difference in lactoferrin concentration between referred and healthy foals. For referred foals, there was no significant association between the serum lactoferrin concentration and the sepsis score, blood culture, or survival. Although both serum IgG and lactoferrin concentrations increase in healthy foals after ingestion of colostrum, only serum IgG is significantly correlated with the sepsis score and outcome.

#### **ABSTRACT # 107**

ALPHA-MELANOCYTE STIMULATING HORMONE RELEASE IN RESPONSE TO THYROTROPIN RELEASING HORMONE ADMINISTRATION IN HEALTHY HORSES. Dianne McFarlane, Alastair Cribb. Atlantic Veterinary College, Charlottetown, PEI, Canada.

Thyrotropin releasing hormone (TRH) administered to horses with pituitary pars intermedia dysfunction (PPID) results in an increase in serum cortisol concentration of greater than 50% for 15 – 90 minutes. Normal horses do not show a change in cortisol concentration following TRH. While this phenomenon has proven useful in the diagnosis of PPID, the physiological basis of this response is unknown. A more complete understanding of the physiological regulation of the pars intermedia (PI) may provide insight into the dysregulation observed in PPID. We hypothesized that TRH is a physiological releasing factor of the PI. To test this hypothesis, we compared alpha-melanocyte stimulating hormone ( $\alpha$ -MSH) concentrations in plasma collected from healthy horses without evidence of PPID ( $n=10$ ) before and 30 minutes after TRH administration. Plasma ACTH concentration was also measured in 5 horses. Both hormones were measured by radioimmunoassay. Controls included 5 horses that received saline only.

There was a significant increase in plasma  $\alpha$ -MSH following TRH in all horses, with a median increase of 409 % and a range of 155-5829% ( $p<0.001$ , Kruskal-Wallis Test). There was no significant difference in plasma ACTH concentration following TRH ( $p= 0.55$ ). There was no difference in  $\alpha$ - MSH concentration before or after saline administration.

Using  $\alpha$ -MSH as a marker of PI response, and ACTH as a marker of pars distalis response, we demonstrated TRH stimulates the PI in healthy horses. This is the first evidence that TRH is a releasing factor of the equine PI. ACTH is also a minor product of the equine PI, however we did not detect an increase in ACTH following TRH. This is consistent with the finding that serum cortisol does not increase following TRH administration in normal horses. In horses with PPID, the PI is hyperplastic and produces large amounts of POMC-derived peptides, including  $\alpha$ - MSH and ACTH. When horses with PPID are given TRH, the presence of excessive numbers of TRH-responsive melanotropes may release sufficient ACTH to evoke a measurable cortisol release from the adrenal glands.

#### **ABSTRACT #108**

EFFECTS OF ORAL LEVOTHYROXINE SODIUM ON SERUM CONCENTRATIONS OF THYROID HORMONES AND THYROID STIMULATING HORMONE (TSH) IN MARES. Carla Somnardahl<sup>1</sup>, Nicholas Frank<sup>1</sup>, Sarah Elliot<sup>1</sup>, Latisha Webb<sup>1</sup>, Don Thompson<sup>2</sup>, Kent Refsal<sup>3</sup>, Joe Denhart<sup>4</sup>. <sup>1</sup>University of Tennessee College of Veterinary Medicine, Knoxville, TN; <sup>2</sup>Louisiana State University, Baton Rouge, LA; <sup>3</sup>Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI; <sup>4</sup>Lloyd Inc., Shenandoah, IA.

Synthetic thyroid hormone (levothyroxine sodium) therapy is often initiated when clinical signs commonly attributed to hypothyroidism are recognized, or when lower than normal serum total triiodothyronine (tT3) and total thyroxine (tT4) concentrations are detected. The purpose of this study was to investigate the effects of levothyroxine therapy on body weight (BW), physical parameters, serum concentrations of thyroid stimulating hormone (TSH) and thyroid hormones; and on the hormonal responses to thyrotropin-releasing hormone (TRH) injection. Levothyroxine sodium (Thyro-L®) was administered orally to 8 mares for 8 weeks according to an incrementally increasing dosing regimen of 2, 4, 6, or 8 teaspoons (tsp)/d, with each dose administered for 2 weeks (1 tsp = 12 mg levothyroxine). Four additional mares were given a placebo and served as controls. Physical parameters including rectal temperature, heart rate, and respiratory rate were measured weekly. Blood samples were collected for serum thyroid hormone measurements and intravenous TRH challenges were performed at the beginning of the study and at 2 wk intervals afterwards. Hormonal responses to intravenous administration of 1.2 mg TRH were examined by comparing pre-injection values with serum tT<sub>3</sub> and fT<sub>3</sub> concentrations at 2h post-injection, tT<sub>4</sub> and fT<sub>4</sub> concentrations at 4h post-injection, and TSH concentrations at 45min and 2h post-injection. Mean  $\pm$  SD body weight decreased ( $P < 0.01$ ) by  $19 \pm 12$  kg over 8 weeks in treated mares and these horses appeared to be more excitable during the 2 weeks when 8 tsp/d was administered, but measured physical parameters were not altered by levothyroxine therapy. Significant ( $P < 0.01$ ) treatment x time effects were detected by repeated measures analysis of variance for serum tT<sub>3</sub>, tT<sub>4</sub>, fT<sub>3</sub>, fT<sub>4</sub>, and TSH concentrations. In treated mares, serum tT<sub>3</sub> ( $r = 0.33$ ;  $P = 0.04$ ), tT<sub>4</sub> ( $r = 0.95$ ;  $P < 0.01$ ), fT<sub>3</sub> ( $r = 0.68$ ;  $P < 0.01$ ), and fT<sub>4</sub> ( $r = 0.76$ ;  $P < 0.01$ ) concentrations were positively correlated with the dose of levothyroxine sodium administered. Serum TSH ( $r = -0.44$ ;  $P < 0.01$ ) concentrations were negatively correlated with dose. Hormonal responses to TRH injection were significantly altered by levothyroxine sodium administration. Significant ( $P < 0.01$ ) treatment x time effects were detected for the tT<sub>3</sub>, tT<sub>4</sub>, fT<sub>3</sub>, and TSH responses examined and the magnitudes of these responses were negatively correlated with dose in treated horses. Results of this study show that levothyroxine sodium significantly reduces body weight, increases serum thyroid hormone concentrations proportional to the dose administered, lowers serum TSH concentrations, and blunts hormonal responses to intravenous TRH administration.

#### **ABSTRACT #109**

EFFECTS OF ORAL LEVOTHYROXINE ON GLUCOSE DYNAMICS IN MARES. Nicholas Frank<sup>1</sup>, Carla Somnardahl<sup>1</sup>, Ray Boston<sup>2</sup>, Hugo Eiler<sup>1</sup>, Latisha Webb<sup>1</sup>, Sarah Elliott<sup>1</sup>, Joe Denhart<sup>3</sup>. <sup>1</sup>University of Tennessee College of Veterinary Medicine, Knoxville, TN; <sup>2</sup>University of Pennsylvania, Kennett Square, PA; <sup>3</sup>Lloyd Inc., Shenandoah, IA.

Intravenous glucose-insulin tolerance tests (IVGITT) were performed to test the hypothesis that levothyroxine sodium administration alters glucose metabolism in horses. Eight mares received levothyroxine (Thyro-L®, Lloyd, Inc., Shenandoah, IA) orally for 8 weeks according to an incrementally increasing dosing regimen of 2, 4, 6, or 8 teaspoons (tsp)/d, with each dose administered for 2 weeks (1 tsp = 12 mg levothyroxine). Four additional mares were given a placebo and served as controls. Glucose dynamics were assessed by IVGITT at the beginning and end of the 8-week period. Mares were acclimatized to stall confinement for 12 hours prior to testing and provided with grass hay and water before and during the test. Testing was performed by infusing 150 mg/kg glucose as a 50% dextrose solution, immediately followed by 0.1 units/kg regular insulin. Blood samples were collected at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135, and 150 minutes post-injection. Blood glucose and insulin concentrations

were measured and data from each IVGITT were analyzed using the minimal model to generate values for glucose effectiveness, insulin sensitivity (SI), and net insulin response (NIR). Insulin disposal rates (IDR) were also calculated by linear regression analysis of insulin concentrations after conversion to natural logarithmic values. Normality was assessed using the Shapiro-Wilk statistic and effects of time were analyzed independently for each treatment group using Wilcoxon signed rank tests. Means  $\pm$  standard deviations are reported. Data were successfully modeled for 3 of 4 mares in the placebo group and all treated mares. Body weight decreased ( $P < 0.01$ ) by  $19 \pm 12$  kg over 8 weeks in treated mares. Insulin sensitivity increased ( $P < 0.01$ ) by greater than 2-fold in levothyroxine-treated mares (from  $1.20 \pm 0.67 \times 10^{-4}$  L·mU<sup>-1</sup>·min<sup>-1</sup> at week 0 to  $2.61 \pm 1.04 \times 10^{-4}$  L·mU<sup>-1</sup>·min<sup>-1</sup> at week 8). In contrast, SI did not differ significantly between weeks 0 and 8 in the placebo group ( $4.34 \pm 6.92$  and  $2.26 \pm 0.22 \times 10^{-4}$  L·mU<sup>-1</sup>·min<sup>-1</sup> respectively). Glucose effectiveness did not differ between weeks 0 and 8 in the levothyroxine ( $4.39 \pm 3.73$  and  $2.34 \pm 0.21 \times 10^{-2}$  min<sup>-1</sup> respectively) or placebo ( $1.73 \pm 1.00$  and  $2.23 \pm 0.47 \times 10^{-2}$  min<sup>-1</sup> respectively) groups. Net insulin response also did not differ between weeks 0 and 8 in the levothyroxine ( $124.88 \pm 15.57$  and  $116.20 \pm 49.06$  mU·min·L<sup>-1</sup> respectively) or placebo ( $120.76 \pm 40.73$  and  $143.21 \pm 46.45$  mU·min·L<sup>-1</sup> respectively) groups. Insulin disposal rate increased ( $P = 0.01$ ) in treated mares. Blood insulin concentrations decreased at a rate of  $9.3 \pm 1.3$  %/min at week 8, compared with  $7.2 \pm 1.1$  %/min at week 0. Placebo group IDR values did not differ with time. Results show that levothyroxine administration increases insulin sensitivity and accelerates insulin disposal. Effects of levothyroxine cannot be distinguished from those of weight loss in this study, but results suggest that this drug may be useful for the treatment of insulin resistance in horses.

#### ABSTRACT #110

CORRELATION BETWEEN PLASMA ALPHA-MELANOCYTE STIMULATING HORMONE CONCENTRATION AND BODY MASS INDEX IN HEALTHY HORSES. M.T. Donaldson, D. McFarlane\*, A. Jorgensen, J. Beech. School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA and \*Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island.

We investigated the relationship between  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) and body condition in horses. In other species, activation of the melanocortin-4 receptor (MC4R) is necessary for maintaining normal body weight. Therefore, one would expect that defects in this receptor would result in obesity and elevated levels of its primary agonist,  $\alpha$ -MSH, in an effort to maintain homeostasis. We postulated that horses are similar and that variability in the function of the MC4R receptor could lead to alterations in body mass index (BMI) and plasma  $\alpha$ -MSH concentration. Specifically, we hypothesized there would be a positive correlation between plasma  $\alpha$ -MSH concentration and BMI. The correlation between body condition score (BCS) and BMI was evaluated because BMI has not been previously used as an index of obesity in horses.

The sample population was comprised of 82 healthy horses examined during routine preventative health care examinations in the equine ambulatory service of the School of Veterinary Medicine, University of Pennsylvania. The median age was 10 years (range 1-23 years). There were 43 geldings, 37 mares and 2 intact males. Breeds represented included Thoroughbred (n = 35), Quarter Horse (16), European Warmblood (8), Arabian (6), Standardbred (4), Cleveland Bay (2), Rocky Mountain Horse (2), Tennessee Walking Horse (2) and a variety of other breeds (7). Samples were collected from January to May 2003 and were collected from 0900 to 1700 hours. Plasma  $\alpha$ -MSH concentration was determined by RIA. At the time blood was collected BCS was determined, and girth circumference, body length and height were measured. Weight was

estimated by the formula weight (kg) = [girth (cm)<sup>2</sup> x length (cm)] / 11877. Body mass index was calculated as estimated weight (kg) / height (m)<sup>2</sup>.

Body mass index and BCS were positively correlated (Spearman  $r = 0.60$ , 95% CI 0.44 to 0.73,  $P < .001$ ). Plasma  $\alpha$ -MSH concentration was positively correlated with BMI (Spearman  $r = 0.25$ , 95% CI 0.03 to 0.45,  $P = .02$ ) and BCS (Spearman  $r = 0.26$ , 95% CI 0.04 to 0.46,  $P = .02$ ). Plasma  $\alpha$ -MSH concentration and BMI were positively correlated in horses 10 years old and older ( $r = 0.49$ , 95% CI 0.20 to 0.69,  $P = .001$ ) but not in horses less than 10 years old (Spearman  $r = -0.04$ ,  $P = .80$ ). Plasma  $\alpha$ -MSH concentration in horses with a BCS of 7, 8 or 9 ( $12.5 \pm 16.4$  pmol/L) was significantly greater than in horses with a BCS of 2, 3 or 4 ( $6.6 \pm 2.2$  pmol/L,  $P = .02$ ) The positive correlation between plasma  $\alpha$ -MSH concentration and clinical indices of obesity is consistent with metabolic syndromes in people due to MC4R defects.

#### ABSTRACT #111

VARIATION IN PLASMA ACTH CONCENTRATION AND DEXAMETHASONE SUPPRESSION TEST RESULTS IN ASSOCIATION WITH SEASON, AGE AND SEX IN HEALTHY PONIES AND HORSES. M.T. Donaldson<sup>1</sup>, S.M. McDonnell<sup>1</sup>, B.J. Schanbacher<sup>2</sup>, S.V. Lamb<sup>2</sup>, D. McFarlane<sup>3</sup>, J. Beech<sup>1</sup>. <sup>1</sup>School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; <sup>2</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY; <sup>3</sup>Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island.

The purpose of this study was to evaluate the variation in plasma ACTH concentration and dexamethasone suppression test (DST) results associated with season, age and sex in healthy Shetland type pony mares (n = 15) and stallions (n = 14) living under semi-feral conditions and horse mares (n = 10) living in a pasture. Plasma ACTH concentrations were measured in September 2002, and in January, May and September 2003. DSTs were performed in January and September 2003. Plasma ACTH concentration of all subjects was compared at each season with the Friedman nonparametric repeated measures test. Plasma ACTH concentration in pony mares, pony stallions and horses was compared using the Kruskal-Wallis ANOVA. The Spearman correlation was used to evaluate the association between age and cortisol or plasma ACTH concentration. Summary statistics are presented as median and range.

Plasma ACTH concentrations in September 2002 (53.3 pg/ml, 25.6 - 479.0) and September 2003 (52.1 pg/ml, 25.6 - 192.0) were significantly greater than in January (17.0 pg/ml, 8.1 - 36.9) and May (18.7 pg/ml, 12.0 - 33.1,  $P < .001$ ). Plasma ACTH concentration was within the reference range for 38 of 39 (97%) subjects in January, for 39 of 39 (100%) subjects in May 2003, and for 2 of 39 (5%) subjects in both September 2002 and September 2003. Dexamethasone suppression test results were normal in January for all subjects and were normal for 29 of 39 (74%) subjects in September 2003. In January, age and plasma cortisol concentration were positively correlated at the end of the DST ( $r = 0.42$ , 95% CI 0.11 to 0.65,  $P = .008$ ) but not at the beginning of the DST ( $r = 0.30$ , 95% CI -0.02 to 0.57,  $P = .06$ ). In September, age and plasma cortisol concentration were positively correlated at the beginning ( $r = 0.32$ , 95% CI 0.01 to 0.58,  $P = .05$ ) and at the end of the DST ( $r = 0.43$ , 95% CI 0.12 to 0.66,  $P = .006$ ). Age and ACTH concentration were positively correlated in January ( $r = 0.35$ , 95% CI 0.03 to 0.60,  $P = .03$ ), September 2002 ( $r = 0.43$ , 95% CI 0.12 to 0.66,  $P = .007$ ) and September 2003 ( $r = 0.35$ , 95% CI 0.03 to 0.61,  $P = .03$ ) but not in May ( $P = .15$ ). Within season, plasma ACTH concentrations of pony mares, pony stallions and horse mares were not significantly different ( $P > .05$ ).

In summary, clinically important seasonal differences in plasma ACTH concentrations and DST results were present. Plasma ACTH concentration and DST results were similar in pony stallions, pony

mares and horses. Seasonal variations in endocrine function test results should be considered when testing horses and ponies for pituitary pars intermedia dysfunction.

#### **ABSTRACT #112**

COMBINED INTRAVENOUS INSULIN AND GLUCOSE TEST: A METHOD FOR PHYSIOLOGICAL ASSESSMENT OF GLUCOSE HOMEOSTASIS IN THE HORSE. Hugo Eiler, (1) Nicholas Frank, (2) Frank M. Andrews, (2) Jack W. Oliver, (1) and Kellie A. Fecteau (1). From the (1) Department of Comparative Medicine, and the (2) Department of Large Animal Clinical Sciences, The University of Tennessee, College of Veterinary Medicine, Knoxville, Tennessee.

The objective of this research was to characterize the glycemia response to simultaneous administration of glucose and insulin for the purpose of developing a combined glucose-insulin test for comprehensive assessment of glucose homeostasis in a single and simple procedure. Six healthy horses were given a combined glucose-insulin test (150 mg/kg + 0.10 U/kg, IV, respectively), and results were compared to both the singular intravenous glucose tolerance test (150 mg/kg) and the singular intravenous insulin sensitivity test (Humulin-R 0.10 U/kg). Blood samples (14) were collected (jugular catheter) within 150 min. Selected horses suffering from pars intermedia pituitary adenoma (endocrine condition), urolithiasis (non-endocrine condition), and treated with xylazine (inhibitor of insulin secretion) were tested along with healthy horses. As expected, in either the glucose tolerance test or insulin sensitivity test, a single phase curve resulted: a positive hyperglycemic phase or a negative hypoglycemic phase, respectively. In contrast, in the combined glucose-insulin test, glycemia resulted in a clearly biphasic curve: a positive or hyperglycemic phase (peak 250% baseline, at 1 minute and returned to baseline by 30 min) followed by a negative or hypoglycemic phase (the nadir was approximately 50% baseline at 40 minutes and returned to baseline by 140 min). Disease and experimental disruption of the glucose homeostasis loop resulted in either absence (blunting) of negative phase or a shift to the left (increased insulin sensitivity). Physical stress caused an insulin resistance profile (sustained positive phase). The combined test provided at least three consistent and temporal correlated elements for the evaluation of the glucose homeostasis loop: baseline value, positive phase profile, and negative phase profile. The combined test has the potential to identify and perhaps assess relative degree of insulin-secretion or insulin sensitivity failure. The result is promising and warrants further clinical research.

#### **ABSTRACT #113**

INGESTION OF HIGH-GLYCEMIC MEALS AFTER EXERCISE INCREASES GLUCOSE FLUX BUT FAILS TO ENHANCE MUSCLE GLYCOGEN SYNTHESIS IN HORSES. Eduard Jose-Cunilleras, Kenneth W. Hinchcliff, Veronique A. Lacombe, Richard A. Sams, Catherine W. Kohn, Lynn E. Taylor and Steven T. Devor. The Ohio State University, Dept. Veterinary Clinical Sciences and Section of Sport and Exercise Science Program, Columbus, OH, and Otterbein College, Dept. Equine Science, Westerville, OH.

After an athletic event horses require substrate for muscle glycogen synthesis. Rates of muscle glycogen synthesis depend on glucose availability. The present study was undertaken to determine the effect of withholding feed and of ingestion of isocaloric meals of hay or grain on glucose turnover rates and muscle glycogen synthesis after exercise.

In each trial, horses underwent 3 consecutive days of strenuous exercise designed to deplete muscle glycogen by ~66%. In the 3<sup>rd</sup> day during the 8 h following exercise, in a randomized cross-over design, each of 6 horses were either not fed (NF), fed half of the daily energy requirements (~15 Mcal DE) as mixed alfalfa and grass hay (H), or

fed an isocaloric amount of corn (C) immediately and 4 h after exercise. Horses received a primed constant infusion of deuterated glucose before, during, and for 8h after the third bout of exercise to allow estimation of rates of plasma glucose appearance (index of gluconeogenesis, hepatic glycolysis, and gastrointestinal absorption of glucose) and rates of plasma glucose disappearance (index of glucose disposal by tissues). Blood and muscle biopsy samples were obtained intermittently for 8 h for determination of plasma glucose, serum immunoreactive insulin and deuterated glucose, and muscle glycogen concentrations. Statistical analysis was performed by 2-way ANOVA with repeated measures.

Plasma glucose concentrations at 2-8 h following exercise were higher in horses fed C, when compared with H or NF (4.7±0.2, 5.1±0.2, 5.7±0.2 mmols/L for NF, H and C, respectively,  $P<0.01$ ). Serum immunoreactive insulin concentrations at 2-6 h after exercise were higher in horses fed C, when compared with H or NF (5.4±0.32, 7.5±0.8, 11.1±1.3  $\mu$ IU/ml for NF, H and C, respectively,  $P<0.05$ ). The rates of plasma glucose appearance and disappearance from blood at 1-8 h after exercise were ~2 and ~3 fold greater in horses fed H and C, respectively, when compared with NF (5.2±0.5, 11.0±0.8, 15.4±1.4  $\mu$ mol/kg/min for NF, H and C, respectively,  $P=0.05$ ). However, despite a 3-fold greater glucose availability to muscle in corn fed horses when compared to withholding feed, muscle glycogen concentrations immediately after and 8 h after exercise were not different amongst the 3 interventions (501±32, 558±36, 563±53 before exercise, 171±19, 205±37, 170±56 immediately after exercise and 225±34, 233±27, 247±23 mmol/kg dw by 8 h after exercise for NF, H and C, respectively,  $P=0.6$ ). In conclusion, this study suggests that in horses, unlike that observed in humans and rodents, increasing glucose availability to skeletal muscle by feeding high-glycemic meals after exercise does not hasten muscle glycogen synthesis during the first 8 h after exercise. Increased glucose availability may instead be preferentially directed towards hepatic glycogen synthesis and/or increased metabolic rate after exercise.

#### **ABSTRACT #114**

ASSESSMENT OF GLOMERULAR FILTRATION RATE USING PLASMA EXOGENOUS CREATININE CLEARANCE TEST: PRELIMINARY RESULTS IN A HEALTHY CANINE POPULATION. Lefebvre HP\*, Jeunesse E\*, Concordet D\*, Ferre P\*, De La Farge F\*\*, Laroute V\*, Giraudel J\*, Watson ADJ# \*UMR 181 Physiopathologie et Toxicologie Expérimentales INRA-ENVT, National Veterinary School, \*\* Biochimie IV, Rangueil Hospital, Toulouse, France # Veterinary Medicine, The University of Sydney, Australia.

Plasma exogenous creatinine clearance test has been recently proposed to assess glomerular filtration rate (GFR) and validated against classical methods in healthy and renal-impaired Beagles (Watson et al., J Vet Intern Med, 2002, 16:22). The aim of this study was to test the practicability of this new approach and to evaluate the variability of GFR in a large cohort of healthy dogs.

113 female pure-breed dogs were involved. The animals were 4.4 +/- 3.6 years old (range: 0.5-14 y) and weighed 21 +/- 14.6 kg (range: 5.2-62.1 kg). All animals were considered to have normal renal function based upon physical examination and plasma biochemistry. Plasma creatinine and urea values in all dogs were within the reference ranges. Exogenous creatinine solution (80 mg/mL) was administered at the nominal dose of 40 mg/kg by i.v. bolus. 1-mL blood samples were collected just before administration, and at 5 et 10 min, 1, 2, 3.5, 6 and 8 h after administration. Plasma creatinine was assayed using an enzymatic method. Plasma creatinine clearance (Cl) was determined using a non compartmental approach. Steady state volume of distribution (Vss) of creatinine and half-life of elimination (t1/2) were also calculated. The daily endogenous production (Q) of creatinine was determined by the product of Cl and

the area under the basal plasma concentration over 24 h. Effect of age and body weight (BW) was analyzed by a general linear model.

The procedure was well tolerated in all animals. Few animals showed some mild local skin inflammation due to hair clipping and/or repeated blood sampling. The mean +/- SD values for CI (i.e. GFR), Vss, t1/2 and Q were 3.0 +/- 0.68 mL/kg/min, 575 +/- 79.2 mL/kg, 3 +/- 0.5 h and 36 +/- 6.8 mg/kg/d. A significant effect (P<0.001) of BW was observed on CI, Vss, and t1/2. Higher values of CI, Vss and lower values of t1/2 were observed in dogs with lower body weight. A significant effect of age (P<0.001) was observed on Vss and Q, lower values of these variables being observed in older dogs.

In conclusion, this study demonstrates that the test can be easily managed in field conditions and offers a practical alternative to assess GFR for prospective studies in large cohort of dogs.

#### ABSTRACT #115

THE AFFECTS OF FLUIDS AND DIURETIC THERAPIES ON GLOMERULAR FILTRATION RATE, RENAL BLOOD FLOW AND URINE OUTPUT IN HEALTHY CATS. JR McCabe, RE Goldstein<sup>1</sup>, LD Cowgill<sup>2</sup> and HN Erb<sup>1</sup>. 1. Cornell University, Ithaca, NY 2. University of California, Davis, CA.

Acute renal failure and acute deterioration in the renal function of cats with chronic renal disease (CRD) are widely treated with fluids and diuretics. The goals of this treatment are to increase glomerular filtration rate (GFR), renal blood flow (RBF) and urine output (UO). Although various diuretics are commonly added to fluid therapy for these purposes their efficacy has not been assessed in cats. The goals of this study were to assess GFR, RBF and UO in healthy awake cats receiving maintenance fluids, high rate IV fluids, or IV fluids and different diuretic therapeutics.

Eight healthy cats were randomly assigned to 4 treatment (Tx) crossover groups: 1-Maintenance fluids (3mls/kg/hr); 2-High rate fluid therapy (9mls/kg/hr); 3-High rate fluid therapy and mannitol (0.5g/kg IV bolus then 1mg/kg/min constant rate infusion (CRI)); and 4-High rate fluid therapy, dopamine CRI (2.5µg/kg/min) and furosemide CRI (0.25mg/kg/hr). Each study period lasted 8 hours. All cats were awake during the study. There was at least 1 week washout period between studies. GFR (ml/kg/min) was calculated using both m99Tc-DTPA nuclear scintigraphy and renal inulin clearance. RBF (ml/kg/min) was calculated using renal clearance of para-amino hippuric acid. UO (ml/kg/hr) was measured as the rate of urine production during the study period. Non-parametric statistical analysis was performed. GFR, RBF and UO were compared between the 4 treatment groups using Friedman's test. Individual groups were compared using Wilcoxon's signed rank test. Significance was set at p<0.05. All results are reported as mean ± SD.

Tx group	1	2	3	4	p value
GFR-DTPA	2.97 ± 0.89	3.27 ± 0.99	3.37 ± 0.86	2.07 ± 1.14	0.01
GFR-inulin	2.94 ± 0.95	3.52 ± 1.29	3.64 ± 1.07	2.58 ± 1.03	0.09
RBF	8.47 ± 2.55	10.77 ± 3.64	11.64 ± 3.14	9.45 ± 4.27	0.05
Urine output	0.80 ± 0.08	2.42 ± 0.46	2.88 ± 0.27	6.42 ± 0.73	0.001

Statistical differences existed for 3 of 4 parameters when comparing all 4 groups. For paired group comparisons: group 2 had higher UO than group 1 (p=0.01); group 3 was not different than group 2 in any parameter; group 4 had lower GFR-DTPA (p=0.02) and higher UO (p=0.01) than group 2; group 4 had a lower GFR-DTPA (p=0.01), GFR-inulin (p=0.01), and RBF (p=0.04), and higher UO (p=0.01) than group 3. In healthy cats furosemide and dopamine induced the largest amount of urine production but did not cause an increase in GFR or RBF. This therapy caused a decrease in GFR when compared to fluid therapy alone and a decrease in GFR and RBF when compared to fluids and mannitol. Additional studies are required to assess these parameters in cats with CRD. In healthy cats it appears that the renal affects of mannitol are superior to those of furosemide and dopamine together when used as an adjunct to fluid diuretic therapy.

#### ABSTRACT #116

COMPLEMENT C3 AND ANTIGEN-ANTIBODY LEVELS IN DOGS WITH PROTEIN-LOSING NEPHROPATHIES. Acierno MJ, Stern L, Labato MA, Mukherjee J, Jakowski R, Ross L. Tufts University School of Veterinary Medicine, North Grafton MA.

The purpose of this study was to determine complement C3 and immune complex levels in the serum of dogs with protein-losing nephropathies, compare these levels with normal dogs, and determine whether these hematological parameters could be utilized as prognostic indicators. Thirty healthy dogs were identified and served as controls. Blood was collected from each dog and the serum was separated and frozen. Thirty-two dogs with naturally occurring protein-losing nephropathies (PLN) were identified as they presented to Tufts Foster Hospital for Small Animals between July 1, 2002 and July 1, 2003. Blood was collected from each patient and the serum was separated and frozen. The clinical course of the patients was followed from the time they were identified until December 1, 2003. Renal histopathology was performed on six PLN patients and each was consistent with glomerulonephritis.

Sandwich ELISAs were developed for the detection of C3 and immune complex (IC) levels. Serum was serially diluted 1:2 and the C3 and IC levels (titer) were defined as the greatest dilution at which the absorbance at 490 nm was greater than or equal to twice the background absorbance reading. The inverse of the log<sub>10</sub> of the titer was calculated and used for statistical analysis. No statistical difference in complement C3 level was detected between the healthy (6.5±0.0409) and PLN dogs (6.3±0.0797) (p<0.4355). Dogs with PLN did have a statistically significant (p<0.001) elevation in IC levels (3.86±0.0783 vs. 3.14±0.0502). In order to determine if IC level was prognostic, the PLN group was divided into two subgroups based on the mean IC level for all PLN dogs. There were 15 PLN patients with above average IC levels and 14 with lower than average IC levels. Survival curves for the two sub groups were plotted. While PLN dogs with a lower than average IC level had a longer median survival (78 days) than those with higher than average IC levels (5.5 days), the difference was not statistically significant (p<0.3189) due to the size of the sub-populations. Therefore, a larger study is necessary to evaluate the relationship between IC level and survival in PLN dogs.

#### ABSTRACT #117

USE OF HEMODIALYSIS IN UREMIC AND NON-UREMIC DOGS WITH ETHYLENE GLYCOL TOXICITY. CE Rollings, T Francey, LD Cowgill. School of Veterinary Medicine, University of California, Davis, CA.

Ethylene glycol (EG) toxicity is an important cause of acute renal failure in dogs and carries a poor prognosis in patients that progress to oligo-anuria. Hemodialysis (HD) is very effective at removing EG and its metabolites and for the management of the resulting uremia, but its use in dogs has not been well documented in this setting. We analyzed presenting biochemical variables and EG and glycolic acid (GA) concentrations, as well as urea, EG, and GA reduction ratios (URR, EGRR, and GARR, respectively) and outcome in dogs with positive serum [EG] that underwent HD. Dogs were divided into azotemic (A) and non-azotemic (NA) groupings for comparison. All data are reported as medians (25-75%).

Records from 26 dogs meeting the above criteria were available for evaluation. BUN, creatinine, and phosphorus in A dogs were 85 mg/dl (56-113), 6.6 mg/dl (4.1-10.0), and 10.5 mg/dl (8.3-14.4), respectively. These values were normal in all NA dogs. All NA and 20% of A dogs achieved non-dialysis-dependent survival. No dog in the NA group became azotemic on follow-up. Of the dogs that died from EG toxicity, median survival was 1 month (0.2-2.6) during which time all remained dialysis-dependent. A comparison between A and NA dogs follows:

Parameter	Non Azotemic (n=6)	P	Azotemic (n=20)
<b>Serum Chemistries</b>			
Total Calcium (mg/dl)	9.6 (8.2-11.0)	0.46	9.2 (8.3-9.6)
Ionized Calcium (mmol/L)	1.29 (1.28-1.30)	<0.01	0.94 (0.82-1.08)
Potassium (mmol/L)	4.1 (3.9-4.2)	0.43	4.2 (3.8-4.6)
Bicarbonate (mmol/L)	19.5 (13.8-22.3)	0.16	13.0 (10.0-17.2)
pH	7.40 (7.39-7.41)	0.02	7.29 (7.22-7.32)
Plasma osmolality (mosm/L)	445 (317-498)	0.08	364 (333-394)
Osmolal gap	10 (9-70)	0.44	30 (18-44)
Anion gap	20 (19-22)	<0.01	34 (31-43)
<b>Toxin Removal</b>			
Presenting [EG] (ppm)	2560 (190-6250)	0.01	658 (72-1019)
Post-dialysis [EG] (ppm)	12 (6-27)	0.78	41 (19-76)
Presenting [GA] (ppm)	640 (325-690)	0.56	640 (158-980)
Post-dialysis [GA] (ppm)	0 (0-0)	0.23	23 (0-58.8)
URR	0.79 (0.75-0.86)	0.96	0.87 (0.73-0.94)
EGR	0.97 (0.96-0.99)	0.17	0.93 (0.79-0.97)
GARR	>0.99 (>0.71->0.99)	NA	0.96 (0.92->0.98)

HD is very effective at removing both EG and GA. URR is a good predictor of GA and EG removal. Prompt dialytic therapy (pre-azotemia) can be curative for EG toxicity. HD can be used in more advanced cases to manage uremia while waiting for possible return of kidney function.

### ABSTRACT #118

RETROSPECTIVE, ACUTE RENAL FAILURE IN CATS: 25 CASES (1997-2002). Worwag S and Langston C, Animal Medical Center, New York, NY.

Background: Acute renal failure is caused by a variety of disorders, including toxic, ischemic, and infectious etiologies. The mortality rate for acute renal failure (ARF) is high, with estimates of 50% in people, despite the ready availability of dialytic therapies, and almost 60% mortality in dogs. Information on cats with acute renal failure is limited to small case series of cats with ARF from specific etiologies. The purpose of this study is to better characterize clinicopathologic factors and outcome of cats with ARF of a variety of causes.

Methods: Medical records were reviewed for a diagnosis of acute renal failure in cats that presented to the nephrology and critical care service at the Animal Medical Center from 1997 to 2003. Criteria for ARF were defined as: 1) clinical signs for  $\leq 7$  days, 2) azotemia (BUN  $\geq 35$ mg/dl, creatinine (SCr)  $\geq 2.4$ mg/dl) in conjunction with a urine specific gravity of  $\leq 1.025$  or anuria, 3) normal to large kidneys on palpation or imaging, and 4) no evidence of chronic renal disease. Cases with renoliths, ureteroliths, and post renal failure, or exclusively pre renal failure cases were excluded.

Results: Twenty-five cats with acute renal failure were identified. Twenty cats were domestic short hair breed, 2 domestic long hair cats and one of each of the following breeds: Siamese, Himalayan, and Abyssinian. There were 15 castrated males, 3 intact males, 6 spayed females and one female cat of unknown reproductive status. Fourteen cats (56%) were either euthanized (9 cats) or died (5 cats). Eleven cats (48%) left the hospital of which 6 cats had normal renal function at discharge and 5 cats had chronic renal disease. Two major categories for the etiology of acute renal failure were established. Twelve cats (44%) had a nephrotoxic cause of which only 3 cats lived; 13 cats (52%) had "other" etiologies that include ischemic causes (4 cats), known or suspected infectious causes, or undetermined causes. Logistic regression analysis was used to examine if initial SCr, BUN and potassium levels, or duration of stay were associated with outcome. Urine production and etiology were analyzed by Fisher's exact test. The initial SCr, BUN and potassium values have no statistical significance in association with outcome. Eighteen cats (72%) were either anuric or oliguric during the initial phase of the renal failure. Five cats (20%) were nonoliguric and 2 cats (8%) were polyuric. All nonoliguric and polyuric cats survived. All anuric or oliguric cats that also had a nephrotoxic etiology did not survive (p=0.041). Eight cats (32%) received hemodialysis treatments during their hospital stay. Five of these cats died, one was euthanized and 2 left the hospital with chronic renal disease.

Conclusion: ARF is a severe disease with high mortality. Despite this, longterm survival is possible. Severity of azotemia does not predict outcome. All nonoliguria and polyuric cats did survive.

Nephrotoxicity and anuria by itself was not fatal in this study, but both of them together were.

### ABSTRACT #119

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL LIGHT MICROSCOPIC FEATURES OF CHRONIC FELINE IDIOPATHIC CYSTITIS. A Specht<sup>1</sup>, JM Kruger<sup>1</sup>, SD Fitzgerald<sup>2</sup>, M Kiupel<sup>2</sup>, J Hauptman<sup>1</sup>. Small Animal Clinical Sciences<sup>1</sup> and Pathobiology and Diagnostic Investigation<sup>2</sup>, Michigan State University, East Lansing, MI.

Identification of etiopathologic factors associated with feline idiopathic cystitis (FIC) has been hindered by lack of comprehensive histopathologic descriptions of urinary bladder lesions in affected cats. In a previous study, hematoxylin and eosin stained urinary bladder biopsy specimens from cats with chronic FIC showed mucosal erosions/ulcerations, and submucosal edema, fibrosis, hemorrhage, neovascularization, mastocytosis, and mononuclear cell infiltration. These changes were significantly different from those observed in specimens from clinically normal cats, but not different from those observed in specimens from cats with urolithiasis. The purpose of this study was to utilize specialized histochemical and immunohistochemical stains to further characterize the inflammatory (lymphocyte subpopulations and/or mast cells), connective tissue (neovascularization and/or fibrosis), and uroepithelial changes associated with chronic FIC. Eighty urinary bladder biopsy specimens from 40 cats with chronic FIC, 15 cats with urolithiasis, and 25 clinically normal cats, submitted to Michigan State University, were stained immunohistochemically with markers for T (CD3) and B (CD79a) lymphocytes, mast cells (c-kit), neovascularization (Factor VIII-related antigen), and uroplakin (uroplakin III), and histochemically for collagen (trichrome) and glycosaminoglycans (GAG; alcian blue), and examined by light microscopy. The degree of positive staining was scored using a standardized scale of 0 to 3.

	T Cells	B Cells	Mast cell	Collagen	Neovasc.	GAG	Uroplakin
FIC	98 / 1.5	83 / 0.8	98 / 1.5	100 / 2.2	100 / 2.9	100 / 2.4	97 / 1.2
Uroliths	92 / 1.3	92 / 0.9	92 / 1.8	100 / 2.0	100 / 2.7	100 / 1.8	75 / 0.7
Normal	0 / 0	8 / 0.04	48 / 0.3	100 / 1.0	100 / 2.1	92 / 1.5	100 / 1.6

Urinary bladder lesions observed in cats with chronic FIC and urolithiasis were significantly different from those observed in normal cats with respect to the frequency and degree of lymphocyte infiltration, degree of mast cell infiltration, fibrosis, neovascularization, and uroplakin staining (p < 0.004). However, the frequency of and degree of lymphocyte and mast cell infiltration and fibrosis and neovascularization were not different between the chronic FIC and urolithiasis groups. The degree of Alcian blue (GAG) staining was significantly greater in cats with chronic FIC compared to normal cats or cats with urolithiasis (p < 0.003). The degree of staining for urothelial apical membrane protein uroplakin III was significantly less in cats with chronic FIC compared to normal cats (p < 0.0001), but not significantly greater compared to cats with urolithiasis. Morphologic changes observed in urinary bladder biopsy specimens from cats with chronic FIC warrant further investigation.

### ABSTRACT #120

USE OF GLUCAGON IN THE MANAGEMENT OF ACUTE URETERAL OBSTRUCTION IN 25 CATS. MA Forman, T Francey, JR Fischer, and LD Cowgill, School of Veterinary Medicine, University of California, Davis, CA.

Acute ureteral obstruction (AUO) has emerged as a common cause of acute uremia in cats. Intravenous glucagon has been proposed to decrease ureteral peristalsis and promote passage of ureteroliths in humans. The aim of this retrospective study was to evaluate glucagon use in the management of AUO in cats.

Cats treated with glucagon as part of medical or surgical therapies for AUO between February 2002 and January 2004 were evaluated and assigned to 3 groups: GR1, glucagon alone; GR2, glucagon and diuretics initiated simultaneously; GR3, glucagon initiated following pre-treatment with diuretics. Short-term effects were assessed by changes in chemistries in non-dialysis treated cats (n=8) and urine output (UOP)(n=11). Long-term effects were assessed by the need for surgery and survival. All data are presented as median (range).

Twenty-five cats (1 M, 11 MC, 13 FS), age 6 (2-16) years were evaluated. Palpable renal pain or asymmetry was noted in 13 (52%) and 18 (72%) cats, respectively. AUO was bilateral in 6 cats (24%) and unilateral in 19 (76%). AUO was caused by ureteroliths in 18 cats (72%), stricture in 2 (8%) and unknown causes in 5 (20%). A total of 113 glucagon administrations, 4 (1-12) per cat, were given within the initial 72 hours. Twenty-two cats received an initial intravenous dose of 0.1mg per cat and 3 cats received 0.05mg per cat.

	GR1 (n=5)	GR2 (n=7)	GR3 (n=13)
# of glucagon tx	18	30	65
UOP (ml/kg/hr) 4hr pre	0.94+	0 (0-3.21)†	0.19 (0-7.56)‡
4hr post	2.26	0 (0-4.48)	1 (0-11.25)
BUN (mg/dl) pre	30-54*	47-198*	58 (28-186)^
post	17-54	24-210	82 (29-185)
CREA (mg/dl) pre	1.6-2.7*	4.2-20.2*	6.3 (1.9-16.8)^
post	1.2-2.7	2-21	8.4 (2.4-18.4)
Surgery (%)	60	29	85
Survival (%)	100	71	69

Number cats per observation: +, 1; \*, 2; †, 3; ^, 4; ‡, 7

Following treatment, 8 anuric cats urinated (32%) (GR1: 1, GR2: 4, GR3: 3) and 4 cats (GR1: 1, GR2: 1, GR3: 2) demonstrated movement of ureteroliths based on ultrasound. Associated adverse events were vomiting/diarrhea, tachypnea, and dyspnea (2 cats each).

On the basis of these data, glucagon therapy as administered, may promote urination in individual cats; however short and long-term benefits were not demonstrated. As clinically significant side effects may result, additional studies will be necessary to justify the routine use of glucagon in cats with AUO.

#### ABSTRACT #121

EFFECT OF DIETARY MODIFICATION ON HEALTH-RELATED QUALITY OF LIFE (HRQL) IN DOGS WITH SPONTANEOUS CHRONIC RENAL FAILURE (CRF). F. Jacob, C. Osborne, D. Polzin, J. Neaton, C. Kirk, T. Allen, L. Swanson. Small Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

We reported that a therapeutic renal failure diet (RF) reduced risks for uremic crises, slowed renal failure progression, and prolonged survival in dogs with CRF. Although prolonging the quantity of life supports the recommendation for initiating appropriate diet therapy, impact of dietary therapy on patient's HRQL must be considered. As part of a double-masked, randomized, controlled clinical trial on diets and CRF, we compared the effect of RF versus an adult maintenance diet (MF) on HRQL of CRF dogs.

Thirty-eight dogs with similar clinical, biochemical and hematological characteristics were randomly assigned to RF (n= 21) or MF (n= 17) groups and evaluated for 24 months. Using a content-validated questionnaire, effect of diets on owner's assessment of HRQL was compared. We also assessed nutritional status, measured by serial evaluations of body weights, body condition scores (BCS), hematocrits and serum albumin concentrations, as an indirect measure of HRQL.

Good HRQL was sustained in dogs fed RF, while HRQL declined in dogs fed MF (P<0.05). Hematocrits were maintained in the RF group but significantly decreased in the MF group. Body weights, BCS, and serum albumin concentrations remained stable in both groups during the course of the study.

The renal failure diet used in this study was superior to a maintenance diet in sustaining HRQL in dogs with spontaneous CRF. Nutritional status of dogs in the RF group remained stable as

evidenced by stable body weights, BCS, hematocrits, and serum albumin concentrations.

#### ABSTRACT #122

ASSOCIATION OF INITIAL PROTEINURIA WITH MORBIDITY AND MORTALITY IN DOGS WITH SPONTANEOUS CHRONIC RENAL FAILURE. F. Jacob, D. Polzin, C. Osborne, J. Neaton, C. Kirk, T. Allen, L. Swanson. Small Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

A cohort study was designed to test the hypothesis that initial urine protein/creatinine (UP/C) ratio  $\geq 1.0$  at the time of initial diagnosis of chronic renal failure (CRF) in dogs was a risk factor associated with increased risk of uremic crises, mortality, and decline in renal function.

Forty-five dogs were prospectively divided into 2 groups on the basis of initial UP/C ratio measurements (UP/C < 1, n = 20; and UP/C  $\geq 1$ , n = 25). Kaplan-Meier and Cox's proportional hazards methods were used to estimate the association of the magnitude of proteinuria with uremic morbidity and mortality. Changes in reciprocal of serum creatinine concentration were used to estimate the decline in renal function.

At the initial visit, dogs had similar clinical, hematological, and biochemical characteristics with exception of systolic blood pressures and UP/C ratios. The relative risk for uremic crises and mortality was approximately 3 times higher in dogs with UP/C  $\geq 1.0$  compared to dogs with UP/C ratio < 1.0. In addition, the risk of adverse outcomes was approximately 1.5 times greater for every 1-unit increment in UP/C ratio. Decline in renal function of a greater magnitude was observed in dogs with higher UP/C ratios.

Higher initial UP/C ratios in dogs with CRF were associated with an increased risk of uremic morbidity, and mortality. Therefore, initial measurements of UP/C ratios in dogs with spontaneous CRF may be of value in refining prognoses.

#### ABSTRACT #123

PREDICTORS OF SURVIVAL IN HEALTHY, NON-AZOTAEMIC CATS. D Walker, HM Syme, P Markwell, J Elliott. Royal Veterinary College, London, UK.

Proteinuria is an independent predictor of survival in humans and cats with naturally occurring renal disease. Additionally, proteinuria and particularly albuminuria may herald incipient nephropathy before development of azotaemia in humans. The aim of this study was to determine if measurements of protein excretion would predict survival of clinically healthy cats.

The study was conducted in a retrospective manner, analyzing samples collected from apparently healthy cats as part of a routine monitoring program.

To be considered healthy the cats had to have been normal on physical examination, have systolic blood pressure (SBP)<175 mmHg, and have no significant abnormalities on plasma biochemical analysis. Hyperthyroid cats were excluded from the study. Total protein and albumin were measured in feline urine samples and indexed to urine creatinine concentrations to yield urine protein-to-creatinine (UPC) and albumin-to-creatinine (UAC) ratios respectively. Albumin quantification was by species-specific ELISA method as described previously. UPC and UAC values were log transformed for statistical analysis. Survival analysis (time to death due to any cause) was performed by Cox's regression. Cases were censored if they were alive at the conclusion of the study or lost to follow up (LTFU). Potential predictive variables that were investigated were SBP, age, urine specific gravity (USG) plasma creatinine concentration and either log UPC or log UAC (in separate models due to co-linearity of the variables). Data are reported as median [25<sup>th</sup>, 75<sup>th</sup> percentile].

Time until death was 357 [280, 730] days (n=15) and length of follow-up for cats that were censored was 507 [112, 801] days (alive

n= 36, LTFU n= 10). UPC was 0.30 [0.26, 0.37] in the cats that died, compared with 0.11 [0.16, 0.21] in the cats that were censored. UAC was 50 [21, 95] mg/g in the cats that died compared with 17 [7, 33] mg/g in the cats that were censored. SBP, USG and plasma creatinine were not predictive of survival and so were excluded from the final analyses. In the first model logUPC was inversely associated with survival (p=0.017), and age approached, but did not reach, statistical significance (p=0.064). With UAC in the model both age (p=0.033) and log UAC (p=0.007) were inversely associated with survival.

Proteinuria is associated with reduced survival times in non-azotaemic cats. Further studies are now warranted to determine whether interventions that decrease proteinuria, such as treatment with ACE-inhibitors, will improve long-term survival.

#### ABSTRACT #124

THE PREVALENCE OF MICROALBUMINURIA IN DOGS AND CATS IN AN INTENSIVE CARE UNIT. CA Turman,<sup>1</sup> SL Vaden,<sup>1</sup> TL Harris,<sup>1</sup> WA Jensen. <sup>1</sup>NCSU- CVM, Raleigh, NC, and <sup>2</sup>Heska Corporation, Fort Collins, CO.

Microalbuminuria (MA) is an early predictor of nephropathy. Transient MA occurs in people with acute inflammatory conditions, lasting only 1-48 hours. It is believed to be due to changes in systemic vascular permeability associated with the acute inflammatory response. The degree of MA appears to be proportional to the severity of the condition. The purpose of this study was to evaluate the prevalence, duration and causes of MA in dogs and cats admitted to an intensive care unit (ICU).

Urine samples were collected from dogs and cats within 24 hours of admission to ICU over a 10-week period. Animals with gross hematuria or known urinary tract infections were excluded. Samples were tested for increased albumin concentrations using a point-of-care immunoassay (E.R.D.-HealthScreen™ Test®, Heska Corporation). Urine albumin concentrations were quantified using an ELISA in those samples that were positive via the point-of-care test. When possible, subsequent urine samples were obtained from those animals that had increased urine albumin concentrations in the first urine sample. Final diagnoses were recorded for all animals.

Samples were collected from 107 dogs admitted to ICU. MA was detected in 65 (61%) of these dogs: 4 (100%) with infectious/inflammatory diseases, 3 (100%) with trauma, 5 (83%) with cardiac disease, 7 (78%) with neoplasia, 5 (71%) with neurological disease, 2 (67%) with gastrointestinal disease, 38 (54%) admitted for post-operative care and 1 (50%) with metabolic disease. Subsequent samples were obtained from 26 dogs 48 +/- 26 hrs later. Of these, 20 (77%) had decreases in the urine albumin concentrations when compared with the first sample; 5 (19%) were negative for urine albumin. 13 of the 14 dogs (93%) that were euthanized or died within 3 days of admission to ICU had MA.

Samples were collected from 23 cats admitted to ICU. MA was detected in 16 (70%) of these cats: 5 (100%) with renal failure, 2 (100%) with metabolic disease, 1 (100%) with neurological disease, 2 (67%) with infectious/inflammatory diseases, 2 (67%) with cardiac disease, 3 (50%) admitted for post-operative care and 1 (33%) with neoplasia. Subsequent samples were obtained from 6 cats 34 +/- 15 hours later. Of these, 4 (67%) had a decreases in the urine albumin concentrations when compared with the first sample; 1 (17%) was negative for urine albumin. 3 of the 4 cats (75%) that were euthanized or died within 3 days of admission to ICU had MA.

The prevalence of MA in dogs and cats admitted to ICU is higher than previously reported in hospitalized populations and appears to vary with different classifications of disease. Transient MA occurred in some patients. A large percentage of patients being euthanized or dying had MA suggesting, that as in people, the presence of MA may be a negative prognostic indicator.

#### ABSTRACT #125

APPLICATION OF HEMODIALYSIS FOR THE MANAGEMENT OF ACUTE UREMIA IN CATS: 119 CASES (1993-2003). V. Pantaleo, T. Francey, J.R. Fischer, L.D. Cowgill. School of Veterinary Medicine, University of California, Davis, CA.

Hemodialysis (HD) has been applied in the management of acute uremia (AU) in cats for a decade, but there has been little documentation of this therapeutic modality. We retrospectively evaluated the case records of all cats diagnosed with AU that were treated with HD between January 1993 and December 2003. The diagnosis of AU and differentiation of underlying etiologies (see table) was based on conventional clinical, laboratory, and imaging criteria and histopathology. All HD treatments were performed using conventional methods. For outcome analysis cats were considered survivors if they were alive at least 10 days post discharge. Data are presented as median [range]. A total of 532 HD treatments were delivered to 119 cats (3 treatments per cat, range 1-36). Gender distribution was MC = 54%, FS = 41%, M = 3%, F = 2%. Blood pressure at the initial HD treatment was 142 mmHg [74-250], systolic; 87 mmHg [36-193], diastolic; and 107 mmHg [27-198], mean. The BUN and the serum creatinine concentrations for all cats at presentation were 222 mg/dl [57-456] and 16.8 mg/dl [3.6-41.2], respectively.

Evaluations based on etiology are presented below:

Etiology	n (%)	BUN (mg/dl)	Creatinine** (mg/dl)	Systolic BP (mmHg)	HD Tx** n	Survival** n(%)
Obstructive	44 (37%)	237 [68-456]	17.2 [8.4-34.4]	140 [86-220]	3 [1-8]	33 (75%)
Toxic	23 (19%)	207 [69-400]	16.2 [4.9-41.2]	151 [90-191]	4 [1-36]	8 (35%)
Infectious	7 (6%)	252 [150-309]	21.1 [13.8-24.2]	143 [86-168]	4 [2-16]	4 (58%)
Metabolic / Hemodynamic	7 (6%)	144 [57-228]	10.4 [3.6-14.9]	125 [70-146]	2 [1-4]	5 (72%)
Neoplastic	4 (3%)	256 [96-366]	19.0 [6.3-20.7]	144 [120-160]	3 [2-4]	2 (50%)
Unknown	34 (28%)	234 [116-341]	16.0 [8.4-26.2]	141 [85-250]	3 [1-17]	10 (29%)

\*\* , P<0.05 for comparison between etiologic groups (ANOVA, Chi-square).

At presentation, 52% of the cats were normotensive, 40% hypertensive and 8% hypotensive. No significant difference were observed in BUN and creatinine between survivors (222 [57-456] and 16.9 [3.6-34.4]) and non survivors (225 [68-408] and 16.6 [3.8-41.2]) with P= 0.8 and P=0.9, respectively. Overall, 62 cats (52%) survived, including 3 cats with renal transplant, 31 (26 %) were euthanized, and 26 (22%) died. Adjunctive HD in cats with severe AU is associated with higher survival than would be predicted for conventional management. However survivability is highly contingent on underlying etiology.

#### ABSTRACT #126

HYPERTENSION IN DOGS WITH SEVERE ACUTE RENAL FAILURE. T Francey, LD Cowgill. Dept of Medicine & Epidemiology, University of California, Davis, CA.

Hypertension (HT) is a common and potentially severe complication of acute renal failure (ARF), but its occurrence in dogs has received only limited review. We analyzed the influence of etiology, hydration, and hemodialysis (HD) on the prevalence of HT in dogs with severe ARF.

All dogs diagnosed with severe ARF requiring HD between 1990-2003 were included if initial blood pressure (BP) was measured before the use of vasoactive drugs or HD. BP was measured indirectly using oscillometric or Doppler methods. Diagnosis and etiology of ARF was based on conventional criteria and dogs were classified in 4 groups: leptospirosis (L), ethylene glycol toxicosis (EG), hemodynamic/metabolic nephrosis (H/M), and other causes (O). HT was defined as systolic BP (SBP) >150 mmHg or diastolic BP (DBP) >95 mmHg. Hydration was assessed using clinical signs and changes in body weight. Changes in blood volume during HD

were measured with an in-line blood volume monitor (Critline®). Data are reported as median [25-75%].

Of 153 dogs treated for severe ARF, 54 satisfied the selection criteria. The etiology groups consisted of L: 18 dogs (33%), EG: 11 (20%), H/M: 7 (13%), and O: 18 (33%). At presentation, 7 dogs (13%) were dehydrated, 12 (22%) were normal, and 35 (65%) were overhydrated. 29 dogs (31%) were oligo-anuric and 25 (69%) were non-oliguric. SBP of all dogs was 172 mmHg [156-198] and DBP was 115 [103-127], with no difference between etiologies ( $P=0.6$ ). Of all dogs, 78% had systolic HT, 84% had diastolic HT, and 87% had either systolic or diastolic HT. Prevalence of HT (systolic or diastolic) was 78% in L, 100% in EG, 86% in H/M, and 89% in O ( $P=0.4$ ). SBP was 166 mmHg [129-180] in dehydrated dogs, 170 [154-191] in normal dogs, and 178 [163-199] in overhydrated dogs ( $P=0.3$ ), with corresponding prevalences of HT at 71%, 83%, and 91%, respectively ( $P=0.3$ ). SBP was 170 mmHg [160-198] in oligo-anuric dogs and 178 [150-198] in non-oliguric dogs ( $P=0.7$ ), with prevalences of HT at 90% and 84%, respectively ( $P=0.9$ ). The first HD treatment significantly decreased SBP from 165 mmHg [154-181] to 149 [129-166] ( $P<0.01$ ) and DBP from 111 [93-127] to 100 [82-116] ( $P=0.03$ ). This decrease was not correlated with changes in body weight (-1.7% BW [-4.4 to 0]) or blood volume (-7.5% [-10.0 to -1.2]) caused by HD. BP and clinical data for dogs with and without HT are shown in the table below:

	Hypertensive (n=47)	Non-Hypertensive (n=7)	P
SBP (mmHg)	178 [165-200]	135 [119-141]	<0.01
DBP (mmHg)	116 [107-127]	92 [87-95]	<0.01
Hydration (% BW)	+9 [0 to +11]	+3 [-2 to +5]	0.06
Survival (n [%])	21 [45%]	5 [71%]	0.19

HT is a common feature of dogs with severe ARF. It is not significantly related to etiology, hydration or urine output, and does not alter survival. HD effectively decreases BP by mechanisms other than normalization of hydration.

#### ABSTRACT #127

COMPARISON OF CONVENTIONAL URINE PROTEIN TEST STRIP METHOD AND A QUANTITATIVE ELISA FOR THE DETECTION OF CANINE AND FELINE ALBUMINURIA. G.F. Grauer,<sup>1</sup> L.E. Moore,<sup>1</sup> A.R. Smith,<sup>1</sup> W.A. Jensen.<sup>2</sup> College of Veterinary Medicine, Kansas State University, Manhattan, KS<sup>1</sup> and Heska Corporation, Ft. Collins, CO.<sup>2</sup>

Previous work assessing the prevalence of microalbuminuria in dogs with a canine albumin specific ELISA suggested there is a high rate of false positive results associated with conventional urine protein test strip methodology (JVIM 15:300, 2001). The purpose of this study was to assess the sensitivity and specificity of the urine protein test strip method compared with a quantitative ELISA for detection of albuminuria in canine and feline urine samples.

As part of a larger longitudinal study assessing the prevalence of microalbuminuria in healthy dogs and cats, 185 urine samples from 79 dogs and 113 urine samples from 49 cats were analyzed by conventional urine protein test strip method (Multistix® Reagent Strips, Bayer Corporation, Elkhart, IN) and a canine or feline albumin specific quantitative ELISA (Heska Corporation, Ft. Collins, CO). A complete urinalysis was also performed on all samples. Urine samples were collected by free-catch voiding, urethral catheterization, or cystocentesis. Initially, urine samples were not excluded on the basis of urine pH or sediment findings. To account for varying urine concentrations, urine samples were normalized to a urine specific gravity of 1.010 prior to the ELISA. Canine and feline urine containing less than 1.0 mg/dl canine and feline albumin, respectively were considered negative for albuminuria by ELISA. Urine protein test strip results greater than or equal to a trace reaction were considered positive.

Sensitivity for the conventional urine protein test strip for albuminuria in canine and feline urine was 54% and 60%,

respectively. Urine protein test strip specificity for canine and feline albuminuria was 69% and 31%, respectively. If urine samples with an alkaline pH ( $\geq 7.5$ ) and/or hematuria ( $\geq 10$  RBC/hpf), pyuria ( $\geq 5$  WBC/hpf), or bacteriuria were excluded, urine protein test strip specificity for canine and feline albuminuria increased to 84% and 55%, respectively.

These data demonstrate that conventional urine protein test strips have a high percentage of false negative and false positive results for detection of albuminuria in canine and feline urine when compared with an ELISA. False positive results on the urine protein test strip are more common with feline urine compared with canine urine. Urine protein test strip false positive results in both species can be decreased by excluding alkaline urine and urine with hematuria, pyuria, and/or bacteriuria from analysis.

#### ABSTRACT #128

SAFETY AND EFFICACY OF EMLA (LIDOCAINE/PRILOCAINE) TOPICAL ANESTHETIC CREAM FOR PLACEMENT OF JUGULAR CATHETERS IN HOSPITALIZED CATS. KA Wagner, KJ Gibbon, LA Trepanier. University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI.

In human pediatric patients, the use of a topical anesthetic cream containing a mixture of 2.5% lidocaine and 2.5% prilocaine (EMLA cream®) has been shown to reduce pain and discomfort associated with procedures such as venipuncture, catheter placement, and skin biopsies. Our recent study showed that EMLA cream, applied as 1 gram to a 10 cm<sup>2</sup> area of skin, is without systemic absorption or adverse effects in healthy cats (Gibbon et al., J Vet Pharm Ther, 2003). The purpose of the present study was to evaluate, in hospitalized sick cats, the safety and efficacy of EMLA cream in decreasing signs of discomfort and facilitating the placement of jugular catheters without sedation. 25 hospitalized cats, with a variety of diseases and requiring jugular catheter placement, have been enrolled to date. Cats were scored for severity of illness and temperament, and then randomly assigned to receive either placebo vehicle or EMLA cream (1 gram to a 2 x 5 cm area of skin overlying one jugular vein). After one hour of occlusion, the cream was removed and a single lumen 19 g., 8 inch jugular catheter was placed on the treated side. The safety of EMLA cream was assessed by observing for evidence of local skin irritation; cardiopulmonary, neurologic, or GI side effects; or detectable plasma lidocaine and prilocaine levels up to 6 hours after cream application. Cats were scored for struggling, aggression, or vocalization (each on a discomfort scale of 0-3) during catheter venipuncture. If individual scores of 2 or higher were obtained, routine sedation was administered to complete catheter placement. Ten cats have received EMLA cream, and 15 cats placebo. There have been no signs of adverse effects in either group. The mean discomfort score for EMLA cats was 2.9, and the mean score for placebo cats was 4.1. 60% of cats receiving EMLA, and 40% of cats receiving placebo, had jugular catheters placed without sedation. Neither of these differences reached statistical significance in this sample size. We conclude from the initial results of this study that EMLA is safe to use as described in ill cats, and that EMLA may provide local analgesia that is sufficient to allow catheter placement in some cats without sedation. However, other factors, such as fear, fractious temperament, and resistance to restraint, may override the benefit of local analgesia in allowing catheter placement without sedation.



### ABSTRACT #129

PLASMA AND INTERSTITIAL FLUID PHARMACOKINETICS OF FLUOROQUINOLONES AFTER ORAL ADMINISTRATION AND A CONSTANT RATE INTRAVENOUS INFUSION IN DOGS. Tara L. Bidgood, Mark, G. Papich, North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina.

Because most bacterial infections are extracellular and antimicrobial pharmacokinetic studies are usually limited to examining the plasma or serum concentrations, this study was undertaken to investigate the concentrations in extracellular tissue fluid (interstitial fluid (ISF)) using an ultrafiltration device for ISF collection in the dog. Enrofloxacin (Baytril) and marbofloxacin (Zeniquin) are fluoroquinolones registered for use in dogs and cats in the US. Tissue distribution of fluoroquinolones in dogs has been determined in studies using tissue biopsies and tissue cages. Both of these techniques are invasive and they have not determined the protein-unbound drug concentrations in the ISF. Only the protein-unbound fraction of antimicrobials is microbiologically active. Our goal also was to determine the influence of lipophilicity and protein binding on the distribution of fluoroquinolones into tissue ISF.

Enrofloxacin and marbofloxacin were administered to six healthy dogs in separate crossover experiments as a single oral dose (5 mg/kg) and as a constant rate IV infusion (CRI) (1.24 mg/kg/h and 0.12 mg/kg/h, respectively) following a loading dose (4.47 mg/kg and 2 mg/kg, respectively) to achieve a steady state concentration of approximately 1 µg/mL for 8 hours. The ISF was collected with an ultrafiltration device simultaneously with plasma to assess the dynamics of drug distribution. Plasma and ISF were analyzed for enrofloxacin, its active metabolite ciprofloxacin, and marbofloxacin with a high performance liquid chromatography (HPLC) assay developed in our laboratory. Lipophilicity was determined from the octanol-water partition coefficient. Protein binding was measured using the ultrafiltration device *in vitro*. Lipophilicity and protein binding of enrofloxacin were higher than marbofloxacin and ciprofloxacin. At steady state during the CRI, marbofloxacin and enrofloxacin had similar distribution into tissue fluid (similar ISF and plasma protein-unbound concentrations) despite a higher lipophilicity and volume of distribution of enrofloxacin. Compared to enrofloxacin, marbofloxacin had a longer half-life, higher  $C_{max}$ , and larger AUC in plasma and ISF after the same oral dose.

Our study has provided an understanding of the distribution of fluoroquinolones into the ISF in dogs. Protein binding rather than lipophilicity is the primary determinant of the extent of drug distribution from the capillaries into the ISF. Plasma and ISF concentrations of both drugs after oral dosing (5mg/kg) were above the surrogate markers ( $C_{max}/MIC$  and  $AUC/MIC$ ) recommended for clinical efficacy against susceptible gram-negative bacteria. This study demonstrated that ISF collection could be performed with an *in vivo* ultrafiltration device easily and without discomfort to dogs. This technique offers advantages over tissue cages and tissue biopsies to determine antimicrobial protein-unbound concentrations at the infection site and provides therapeutically valuable antimicrobial drug concentration data.

### ABSTRACT #130

PHARMACOKINETICS OF MORPHINE AND IT ACTIVE METABOLITE MORPHINE-6-GLUCURONIDE FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF MORPHINE TO DOGS. Butch KuKanich, Mark Papich, and Duncan Lascelles, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Morphine is considered the prototypical opiate analgesic in human and veterinary medicine. Morphine primarily interacts with mu opiate receptors, but also exhibits affinity for kappa opiate receptors. Despite its common usage in dogs, very few pharmacokinetic studies have been performed and no pharmacodynamic studies have

established effective dosages or plasma concentrations in dogs. Additionally, morphine-6-glucuronide has shown analgesic activity, but its contribution to the effects of morphine administration in dogs has not been evaluated.

The purpose of the study was to evaluate the pharmacokinetics of morphine administered intravenously and orally to dogs in a randomized crossover design as part of an ongoing pharmacokinetic - pharmacodynamic study. Morphine (0.5 mg/kg) was administered as an i.v. bolus to 6 dogs. Pilot studies determined immediate release morphine tablets could not be administered in high enough doses to achieve plasma concentrations without gastrointestinal adverse effects (primarily vomiting), so morphine extended release tablets were dosed orally to dogs as whole tablets (1.6 ± 0.3 mg/kg). Only 5 dogs were included in the oral study due to repeated vomiting upon administration in 1 of the dogs. Concentrations of morphine were determined in plasma with a high pressure liquid chromatography with coulometric detection assay developed in our laboratory. Intravenous plasma samples were analyzed by compartmental and noncompartmental analysis with commercially available computer software.

Pharmacokinetic analysis of morphine was not possible following oral administration of morphine extended release tablets due to its poor oral bioavailability and inconsistent plasma profile. The oral bioavailability could not be calculated due to low levels achieved and erratic absorption. Morphine-6-glucuronide was not detected in any samples following oral or i.v. administration of morphine with a 10 ng/mL limit of detection. Mean ± SD values for half-life, volume of distribution, and clearance after IV morphine administration were 1.16 ± 0.36 hours, 7.49 ± 1.04 L/kg, and 83.00 ± 33.89 ml/min/kg, respectively. Mean ± SD for time to plasma concentrations dropped below 20 ng/mL, minimum effective concentrations in humans, was 2.06 ± 0.80 hrs. The elimination half-life was markedly shorter and clearance rate higher than previously reported in dogs, primarily due to previous studies examining concentrations far below therapeutic concentrations.

In conclusion morphine cannot be dosed practically to dogs orally, and must be administered parenterally either by a constant rate infusion or every 2-3 hours to maintain expected plasma concentrations consistent with analgesia in dogs. Morphine-6-glucuronide was not detected at any time point and is not expected to contribute to analgesic effects of morphine in dogs.

### ABSTRACT #131

CARDIOVASCULAR AND RESPIRATORY SAFETY OF ALFAXAN®-CD RTU IN CATS PREMEDICATED WITH ACEPROMAZINE, MEDETOMIDINE, MIDAZOLAM OR BUTORPHANOL. Heit M, Schnell M, \*Whittem T and \*Pasloske K. Provident Preclinical Inc., Doylestown, PA; \*Jurox Pty. Ltd., Rutherford, Australia.

Alfaxalone in 2-hydroxypropyl-beta-cyclodextrin (CD) is marketed in Australia as Alfaxan®-CD RTU (A®-CD) for induction and maintenance of anesthesia in dogs and cats. The objective of this study was to determine the effective intravenous (IV) dose of A®-CD in cats treated with one of four premedicants and to investigate cardiovascular and respiratory interactions between A®-CD and the premedicants. Cats were randomly assigned to five treatment groups of six animals (3M/3F) each. Cats were injected intramuscularly with either acepromazine (1.1 mg/kg), medetomidine (100 µg/kg), midazolam (0.1 mg/kg), butorphanol (0.4 mg/kg) or 0.9% NaCl (0.1 mL/kg). Approximately 25 minutes later, A®-CD was infused IV at 5 mg/kg at a rate to deliver the entire dose over one minute. The persons administering A®-CD were masked to treatment group and stopped the infusion when the animal could be intubated. Study parameters included clinical observations, body weight, mortality, physical exam, hematology and serum chemistry, cardiovascular and respiratory parameters (body temperature, pulse rate, heart rhythm,

direct blood pressure, hemoglobin saturation and respiratory rate), anesthetic event times (onset and duration of recumbency, anesthesia, and non-responsiveness to noxious stimuli), overall anesthetic score, and A<sup>®</sup>-CD dose. Means and standard deviations were calculated and compared.

The investigator was masked to group during the study. Group code will be broken at the Forum. All animals remained healthy throughout the study, had stable body weights and normal physical examinations. There were no treatment-related changes in hematology. Average increases in AST (~3X) and CPK (~10X) were observed amongst groups. Animals in the treatment group coded 4 (T4) experienced the largest CPK increase (~22X), whereas the pretreatment that resulted in the longest recumbency (T5), caused the smallest (~4X) increase. A time-dependent decrease in body temperature was observed in all groups. T5 caused an approximate 50% decrease in pulse rate (sometimes with sinus arrhythmia) prior to A<sup>®</sup>-CD which remained stable after induction. There were no clinical differences among groups or compared to baseline with regards to respiratory rate or %SpO<sub>2</sub>. Mean arterial pressure decreased after T4 injection and remained stable after A<sup>®</sup>-CD whereas, in all other groups except T5, pressure was unaffected by pretreatment but decreased after induction. Blood pressure was unaffected in T5. T5 pretreatment resulted in emesis, lateral recumbency and in anesthetic event durations that were ~4X longer than the approximately 15, 25, and 45 min noted for non-responsiveness, anesthesia, and recumbency, respectively, in the other groups. Overall anesthetic score, comprised of increasing quality of induction, anesthesia, and recovery scores, was greatest for T4 and T5 and least for T1. Although quality of induction and anesthesia were comparable among groups, recovery was inferior in T1. The induction dose required ranged from 2.1 to 3.3 mg/kg (T5 and T2 respectively).

#### ABSTRACT #132

ANESTHETIC AND CARDIORESPIRATORY EFFECTS OF THE STEROID ANESTHETIC ALFAXAN<sup>®</sup>-CD RTU IN DOGS. Muir WW, Lerche P, Wiese AJ and Whittem T\*. The Ohio State University, Columbus, OH. \* Jurox, Rutherford, Australia.

Neuroactive steroids produce hypnosis and muscle relaxation by enhancing the inhibitory effect of gamma-aminobutyric acid (GABA) on the GABA<sub>A</sub> receptor. Alfaxalone (Alfaxan<sup>®</sup>-CD RTU, Jurox, Australia) is a steroid hypnotic drug developed for both induction and maintenance of anesthesia in dogs. This study was designed to determine the cardiovascular and respiratory effects of Alfaxan<sup>®</sup>-CD RTU at the planned intravenous (IV) label dose of 2 mg/kg and at 6 mg/kg and 20 mg/kg (1, 3 and 10 times the label dose). Four female and four male dogs were instrumented under propofol-isoflurane anaesthesia on the day before study. A thermidilution catheter in the pulmonary artery was used to monitor core body temperature, central venous pressure, pulmonary artery pressure, and cardiac output. A teflon catheter in the right carotid artery was used for determination of aortic blood pressure and for anaerobically collecting arterial blood samples for pH and blood gas (PO<sub>2</sub>, PCO<sub>2</sub>) analyses. Also measured were; heart rate (beats/min), body temperature (°C), SAP, DAP, MAP (mm Hg), mean pulmonary artery pressure (MPAP) (mm Hg), right atrial pressure (RAP) (mm Hg), cardiac output (mL/kg/min), heart rhythm (lead II), respiratory rate (breaths/min), hemoglobin oxygen saturation (SpO<sub>2</sub> %), mucous membrane color and capillary refill time. On the day of study data were collected at -60, -5 and 1, 5, 10, 15 and 30 minutes and every 10 minutes thereafter until the dog recovered from anesthesia. The three doses of Alfaxan<sup>®</sup>-CD RTU were administered IV, in random order with a minimum washout between dosing of 3 hours from full recovery. The investigators were blind to the dose administered. After administration of Alfaxan<sup>®</sup>-CD RTU all dogs were intubated and allowed to breathe room air. If a dog's arterial PO<sub>2</sub> fell below 60 mm

Hg positive pressure ventilation was initiated with 100% oxygen at 6 breaths per minute until spontaneous breathing produced an arterial PO<sub>2</sub> greater than 80 mm Hg. Statistical analysis included analysis of variance for repeated measures with appropriate post t tests for within and between group comparisons. A P < 0.05 was considered significant. .

The administration of Alfaxan<sup>®</sup>-CD RTU produced excellent and uneventful induction, maintenance and recovery from anesthesia and dose dependent changes in cardiovascular, respiratory, pH and blood gas (PO<sub>2</sub>, PCO<sub>2</sub>) values and anesthetic duration. All cardiopulmonary variables returned to within baseline values by 15 minutes (2 mg/kg) and 30 minutes (6, 20 mg/kg) after drug administration. Respiratory depression and apnea times averaging 1 minute were observed only at the two larger doses. Anesthesia was typified by excellent muscle relaxation and good to excellent analgesia as evidenced by a total loss of muscle tone and a lack of response to both mechanical (toe pinch) and electrical (buccal and mucosal stimulation) noxious stimulation. In conclusion, Alfaxan<sup>®</sup>-CD RTU was safe and produced excellent quality induction, anesthesia and recovery scores at clinical and super-clinical doses.

#### ABSTRACT #133

PHARMACOKINETICS OF GEMCITABINE IN DOGS. Martin-Jimenez, T., Freise, K.J.. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Gemcitabine (dFdC) is a relatively new chemotherapeutic agent in humans and its use in canine oncology is being actively explored with mixed results. The few pharmacokinetic (PK) studies done with dFdC in dogs were as pre-clinical studies in humans. The results from these studies are conflicting, and no studies looked at the kinetic linearity of dFdC or at its PKs during an infusion, the standard dosing protocol in human chemotherapy. Without a full understanding of the plasma PKs and its variability, accurate and standardized dosing of dFdC cannot be undertaken. In order to take the first step towards understanding the PKs of dFdC and its main inactive metabolite, difluorodeoxyuridine (dFdU), we examined the plasma concentrations of these compounds with 3 different doses of dFdC both as a bolus dose and a bolus dose with a constant rate infusion (CRI).

Five intact female dogs in an incomplete block crossover design were administered 3, 10, and 30 mg/kg dFdC as an IV bolus in phase I. In phase II using the same design the dogs were administered 10, 30, and 60 mg/kg doses as an IV bolus with a 30 minute CRI so that plasma concentrations remained constant the entire infusion period. Serial blood samples were collected after each dose and plasma analyzed for dFdC and dFdU by HPLC with UV detection. Non-compartmental analysis of the data was performed to examine both dFdC and dFdU dose proportionality. Compartmental analysis of dFdC data was performed to create predictive models of plasma concentrations. Additionally, the effects of dose normalization by body weight vs. body surface area (BSA) were compared.

In phase I the plasma dFdC area under the concentration-time curve (AUC) normalized by dose (D) was not significantly different across doses (p=0.4122), while a trend in AUC/D differences existed for dFdU (p=0.1136). In phase II the effects of dose on dFdC AUC/D was not significant (p=0.7678) while the effect of dose on dFdU AUC/D was significant (p=0.0205). The dFdU terminal elimination t<sub>1/2</sub> was not significantly different across doses (p=0.4434). In both phases the increased doses cause a numerical decrease in dFdU AUC/D. In phase I a 2-compartment model with 1<sup>st</sup> order elimination was selected to best fit the data for the 3 and 10 mg/kg doses with a terminal elimination t<sub>1/2</sub> of 93 and 133 minutes, respectively, while a 3-compartment model was selected to best fit the data for the 30 mg/kg dose with a terminal elimination t<sub>1/2</sub> of 430 minutes. In phase II a 1-compartment model with 1<sup>st</sup> order elimination was selected to best fit the data for all doses with a t<sub>1/2</sub> of 76 minutes. The differences

in the correlations between clearance vs. weight and clearance vs. BSA were not significant for phase I or II ( $p=0.9442$  and  $0.9760$ , respectively).

The results indicate that the kinetics of dFdC across a moderate dose range are linear, while the metabolism of dFdC to dFdu is likely saturable. A 3-compartment model best describes the dFdC plasma kinetics, but due to assay sensitivity and sampling times a 1- or 2-compartment model may appear more appropriate. Normalization of dFdC dose by BSA is not superior to normalization of dose by body weight.

#### ABSTRACT #134

MANAGEMENT OF PERI-OPERATIVE PAIN ASSOCIATED WITH SOFT TISSUE SURGERY IN DOGS TREATED WITH FIROCOXIB. D. Lever<sup>1</sup>, R. Gogolewski<sup>2</sup>, D. Larsen<sup>3</sup>, P.D. Hanson<sup>3</sup>. <sup>1</sup>Barolin Veterinary Hospital, Bundaberg, Queensland, Australia; <sup>2</sup>Merial Pty Ltd, Parramatta, Australia; <sup>3</sup>Merial Limited, Duluth, GA, USA.

Firocoxib is a highly selective inhibitor of the inducible isoform of cyclooxygenase (COX-2) and is a member of the coxib class of nonsteroidal anti-inflammatory drugs (NSAIDs). In this study the peri-operative analgesic efficacy of firocoxib was evaluated when administered orally once daily at approximately 5 mg/kg for 5 days to dogs undergoing surgery (ovariohysterectomy). The study was a negative control, double-blinded, efficacy study using a randomized block design where blocks were replicates. Twenty, client-owned, female dogs of various breeds, weighing 14 to 74 kg with a mean of 26.8 kg and aged 0.5 to 7 years with a mean of 2.9 years, were included. Only clinically normal dogs were enrolled in the study based on physical examination and haematological and biochemical evaluation prior to the study (Day -3). Replicates of two dogs were formed in order of presentation. Within replicates one dog was randomly allocated to treatment with placebo and the other to treatment with firocoxib. Individuals administering treatment, performing surgery, or making assessments were not aware of the treatment assignments. Treatments were administered approximately 3 hours before surgery on Day 0, and on Days 1, 2, 3 and 4 at approximately 24 hour intervals. Ovariohysterectomies were performed following premedication with acetylpromazine, induction with propofol, and maintenance with isoflurane/oxygen. Surgeries for each replicate were performed by the same surgeon who was also responsible for all post-operative assessments for that replicate. The extent of pain was assessed using a visual analog scale (VAS) once on Day -3 (prior to treatment), at approximately 2 and 4 hours after surgery on Day 0 and on Days 1, 2, 3 and 4 at approximately 3 to 5 hours after treatment. To determine the VAS score a mark was made on a 100-mm scale such that 0 corresponded to no pain and 100 corresponded to the worst pain possible. Clinical signs associated with pain in dogs included restlessness, depression, panting, vocalization, looking or licking at the wound excessively, biting, an anxious appearance, reluctance to move, and inappetence. VAS was analyzed using analysis of variance for a randomized block design at each time point. Statistical testing was considered significant if the  $p$ -value was less than 0.05.

The pre-study VAS scores were not different ( $p=0.2377$ ) but after surgery the VAS scores were lower for the firocoxib-treated group at 2 hours (35.3 vs 55.8;  $p=0.0005$ ) and 4 hours after surgery (31.1 vs 46.1;  $p=0.0027$ ) and also on Day 1 (9.1 vs 15.5;  $p=0.0218$ ). On Days 2, 3 and 4 the mean VAS scores for both groups were  $<4.0$  and the differences between groups were not statistically significant ( $p>0.05$ ).

These results indicate that the degree of pain experienced post-operatively at 2 and 4 hours and 1 day after ovariohysterectomy was significantly reduced ( $p<0.05$ ) in firocoxib-treated dogs compared to vehicle-treated controls. These results demonstrate that firocoxib provides significant peri-operative analgesia.

#### ABSTRACT #135

ENERGY RESTRICTION DURING A WEIGHT LOSS PROGRAM MUST BE STRICTER IN FEMALE THAN IN MALE DOGS. Isabelle Jeusette<sup>1</sup>, Vincent Biourge<sup>2</sup>, Patrick Nguyen<sup>3</sup>, Louis Istasse<sup>1</sup>, Marianne Diez<sup>1</sup>. <sup>1</sup>Animal Nutrition Unit, University of Liège, Belgium <sup>2</sup>Royal Canin, Centre de Recherche, de Aimargues, France <sup>3</sup>National Veterinary School of Nantes, France

Obesity is the most common nutritionally related health problem in companion animals. In veterinary practices, at least 60 % of obese dogs are entire or neutered females. The aim of this study was to assess the effect of sex on energy restriction and rate of weight loss in obese dogs.

Twelve chronically obese ( $> 10$  months) Beagles were included in the study. The group consisted of 3 entire and 3 neutered females as well as of 6 castrated males. Mean ( $\pm$  SEM) ages ( $4.8 \pm 0.3$  and  $4.8 \pm 0.5$  yrs), initial body weights (BW) ( $23.4 \pm 0.3$  and  $20.6 \pm 0.5$  kg), optimal BW ( $15.1 \pm 0.1$  and  $14.1 \pm 0.2$  kg) and excess BW ( $55 \pm 2$  and  $46 \pm 3$  %) were similar within the male and female groups. Over a 1 month baseline period during which the dogs were fed a commercial maintenance diet (Royal Canin Adult Premium Croc, crude protein 24.0 %, fat 16.1 %, 4140 kcal Metabolizable Energy/kg), dogs underwent hormonal and biochemical evaluation in order to rule out any primary hormonal or metabolic disorder. Dogs were then fed a high protein and low starch commercial reducing diet (Obesity Program DP 37, crude protein 34.0 %, crude fat 9.5 %, total dietary fibre 27.0 %, Metabolizable Energy 2800 kcal/kg -as is). As a starting point, dogs were fed the same amounts (by weight) of reducing diet than of the maintenance baseline diet. Those amounts were then progressively reduced to induce a weekly weight loss rate of around 1-2 % based on initial BW. During the weight loss period, BW, food consumption and body condition scores were monitored weekly.

Two significantly different ( $P<0.01$ ) levels of energy restriction – 90 % of the maintenance energy requirement (MER) for optimal BW in males and 79 % MER in females- were necessary to induce weight loss in dogs. To reach target BW in females, energy allowance had even to be gradually decreased to reach 72 % MER. Those levels of energy restriction led to a weekly rate of weight loss of 1.40 and 1.21 % for the male and female groups respectively. Target BW and optimal body condition were reached within 21 to 28 weeks for males and 21 to 32 weeks for females.

Our results indicate that energy restriction could be more severe in female than male dogs to induce and maintain similar rates of weight loss. Energy allowance must be regularly adjusted in females to keep a constant rate of weight loss.

#### ABSTRACT #136

ADIPONECTIN AND LEPTIN PLASMA LEVELS: EARLY MARKERS IN THE TIME COURSE OF OBESITY-ASSOCIATED INSULIN RESISTANCE (IR) IN DOGS. C. Gavet<sup>1</sup>, B. Siliart<sup>1</sup>, H. Shibata<sup>2</sup>, T. Honjoh<sup>2</sup>, M. Saito<sup>3</sup> and P. Nguyen<sup>1</sup>. <sup>1</sup>Nutrition and Endocrinology Unit, Nantes National Veterinary School (Nantes, France), <sup>2</sup>Morinaga Institute of Biological Science (Yokohama, Japan), <sup>3</sup>Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University (Sapporo, Japan).

Obese dogs suffer from metabolic disorders especially insulin resistance, which is responsible for type 2 diabetes. The clinical diagnosis of IR is difficult and it is often made after IR already had deleterious effects. The aim of this study was, in the time course of IR, to identify early markers of loss of insulin sensitivity in order to improve both the diagnosis and prognosis of this syndrome.

Eight beagle dogs were given a high-fat diet at twice the NRC recommended energy allowance for about 18 months. Insulin sensitivity was assessed using the euglycemic hyperinsulinemic clamp technique, prior to the initiation of the weight gain, 10 months and 18 months later. Plasma triglycerids (TG), insulin, adiponectin,

leptin, insulin-like growth factor 1 (IGF1) and cortisol were followed during the progression of obesity. Blood was collected every 2 weeks for parameters assays.

The body weight of dogs was 10.7±0.7, 14.3 ±1.2 and 15.6 ±1.3 (mean ±SEM) respectively at the beginning of the study and 10 and 18 months later. At 10 months, insulin sensitivity did not differ from the first evaluation while it was significantly lower at 18 months (glucose infusion rate: 21.26 ±2.76, 16.93 ±2.12, 14.94 ±1.72 mg/kg/min). Plasma concentrations of studied parameters are shown in the following table:

	T0	T0+5 mo	T0+10 mo	T0+15 mo	T0+18 mo
Adiponectin (ng/ml)	94 ±12	90 ±13	60 ±12*	24 ±8*	17 ±6*
Cortisol (nmol/l)	34 ±3	46 ±7	35 ±3	42 ±4	75 ±10*
IGF1 (ng/ml)	90 ±6	85 ±12	139 ±16*	102 ±12	162 ±19*
Insulin (μU/ml)	20 ±2	20 ±4	19 ±3	28 ±4*	27 ±3*
Leptin (ng/ml)	7.3 ±2.5	23.6 ±6.0*	35.5 ±9.1*	57.6 ±13.6*	56.1 ±10.4*
Triglycerids (mmol/l)	0.42 ±0.04	1.01 ±0.13*	1.21 ±0.17*	0.87 ±0.14*	0.93 ±0.17*

Values with an asterisk (\*) significantly differ from initial (T0) values (paired t-test, p<0.05).

These data show that weight gain is associated with IR (Insulin sensitivity was decreased by 30%). Plasma concentrations of adiponectin, IGF1, leptin and TG were altered before the onset of a significant decrease in insulin sensitivity. Among these parameters, adiponectin (the easiest to assay) and leptin would be the most reliable parameters for the diagnosis of IR in obese dogs.

#### ABSTRACT #137

COMPOSITION OF DRY DOG FOOD AND THE RISK OF GASTRIC DILATATION-VOLVULUS IN HIGH RISK BREEDS. Raghavan M, Glickman NW, Glickman LT. Department of Veterinary Pathobiology, Purdue University, West Lafayette, IN, USA.

A nested case-control study was conducted among dogs from 11 large and giant high risk breeds to identify associations between the composition of dry dog foods and the risk of gastric dilatation-volvulus (GDV). The specific hypotheses tested were that the risk of GDV increases with consumption of dry dog food containing predominantly cereal ingredients such as soy, wheat, or corn, and with consumption of dry food containing calcium-rich ingredients such as lamb or other meat meal, fish meal, poultry by-product meal, and meat & bone meal, among the first four label ingredients. The risk of GDV was also evaluated with respect to preservative types (i.e., antioxidants, antimicrobials, fat emulsifiers, stabilizers) and the method of processing (i.e., extrusion vs. baking).

Cases were 85 dogs that developed GDV among 1,634 dogs participating in a 5-year prospective study while controls were 195 dogs in the same cohort without GDV, that were frequency matched to cases by year of GDV onset. Participation in this case-control study was further restricted to dogs reported by owners to have consumed a single brand and variety of commercial dry dog food. Using the list of ingredients on food labels and published references, dogs were identified as those that consumed dry foods containing cereal ingredients or calcium-rich ingredients (e.g., meat/lamb meal, fish meal, chicken by-product meal, meat and bone meal) among the first four ingredients, and dry foods formulated with or without preservatives. Dogs were also categorized as consuming extruded or baked dry foods.

In a multivariate logistic model, increasing age, a history of GDV in first-degree relative, and having a thin or lean body condition were confirmed as host risk factors for GDV ( $P<0.05$ ). Consumption of dry food containing a calcium-rich meat meal product among the first four ingredients was associated with a significantly decreased risk of GDV (Odds Ratio (OR), 0.38; 95% Confidence Interval (CI), 0.19, 0.79;  $P=0.004$ ), while moistening of dry foods containing the preservative citric acid was associated with a significantly increased risk of GDV (OR, 3.81; CI, 1.55, 9.37;  $P=0.001$ ). The presence of

cereal ingredients such as soy, corn, or wheat among the first four ingredients was not associated with GDV risk (OR, 0.98; CI, 0.54, 1.79;  $P = 0.95$ ). Given the relatively small number of dogs that consumed baked dry foods (nine control dogs and no case dogs) it was not possible to determine if there was an association between the processing method and GDV. These findings suggest that consumption of dry dog foods containing calcium-rich meat meals among the first four ingredients may help prevent GDV in high risk dogs as will not moistening dry dog foods prior to feeding, especially if they contain the preservative citric acid.

#### ABSTRACT #138

OBESITY CONTROL BY FEEDING A DIACYLGLYCEROL -CONTAINING DOG FOOD. T Umeda<sup>1</sup>, J.E. Bauer<sup>2</sup>, K Otsuji<sup>1</sup>. <sup>1</sup>Kao Corporation, Tokyo Japan, <sup>2</sup>Companion Animal Nutrition Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX, USA.

Diacylglycerol (DAG) is a component of vegetable oil present in small amounts in typical dietary oils containing triacylglycerols (TAG) and a digestive intermediate of TAG. It has been shown that DAG has characteristics consistent with less accumulation of body fat through recent clinical experiments in animal models and humans. We have examined its inclusion in dog food as an anti-obesity ingredient. Obesity is a serious problem for both human and canine species, and there is a high demand for "anti-obesity" dog foods. Although "anti-obesity foods are available that are reduced in fat or calories they are often less palatable and less satisfying overall. By contrast, if a weight-reducing dog food were to have intrinsic metabolic characteristics due to help lower canine body weight lower without reducing the amount of dietary fat or calories, it would be expected that to be more useful and beneficial to dogs and their owners. Thus, we examined the potential of dietary DAG as an anti-obesity ingredient in dog food using obese beagles. 32 beagles between 2-6 years old were used for the experiment; 15 females and 17 males. They were divided into two groups so that gender, age, weight, and caloric intakes were similar. 7% of TAG or DAG with similar fatty acid compositions was added to a basal food which contained 9% fat from dry ingredients after extrusion. Amounts of the supplemented diets were calculated and fed for a 6 week period to maintain body weight of each dog. Total caloric intakes did not change throughout the experiment. Body weight was measured every week, and serum chemistry evaluations were done before and after the feeding period. Body fat amount was measured before and after the experiment using a deuterium dilution method. There was no difference in the amount of food intake between 2 groups. Differences in body weight were seen after 2 weeks between the groups which was statistically significant after 6 weeks pass ( $p=0.0152$ , ANOVA). The amount of body fat was decreased in the DAG group. Serum total cholesterol value became significantly lower in the DAG group. And beta-hydroxybutyrate concentrations became significantly increased in the DAG group. Based on the above experiment, it was determined that DAG was effective for weight reduction and body fat content in the obese canine.

#### ABSTRACT #139

GENE THERAPY WITH NON-VIRAL PEPTIDE VECTOR ENCODING CANINE CTLA4IG GENE IN CANINE SYSTEMIC LUPUS ERYTHEMATOSUS MODEL. Eun-Wha Choi (1), Il-Seob Shin (2), Hwa-young Youn (2), Dae-Yong Kim (3), Kyung-Won Seo (2), Hang Lee (4), Young-Jin Chae (5) & Chang-Woo Lee (1). (1) Department of Veterinary Clinical Pathology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea. (2) Department of Veterinary Internal Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea. (3) Department of Veterinary Pathology, College of Veterinary

Medicine, Seoul National University, Seoul 151-742, Korea. (4) Department of Veterinary Biochemistry, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea. (5) Rascience, Ilsan-Gu, Goyang-City, Kyunggi-Do 411-380, Korea.

The purpose of this study is to develop canine systemic lupus erythematosus (SLE) model, to construct new therapeutic CTLA4Ig vector, and to apply it in the treatment of experimentally induced canine SLE.

SLE is a systemic autoimmune syndrome defined by clinical and serological features, including arthritis, glomerulonephritis, dermatitis and autoantibodies. SLE was induced in eight normal dogs by immunization with heparan sulfate, the major glycosaminoglycan of glomerular basement membrane. All the heparan sulfate-immunized dogs showed mild to moderate levels of proteinuria and skin disease. Cutaneous signs associated with SLE including alopecia, erythema, crusting, scaling, and seborrhea were observed. Immunohistological examination of the skin lesions revealed deposition of IgM and complement in dermal-epidermal junction. Three out of eight dogs showed lameness. The anti-nuclear antibody tests were positive with the antibody titers higher than 1: 128 in all eight dogs. This experimental SLE model could be useful for studying immune-mediated skin disease and autoimmunity.

Although viral vectors are commonly used for therapeutic gene delivery, their applications are limited due to their specific cell membrane receptor-mediated infection and host immune response. For therapeutic gene construction, the extracellular domain of canine CTLA-4, and the CH2-CH3 domains of canine immunoglobulin alpha constant region were inserted between the cytomegalovirus promoter and poly-adenylation sequence of bovine growth hormone. The constructed fusion gene was ligated to the non-viral synthetic peptide vector and delivered via vein to dogs with SLE. With this treatment, clinical signs of SLE were reduced dramatically. The anti-nuclear antibody titers and urine protein / creatinine ratios recovered to normal values, and the skin regained its normal histological features. As the peptide vector did not show either tissue specific tropism or host's immune response, CTLA4Ig vector in this study can be applicable to the treatment of systemic autoimmune disease.

#### ABSTRACT #140

IgE REACTIVITY TO VACCINE COMPONENTS IN DOGS THAT DEVELOPED IMMEDIATE-TYPE ALLERGIC REACTIONS AFTER VACCINATION. Keitaro Ohmori<sup>1</sup>, Sadatoshi Maeda<sup>1</sup>, Kenichi Masuda<sup>1</sup>, Koichi Ohno<sup>1</sup>, Yukiko Kaburagi<sup>2</sup>, Keigo Kurata<sup>2</sup>, Douglas J. DeBoer<sup>3</sup>, Masahiro Sakaguchi<sup>2</sup>, Hajime Tsujimoto<sup>1</sup>. <sup>1</sup>Department of Veterinary Internal Medicine, Graduate School of Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan, <sup>2</sup>Department of Immunology, National Institute of Infectious Diseases, Tokyo, Japan, <sup>3</sup>Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Allergic reactions including anaphylaxis after vaccination in dogs are problematic matters in small animal practice. However, little is known about these reactions in terms of the pathogenesis and causative allergens included in vaccines. The purpose of this study is to investigate the relationship between IgE reactivity to the vaccines and immediate-type allergic reactions after vaccination in dogs.

Sera from 10 dogs that developed immediate-type allergic reactions such as circulatory collapse, cyanosis, dyspnea, facial edema and vomiting within 1 hour after vaccination (monovalent live parvovirus vaccine or combined vaccines of canine distemper virus, canine adenovirus type 2, canine parvovirus, canine parainfluenza virus, canine coronavirus and/or leptospira) and sera from 50 dogs that did not develop allergic reactions after vaccination were collected in Japan. Serum IgE reactivity to the injected vaccines was measured by fluorometric ELISA using a mouse monoclonal anti-dog IgE antibody. Then, IgE reactivity to fetal calf serum (FCS) and

stabilizer proteins (gelatin, casein and peptone) included in the vaccines was measured in sera that had specific IgE to the vaccines.

Levels of specific IgE to the vaccines in dogs with the immediate-type allergic reactions (59 to 4173 fluorescence unit [FU], mean  $\pm$  SD:  $992.5 \pm 1181.9$  FU) were significantly higher than those in control dogs (38 to 192 FU, mean  $\pm$  SD:  $92.4 \pm 43.3$  FU) ( $P < 0.001$ ). Of the 8 dogs that developed the immediate-type allergic reactions and had high levels of specific IgE to vaccines, 7 had specific IgE directed to FCS. The IgE reactivity to the vaccines in the sera from these dogs was almost completely inhibited by FCS. The remaining 1 dog had IgE directed to gelatin and casein included as stabilizers in the vaccine.

The results obtained in this study suggested that immediate-type allergic reactions after vaccination in dogs were induced by type I hypersensitivity mediated by IgE directed to vaccine components. In addition, FCS, gelatin and casein included in vaccines could be the causative allergens that induced immediate-type allergic reactions after vaccination in dogs.

#### ABSTRACT #141

INVESTIGATION OF INNATE IMMUNE RESPONSES USING A CANINE MACROPHAGE CELL LINE. B. Catchpole, A. Brooke Houghton, C. Wheeler-Jones, Royal Veterinary College, University of London, UK.

Interest in innate immunity has increased dramatically over recent years with the discovery of the Toll-like receptors (TLR) and the realization that these are crucial for cellular responses against foreign organisms. Furthermore, a similar receptor (NOD2) expressed by macrophages which recognizes bacterial lipopolysaccharide (LPS) has been found to be defective in people suffering from Crohn's disease. It has been proposed that a reduced ability to recognize and control enteric bacteria due to such innate immune deficiency can lead to inflammatory bowel disease. Whether the same is true in dogs remains to be elucidated. The aim of the current study was to develop assays using a canine monocyte/macrophage cell line (DH82) which would allow future investigations into innate immune responses in dogs.

Cultured DH82 cells were exposed to various microbial components including yeast zymosan [TLR-2], bacterial LPS [TLR-4 and NOD2] and bacterial DNA [TLR-9] using PMA & ionomycin as a positive control. Cells were lysed at various times after stimulation, RNA isolated (RNeasy Kit, Qiagen) and cDNA synthesized (M-MLV reverse transcriptase, Promega). The response to stimulation was determined by PCR using canine cytokine-specific primers for IL-1 beta, IL-6 and TNF-alpha with GAPDH used as a housekeeping gene control. An attempt was made to quantify the cytokine mRNA by performing real-time PCR (DyNAmo SYBR Green PCR Kit; Opticon 2 DNA engine, MJ Research).

Cytokine gene transcription in DH82 cells was rapidly upregulated following exposure to LPS, peaking after around 60 minutes and was dose dependent. The response to zymosan and bacterial DNA, but not mammalian DNA, was similar. Real-time PCR was successful for quantifying canine IL-1 beta, IL-6 and TNF-alpha.

Stimulation of DH82 cells with microbial components in vitro is a useful model for studying innate immune reactivity of canine macrophages. In the absence of cytokine ELISAs, real-time quantitative PCR for IL-1 beta, IL-6 and TNF-alpha mRNA is an alternative for assessing canine cytokine responses. Future work will focus on adapting these techniques for use with blood monocytes isolated from dogs with suspected innate immune deficiency.

## ABSTRACT #142

COMPARISON OF THE EFFECTS OF DERACOXIB, BUFFERED ASPIRIN, AND PLACEBO ON THE GASTRIC MUCOSA OF HEALTHY DOGS. Sennello KA, Leib MS. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Deracoxib is a cyclooxygenase-2 (COX-2) inhibitor that has recently been marketed for control of pain in dogs with osteoarthritis. Cyclooxygenase-2 specific NSAIDs have been advocated for their anti-inflammatory actions, pain relief and low incidence of gastrointestinal side effects such as vomiting and gastrointestinal ulceration. The purpose of this study was to compare gastroscopic findings of dogs receiving placebo, aspirin and deracoxib for 28 days.

Twenty-four healthy, random source dogs were divided into three groups. Group I received aspirin 25mg/kg PO TID; Group II received deracoxib 1.5mg/kg QD and placebo PO BID, and Group III were given placebo PO TID. Gastroscopy was performed on days -7, 6, 14, 28 of treatment. Four regions of the stomach (pylorus, incisura, cardia, and body) were evaluated separately and visible lesions were scored on a scale of 1 (mucosal hemorrhage) to 11 (perforating ulcer) by an observer unaware of which treatments the dogs received. Dogs were observed every 8 hours for vomiting and diarrhea. Feces were scored from 1-5 (scores <4 were considered diarrhea).

Median total scores for each group were compared at each endoscopy day using a Kruskal-Wallis test. Total dog days of vomiting and dog days of diarrhea in each group were compared using a Wilcoxon rank sums test. Significance was determined at  $p < 0.05$ .

Significant differences in median total scores were found between Groups I and II, and between Groups I and III on days 6, 14, and 28. No significant differences in median total scores were found between Groups II and III on days 6, 14 and 28. Significant differences in dog days of vomiting were found between Groups I and II, no significant differences were found between groups II and III. No significant differences were found between groups for dog days of diarrhea.

In this study, the administration of deracoxib to healthy dogs resulted in significantly lower gastric lesion scores, and days of vomiting compared to dogs receiving aspirin. There was no significant difference in gastric lesion scores between dogs receiving deracoxib and placebo.

## ABSTRACT #143

ANTIBIOTIC RESPONSIVE HISTIOCYTIC ULCERATIVE COLITIS IN NINE DOGS. Roger A. Hostutler<sup>1</sup>, Brian J. Luria<sup>2</sup>, Susan E. Johnson<sup>1</sup>, Steven E. Weisbrode<sup>1</sup>, Robert G. Shering<sup>1</sup>, Jordan Q. Jaeger<sup>3</sup>, Grant G. Guillard<sup>4</sup>. <sup>1</sup>The Ohio State University College of Veterinary Medicine, Columbus, Ohio; <sup>2</sup>University of Florida, Gainesville, FL; <sup>3</sup>Carolina Veterinary Specialists, Charlotte, NC; <sup>4</sup>Massey University, Palmerston North, New Zealand.

Canine histiocytic ulcerative colitis (HUC) is a disease characterized by severe colonic inflammation predominantly with periodic acid-Schiff (PAS) positive macrophages. The inflammation results in colonic thickening, ulceration, and distortion of normal glandular architecture. Resultant clinical signs consist of chronic large bowel diarrhea, tenesmus, and marked weight loss. Conventional therapy for HUC consists of a combination of the following: dietary modifications; antibiotics such as chloramphenicol, metronidazole, and tylosin; and anti-inflammatory or immunosuppressive drugs such as sulfasalazine, prednisone, and azathioprine. Clinical signs often do not resolve with conventional therapy, and affected dogs frequently are euthanized.

We studied 9 dogs (8 boxers and 1 English bulldog) with histologically confirmed HUC that were successfully treated with antibiotic therapy (either enrofloxacin alone or in combination with metronidazole and amoxicillin). Their clinical signs, physical examination findings, laboratory abnormalities, and histological

severity of disease were evaluated. Four dogs were treated with conventional therapy and failed to respond clinically. These dogs then were treated with the antibiotics described using conventional dosages (enrofloxacin, n=1; enrofloxacin, metronidazole, and amoxicillin, n=3) and had resolution of clinical signs within 3 to 12 days. Five dogs were treated solely with antibiotics using conventional dosages at the time of diagnosis (enrofloxacin, n=1; enrofloxacin and metronidazole, n=1; enrofloxacin, metronidazole, and amoxicillin, n=3) and had resolution of clinical signs within 2 to 7 days. Histological scoring of severity of disease before and after treatment in 5 dogs was statistically significant ( $p = .01$ ). Significant weight gain also occurred after treatment ( $p = .01$ ). These clinical observations support a causative role for an infectious agent responsive to antibiotics in the ultimate clinical manifestation of canine HUC, and also support the use of antibiotics in the treatment of this disease.

## ABSTRACT #144

PREVALENCE OF *GIARDIA* SPECIES AND FACTORS ASSOCIATED WITH FECAL SHEDDING OF THE ORGANISM IN DOMESTIC CATS. R. Vasilopoulos, L.G. Rickard, A. Mackin, C. Huston, and G.T. Pharr. College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

Fecal samples from 250 domestic cats from northeastern Mississippi and northwestern Alabama of various breeds, gender, and ages, and with various clinical signs, were examined for the presence of both *Giardia* species cysts and *Cryptosporidium* species oocysts using a direct immunofluorescent assay.

The prevalence for *Giardia* in our local region was 14% (35/250; 95% CI = 9.9-18.9%), and the prevalence of *Cryptosporidium* was slightly lower at 11.6% (29/250; 95% CI = 7.9-16.2%). Twenty-five cats were concurrently shedding cysts and oocysts of both parasites, 10 cats were shedding *Giardia* cysts only, and 4 cats were shedding *Cryptosporidium* oocysts only. The prevalence of *Giardia* in apparently healthy cats was 8% (7/92; 95% CI = 3.1-15.1%) while the prevalence in cats currently showing any gastrointestinal signs was 18% (13/72; 95% CI = 10-28.9%). Factors associated with having a sample positive for *Giardia* cysts included concurrent infections with *Cryptosporidium* ( $p < 0.001$ ; OR = 170.83; 95% CI = 43.61-745.82); the presence of diarrhea within two weeks of sample acquisition ( $p < 0.001$ ; OR = 3.55; 95% CI = 1.5-8.37); age, with kittens (< 6 months) more likely to be shedding *Giardia* cysts than older cats ( $p < 0.001$ ; OR = 6.8; 95% CI = 2.88-16.11); and concurrent enteric infection with coccidian parasites ( $p < 0.001$ ; OR = 6.69; 95% CI = 2.21-20.06). There was, however, no association between the presence of *Giardia* cysts and type of housing, non-gastrointestinal diseases, vomiting, gender, FeLV/FIV status, source, and gastrointestinal parasites other than *Cryptosporidium* and intestinal coccidians.

Our study demonstrates a prevalence of *Giardia* in cats from the northeastern Mississippi and northwestern Alabama region that is higher than has typically been reported elsewhere. The actual prevalence rate may be higher since a single fecal sample was submitted for analysis from each cat and *Giardia* cysts and *Cryptosporidium* oocysts are intermittently shed. This apparent increased *Giardia* prevalence in our local region may be genuine, or it may simply reflect the development of improved methods for detecting the presence of the organism in feces.

Our study also found a strong association between *Giardia* and *Cryptosporidium* infections in cats, supporting the possibility that infection with one protozoal parasite may predispose cats to concurrent infection with the other protozoal parasite or, alternatively, that the two protozoal infections share common risk factors.

#### ABSTRACT #145

GUT MUCOSAL IMMUNOGLOBULIN A DEFICIENCY IN THE GERMAN SHEPHERD DOG. Rebecca M. Littler. Davies White Veterinary Specialists, Beds, UK.

A relative serum IgA deficiency IgA(D) has been linked to the increased incidence of certain immune mediated and infectious conditions in the German shepherd dog (GSD). This study examined i) the existence of a gut mucosal IgA deficiency, by examination of Ig concentration in faecal extracts, from GSDs and controls ii) the contribution of plasma cell number and function in humoral gut mucosal immune responses.

Humoral gut mucosal immunoglobulin production was assessed in faecal extracts from 76 GSDs and 63 mixed breed controls. Immunoglobulin concentrations were measured by ELISA. Faecal immunoglobulin assays were validated against established canine organ culture systems, and precision and stability of assays investigated. The contribution of plasma cell number to gut immunoglobulin concentrations was assessed by immunohistochemically staining gut biopsy sections from IgA(D) GSDs, normal GSDs and controls and measuring the number of positive staining IgA and IgG plasma cells in 3 defined villous areas. Plasma cell function was assessed by measuring the concentration of IgA, IgM and IgG produced in culture supernatants after 7 days culture of peripheral blood mononuclear cells stimulated by poke weed mitogen. Cultures were prepared from IgA(D) and healthy GSDs

IgA concentration was significantly lower, and IgG higher in GSD faecal extracts (n=76) than controls (n=63, p<0.01). Six GSDs had no demonstrable faecal extract and very low serum IgA. Faecal extract IgM concentrations were similar in GSDs and controls albumin concentration was higher in GSDs.

There was no significant difference in gut biopsy IgA or IgG plasma cells in IgA deficient GSDs (n=6), healthy GSDs, (n=10), or controls, n=10. IgA production by peripheral blood mononuclear cells in response to poke weed mitogen was significantly lower in IgA(D) GSDs compared to healthy GSDs (p<0.01).

In conclusion, the GSD shows a relative and total mucosal IgA deficiency. This may lead to reduction in gut mucosal immune barrier function, and derangement in mucosal and systemic humoral immune responses. The deficiency may be due to impaired function of IgA secreting plasma cells.

#### ABSTRACT #146

ASSESSMENT OF SERUM COBALAMIN CONCENTRATIONS AS AN INDICATOR OF CELLULAR COBALAMIN DEFICIENCY IN DOGS WITH GASTROINTESTINAL DISEASE. DA Williams, CG Ruaux, JM Steiner, and J Wright. Gastrointestinal Laboratory, Department of Small Animal Medicine and Surgery, College Station, TX.

We have previously described a correlation between serum cobalamin concentration and cellular cobalamin deficiency in cats with gastrointestinal disease. Serum cobalamin concentrations may not consistently reflect systemic cobalamin availability; other markers such as serum methylmalonic acid (MMA) and serum homocysteine may be more accurate indicators. The objective of this study was to assess the prevalence of cobalamin deficiency in dogs with varying serum cobalamin concentrations.

Serum samples from dogs with suspected gastrointestinal disease (n=158) were obtained from accessions to the GI Laboratory. Serum concentrations of cobalamin and folate were determined using a modified automated chemiluminescence immunoassay system. Serum concentrations of MMA and homocysteine were determined by gas chromatography/mass spectrometry. The upper limit for normal canine serum MMA concentration was defined as the 95<sup>th</sup> percentile of serum MMA concentrations measured in 28 normal dogs, all with serum cobalamin concentrations in the upper half of the

laboratory reference range (290-749 ng/L). Serum MMA concentration was considered the gold standard method for determining cellular cobalamin availability. Dogs with serum MMA greater than the 95<sup>th</sup> percentile of normal dogs were considered cobalamin deficient. Serum homocysteine concentrations were not normally distributed, thus non-parametric methods were used to analyze this data set. Sensitivity and specificity for diagnosis of cobalamin deficiency in dogs using serum cobalamin concentrations was analyzed with a Receiver Operator Characteristic (ROC) curve.

A significant correlation between serum cobalamin and MMA concentrations was observed (Spearman's test: p < 0.001; r = -0.3567). There was no correlation between serum cobalamin and homocysteine concentrations (p = 0.9192). ROC analysis indicated that 226 ng/L is the theoretical optimum serum cobalamin concentration for use as a diagnostic cut-off for cobalamin deficiency in dogs, with a sensitivity of 65% and specificity of 60%. Cobalamin deficiency was common in dogs with serum cobalamin concentrations near the lower end of the reference range (290 ng/L); the negative predictive value for a cut-off value for serum cobalamin of 280 ng/L was only 50%.

We conclude that serum cobalamin concentrations below the lower limit of the reference range are associated with cellular cobalamin deficiency in dogs. Some dogs with serum cobalamin concentrations in the lower part of the currently accepted reference range are actually in a state of cellular cobalamin deficiency.

#### ABSTRACT #147

SERUM PEPSINOGEN A CONCENTRATIONS IN CATS INFECTED WITH *HELICOBACTER* SPECIES AND SIGNS OF GASTRIC INFLAMMATION. U Tress<sup>1</sup>, KW Simpson<sup>2</sup>, CG Ruaux<sup>1</sup>, JM Steiner<sup>1</sup>, and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX; <sup>2</sup>College of Veterinary Medicine, Cornell University, Ithaca, NY.

Pepsinogen (PG) is the zymogen of pepsin, the major gastric protease in vertebrates. In human beings serum concentrations of PG A are utilized as non-invasive markers for specific gastric disorders such as gastric inflammation and ulceration. Gastric infection with *Helicobacter spp.* in human beings is associated with an increase of serum PG A concentrations and the therapeutic success of eradication protocols can be monitored by the measurement of serum PG A concentrations. The goal of this study was to evaluate the clinical usefulness of serum PG A concentrations as a non-invasive marker for infection with *Helicobacter spp.* or gastric inflammation in cats.

Seventy cats were investigated, 57 of which were infected with *Helicobacter spp.*, while 13 cats were not. Sixteen cats showed vomiting and two cats had diarrhea combined with vomiting. In addition, 7 cats had diarrhea, 4 cats were anorectic, and 41 cats did not show any clinical signs. Serum PG A concentrations were measured using a previously developed in-house radioimmunoassay. Inflammation of the gastric mucosa was scored in biopsy samples. Additionally, the number of neutrophils in the gastric mucosa was assessed. Degrees of inflammation and infiltration with neutrophils were rated on a scale from 0 to 3 (0: absent; 1: mild; 2: moderate; 3: severe). Statistical analysis was performed using a statistical software package (GraphPad Prism 4.0). Statistical significance was defined as a p-value <0.05.

The median serum PG A concentration of the 13 *Helicobacter spp.* negative cats was 102.0 µg/L. The median serum PG A concentration of the 57 *Helicobacter spp.* positive cats was 74.0 µg/L. No gastric inflammation was noted in biopsy samples from 15 cats. Thirty-five cats had mild, 18 cats moderate, and two cats severe inflammation. There was no statistically significant difference between the median serum PG A concentration of cats infected with *Helicobacter spp.* and those that were not infected (p-value 0.0561). There was no statistically significant difference in median serum PG A

concentration in cats with gastric inflammation compared to those that had no inflammation (p-value 0.938). The median PG A concentrations of all groups were within the normal range for fPG A (42.8 to 260 µg/l). Two cats that were *Helicobacter spp.* positive and showed signs of gastric inflammation had fPG A concentrations above the reference range. One *Helicobacter spp.* negative cat showing inflammation, twelve *Helicobacter spp.* positive cats showing inflammation and four cats that were *Helicobacter spp.* positive with no signs of inflammation were all below the reference range.

We conclude that measurement of serum PG A concentration in cats is not a useful marker for either detection of gastric *Helicobacter spp.* infections or inflammation of the gastric mucosa.

#### **ABSTRACT #148**

DEVELOPMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE MEASUREMENT OF FELINE ALPHA<sub>1</sub>-PROTEINASE INHIBITOR IN SERUM AND FECES. K Fetz, JM Steiner, CG Ruaux, JS Suchodolski, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Alpha<sub>1</sub>-proteinase inhibitor (α<sub>1</sub>-PI) is a member of a group of proteins that inhibit serine proteinases. The measurement of fecal α<sub>1</sub>-PI has been used for the detection of gastrointestinal protein loss in both human and canine patients. The aim of this study was to develop and validate an ELISA for the measurement of feline α<sub>1</sub>-PI in feces and serum, as a prelude for further clinical studies.

Prior to the development of a capture sandwich ELISA, feline α<sub>1</sub>-PI was purified from cat serum and antiserum against feline α<sub>1</sub>-PI was raised in two rabbits. Monospecific polyclonal antibodies were purified from antiserum by affinity chromatography. Purified antibodies from the first rabbit were used as capture antibodies, while purified antibodies from the second rabbit were biotinylated and used as reporter antibodies. A streptavidin horseradish peroxidase preparation and a horseradish peroxidase substrate were used for color development. The assay was validated by determination of sensitivity, linearity, accuracy, precision, and reproducibility using three fecal and three serum samples. A reference range for serum α<sub>1</sub>-PI was determined from the central 95<sup>th</sup> percentile in 44 cats. A reference range for fecal α<sub>1</sub>-PI was determined from the central 95<sup>th</sup> percentile of the mean of fecal extracts from 3 different bowel movements in 19 cats.

Sensitivity was 0.02 g/L in serum and 0.04 µg/g in feces. Observed to expected (O/E) ratios for five serial dilutions of three serum samples ranged from 100.0 to 129.7% (mean±SD: 112.2±9.9%). O/E ratios for four serial dilutions of fecal extracts ranged from 103.5 to 141.6% (115.6±12.8%). O/E ratios for serum and fecal samples, spiked with seven different concentrations of α<sub>1</sub>-PI, ranged from 82.3 to 107.8% (94.7±7.6%) for serum samples, and 78.5 to 148.7% (96.8±18.2%) for fecal samples. Coefficients of variation for intra-assay variability were 4.9, 6.4, and 7.9% for serum samples, and 5.3, 11.8, and 14.2% for fecal samples. Coefficients of variation for inter-assay variability were 6.8, 10.0, and 12.1% for serum samples, and 7.7, 10.2, and 20.4% for fecal samples. The reference range for serum α<sub>1</sub>-PI was 0.64 to 1.40 g/L. The reference range for fecal α<sub>1</sub>-PI was up to 1.8 µg/g fecal material.

We conclude that the ELISA for the measurement of α<sub>1</sub>-PI in serum and feces of cats is sufficiently sensitive, linear, accurate, precise, and reproducible for clinical evaluation.

#### **ABSTRACT #149**

A RANDOMIZED CONTROLLED TRIAL OF PREDNISONE VERSUS PREDNISONE AND METRONIDAZOLE FOR TREATMENT OF CANINE INFLAMMATORY BOWEL DISEASE. Albert E. Jergens<sup>1</sup>, John Crandell<sup>1</sup>, Michelle Pressel<sup>1</sup>,

Dawn Kingsbury<sup>1</sup>, Rich Evans<sup>1</sup>, Hans Coetzee<sup>1</sup>, Peggy Schmidt<sup>1</sup>, Jörg M. Steiner<sup>2</sup>, David A. Williams<sup>2</sup>, <sup>1</sup>College of Veterinary Medicine, Iowa State University, Ames, Iowa and <sup>2</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, Texas.

Standard therapy for dogs with inflammatory bowel disease (IBD) consists of the administration of glucocorticoids to reduce intestinal inflammation. Drug therapy combining prednisone with metronidazole is often recommended in dogs having moderate-to-severe IBD despite insufficient evidence to support this practice. The objective of this study was to compare the efficacy of prednisone plus metronidazole combination therapy versus prednisone monotherapy in the treatment of canine patients with IBD. In a randomized clinical trial, we enrolled 19 dogs having mild-to-severe IBD and randomly assigned 9 to receive prednisone alone (1-4 mg/kg PO divided BID) and 10 to receive prednisone (same dosage) and metronidazole (10 mg/kg PO BID) for 21 days. Most dogs were fed one of 2 similar commercially-prepared, highly digestible diets during the drug treatment period. The primary efficacy variable was clinical remission, defined as a 75% reduction in the numerical canine IBD activity index (CIBDAI) score and decreased serum concentration of C-reactive protein (CRP) following therapy. The data were subjected to non-parametric analysis using the Wilcoxon rank sum test and Kendall's Tau b analysis.

The lack of remission in 1 prednisone treated dog was attributed to poor owner compliance with treatment recommendations, necessitating its removal from analysis. Remission rates were comparable in the prednisone and combination therapy groups, 89 % and 100 %, respectively. The mean CIBDAI score decreased to a similar extent, from 7.6 and 5.8 to 1.6 and 0.8 in the prednisone and combination therapy groups, respectively. There was no significant ( $P > 0.05$ ) difference of CIBDAI decrease between treatment groups. A similar trend was observed for serum CRP, with median values in the prednisone group decreasing from 9.3 to 3.0 µg/ml, and within the combination therapy group from 4.7 to 3.0 µg/ml subsequent to treatment. Kendall's non-parametric test for correlation between diagnostic tests indicated strong correlation ( $P < 0.0001$ ) between the CIBDAI score and CRP concentration.

These pilot data represent the first reported controlled trial evaluating drug therapy for canine IBD. Compared with prednisone alone, treatment of canine IBD with a combination of prednisone and metronidazole demonstrated no superiority as measured by the CIBDAI and CRP indices. The CIBDAI would appear to be a useful clinical tool for assessing the interventional effect of drug therapy in canine patients with IBD.

#### **ABSTRACT #150**

ASSESSMENT OF CANINE SMALL INTESTINAL BACTERIAL DIVERSITY BY DENATURING GRADIENT GEL ELECTROPHORESIS. JS Suchodolski, CG Ruaux, JM Steiner, K Fetz, and DA Williams. Gastrointestinal Laboratory, Department of Small Animal Medicine and Surgery, Texas A&M University, College Station, TX.

The normal small intestinal bacterial flora in dogs has not been well defined. Previous studies have focused on the enumeration and identification of bacterial species by direct culture of duodenal juice. This technique is considered to be the gold standard for the diagnosis of small intestinal bacterial overgrowth (SIBO). Bacterial culture, however, has limitations for assessing the bacterial diversity in the gut. Samples have to be plated immediately in order to reflect an accurate representation of the microbial flora. It has also been recognized that many microbial species cannot be identified using standard culture techniques. Thus, a culture-dependent approach may underestimate the bacterial diversity of complex microbial communities such as those found in intestinal fluid. Recently, amplification of bacterial 16S rDNA with subsequent separation by denaturing gradient gel electrophoresis (DGGE) has been described



as an approach to assess bacterial diversity in biological samples. The purpose of this study was to evaluate the use of DGGE profiles for assessment of bacterial diversity in canine duodenal juice.

Eight dogs, euthanized for an unrelated project, were used for this study. Samples were collected in duplicate fashion from each dog. Immediately after euthanasia the abdominal cavity was opened, the duodenum isolated, and two samples of approximately 0.5 ml of duodenal juice were aspirated with a syringe from approximately the same collection site. Bacterial DNA was purified by phenol:chloroform:iso-amylalcohol extraction, the variable V6-V8 region of 16S rDNA was amplified with universal bacterial primers F-968-GC and R-1401, and PCR amplicons were subsequently separated by DGGE. The reproducibility of DNA extraction, amplification of bacterial DNA, and the separation of amplicons by DGGE was evaluated by comparing similarity indices (Dice coefficient; 100% represents complete identity) of DGGE profiles between duplicates of each dog and between the samples from all 8 dogs using gel analysis software (BioNumerics 3.0, Applied Maths).

Similarity indices of DGGE profiles between duplicates collected from the same collection site from each dog were 66.7, 76.9, 92.3, 94.7, 96.3, 100.0, 100.0, and 100.0 (mean±SD: 90.9±12.4%). Mean±SD similarity index of DGGE profiles of duodenal juice between the 8 dogs was 36.9±19.6% (range: 0-69.4%). There was a significantly higher variation in DGGE profiles between dogs than between duplicates obtained from the same dog ( $p < 0.0001$ ).

We conclude that DGGE can serve as a reproducible tool for qualitative assessment of small intestinal bacterial diversity in dogs. These DGGE profiles indicate that dogs have a highly diverse small intestinal microflora with marked differences between individual dogs.

#### ABSTRACT #151

FECAL CONSISTENCY AND VOLUME IN DOGS WITH SUSPECTED SMALL INTESTINAL BACTERIAL OVERGROWTH RECEIVING BROAD SPECTRUM ANTIBIOTIC THERAPY OR DIETARY FRUCTO-OLIGOSACCHARIDE SUPPLEMENTATION. CG Ruaux<sup>1</sup>, MA Tetrick<sup>2</sup>, JM Steiner<sup>1</sup> and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, TX. <sup>2</sup>The Iams Company, Lewisburg, OH.

Medical management of dogs with suspected small intestinal bacterial overgrowth (SIBO) usually involves antibiotic therapy. Often, repeated cycles of antibiotic therapy are necessary. Effective dietary therapy may also be useful in the management of these cases. The aim of this study was to compare the effects of broad-spectrum antibiotic therapy and a fructo-oligosaccharide supplemented diet (FOS-diet) on fecal consistency and volume in dogs with suspected SIBO.

Client-owned pet dogs (n=30) were recruited from accessions to the GI Laboratory. Criteria for entry into the trial included clinical signs and history consistent with SIBO, abnormally high serum folate concentration (>14 µg/L), typically with abnormally low serum cobalamin concentration (<290 ng/L), and normal serum TLI concentration. Dogs were randomized to receive either antibiotic therapy for 30 days (tylosin, 15 mg/kg PO BID; n=15) or a diet containing 1% FOS for 60 days (Eukanuba® Low-Residue® Adult Canine, Dry; n=15). Owners kept daily records of appetite, fecal volume and consistency, and number of bowel movements passed. Appetite was scored from 0 (very poor) through 2 (normal) to 4 (excessive). Fecal volume was scored from 0 (normal) to 2 (copious) and fecal consistency was scored from 1 (liquid) through 4 (normal) to 7 (extremely hard). Data were analyzed by two-way ANOVA.

Mean appetite scores were significantly higher in the diet group throughout the study period ( $p < 0.01$ ). Fecal volume scores normalized significantly in both treatment groups ( $p < 0.01$ ), however the effect of the two treatments differed significantly over time ( $p < 0.01$ ). Rapid normalization of fecal volume in the antibiotic group

was followed by deterioration on withdrawal of antibiotics. Fecal volume in the diet group reduced gradually, remaining significantly lower at the end of the trial. Fecal consistency improved significantly with both treatments ( $p < 0.01$ ), but the effects of the two treatments differed significantly over time. Tylosin-treated dogs showed immediate normalization of fecal consistency with deterioration on withdrawal of therapy. The FOS-diet group exhibited a slow normalization of fecal consistency, which was maintained until the end of the trial. The number of daily bowel movements showed no significant change over time with either therapy.

We conclude that both broad-spectrum antibiotic therapy and FOS-supplemented diets are associated with improved fecal character in dogs with suspected SIBO. Beneficial effects of antibiotic therapy show rapid onset but may be transient, while benefits from the FOS-diet are slower in onset but are sustained while the diet is administered.

#### ABSTRACT #152

EFFECT OF DOXORUBICIN ON HEPATIC <sup>13</sup>C-AMINOPYRINE DEMETHYLATION CAPACITY IN DOGS. JM Steiner<sup>1</sup>, K Hahn<sup>2</sup>, SR Teague<sup>1</sup>, and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX; <sup>2</sup>Gulfcoast Veterinary Specialists, Houston, TX.

Hepatic function tests that are both sensitive and specific for estimation of hepatic function in dogs have not been available. Recently, a new diagnostic test, <sup>13</sup>C-aminopyrine demethylation blood test, has been described in dogs. The kinetics of <sup>13</sup>C-aminopyrine demethylation have been described in normal dogs and a time point of 45 minutes after <sup>13</sup>C-aminopyrine administration has been identified as the optimal time point for blood collection. Also, a dose of 2 mg/kg <sup>13</sup>C-aminopyrine given intravenously has been described as optimal. Finally, a <sup>13</sup>C-aminopyrine demethylation blood test has been performed in dogs that underwent hepatic biopsy. Initial results suggest that dogs with more severe hepatic disease have a decreased hepatic demethylating capacity. Doxorubicin is a chemotherapeutic agent that is commonly used for the therapy of dogs with lymphoma and other malignant neoplasias. Little is known about hepatic toxicity of doxorubicin in dogs. Therefore, the goal of this study was to evaluate the effect of doxorubicin on hepatic demethylation capacity.

A <sup>13</sup>C-aminopyrine demethylation blood test was performed in 3 dogs before and after undergoing chemotherapy with doxorubicin for malignant neoplasia. A baseline blood sample of 1 ml was taken, followed by intravenous injection of 2 mg/kg <sup>13</sup>C-aminopyrine and collection of another 1 ml blood sample at 45 minutes after <sup>13</sup>C-aminopyrine administration. Blood samples were immediately placed into vacutainer tubes containing 2 ml of 6M hydrochloric acid and the percent dose of the <sup>13</sup>C administered as <sup>13</sup>C-aminopyrine was determined based on fractional mass spectrometry. Two weeks after doxorubicin treatment the <sup>13</sup>C-aminopyrine demethylation blood test was repeated in the same fashion and the results compared to those obtained prior to chemotherapy by a paired *t*-test. A *p*-value <0.05 was considered statistically significant.

None of the 3 dogs showed any clinically obvious side effects from the <sup>13</sup>C-aminopyrine demethylation blood test before or after doxorubicin administration. The PCD at 45 minutes after <sup>13</sup>C-aminopyrine administration decreased in all 3 dogs after treatment with doxorubicin. It decreased by 84.6% in dog 1, 49.5% in dog 2, and 48.9% in dog 3 with an average of 61.0%. The mean±SD PCD at 45 minutes after <sup>13</sup>C-aminopyrine administration was 0.0468±0.0253 after doxorubicin administration, which was significantly lower when compared to 0.1191±0.0074 before treatment with doxorubicin (*p*-value = 0.03).

We conclude that doxorubicin treatment in dogs leads to a significant decrease in hepatic demethylation capacity. Further

studies are needed in order to study the clinical significance of this finding.

#### ABSTRACT #153

SERUM ALPHA<sub>1</sub>-PROTEINASE INHIBITOR CONCENTRATIONS IN CATS WITH INCREASED SERUM PANCREATIC LIPASE IMMUNOREACTIVITY AND CATS WITH EXPERIMENTALLY INDUCED PANCREATITIS. K Fetz<sup>1</sup>, JM Steiner<sup>1</sup>, CG Ruaux<sup>1</sup>, JS Suchodolski<sup>1</sup>, N Zavros<sup>2</sup>, T Rallis<sup>2</sup>, and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, TX; <sup>2</sup>Aristotle University of Thessaloniki, Greece.

In some species, including human beings, rats and rabbits,  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ -PI) is an acute phase reactant, its plasma concentration increasing three-to fourfold during an inflammatory response. In other species, including mice, dogs, and horses,  $\alpha_1$ -PI does not play an important role in the acute phase response (APR). The role of  $\alpha_1$ -PI as an acute phase reactant has not previously been evaluated in cats. Recently, a specific immunoassay for the measurement of  $\alpha_1$ -PI in cat serum has been developed and validated. The aim of this study was to evaluate serum  $\alpha_1$ -PI concentrations in cats with biochemical evidence of pancreatitis and in cats with experimentally induced pancreatitis. Both of these groups would be expected to undergo an APR and could thus be useful to determine the importance of  $\alpha_1$ -PI as an acute phase reactant in this species.

Fifty serum samples of cats with increased serum pancreatic lipase immunoreactivity (fPLI) were selected from accessions to the Gastrointestinal Laboratory. In these cats serum fPLI concentrations ranging from 12.1 to 1329  $\mu$ g/L were measured (reference range: 2.0-6.8  $\mu$ g/L). An additional set of serum samples was obtained from four cats in which transient acute pancreatitis had been induced by retrograde injection of oleic acid into the pancreatic duct as part of another research project. Samples were collected at 0, 8, 24 and 48 hours after induction of pancreatitis. One sham-operated cat was used as a negative control. Serum feline  $\alpha_1$ -PI (f $\alpha_1$ -PI) concentrations were analyzed with an in-house ELISA. Data were analyzed with a statistical software package (GraphPad Prism 4.0). Correlation of serum fPLI and f $\alpha_1$ -PI concentrations was evaluated with Spearman test. Data from the induced pancreatitis study were analyzed using the Friedman test.

Thirty-six of 50 cats with increased fPLI concentrations had f $\alpha_1$ -PI concentrations within the reference range of 0.64-1.40 g/L, while ten cats had f $\alpha_1$ -PI concentrations above and four cats had f $\alpha_1$ -PI concentrations below this range. There was no correlation between serum f $\alpha_1$ -PI and fPLI concentrations (Spearman  $r=0.025$ ,  $p=0.8631$ ). Three of four cats with induced acute pancreatitis had f $\alpha_1$ -PI concentrations above the reference range at different time points. Feline  $\alpha_1$ -PI concentrations in the sham operated control were within the reference range at all times. A significant increase of f $\alpha_1$ -PI concentrations over a period of 0-48 hours was observed ( $p=0.0329$ ).

We conclude that serum f $\alpha_1$ -PI concentrations increase significantly in cats after induction of pancreatitis. However, this increase was trivial compared to the 3 to 4 fold increases observed in species in which  $\alpha_1$ -PI is a true acute phase reactant. Furthermore, serum  $\alpha_1$ -PI was only increased in 20% of cats with biochemical evidence of spontaneous pancreatitis. Thus, this study provides little evidence for a significant role of  $\alpha_1$ -PI as an acute phase reactant in the cat.

#### ABSTRACT #154

EVALUATION OF EXON 3, INTRON 3 AND THE INTRON 3/EXON 4 BOUNDARY OF THE LIPOPROTEIN LIPASE GENE IN MINIATURE SCHNAUZERS WITH PANCREATITIS. R Schickel<sup>1</sup>, JM Steiner<sup>1</sup>, ML Cox<sup>2</sup>, MA Bishop<sup>1</sup>, and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory and <sup>2</sup>Canine Genetics Laboratory,

College of Veterinary Medicine, Texas A&M University, College Station, TX.

A high incidence of hyperlipidemia and pancreatitis has been described in the Miniature Schnauzer. Lipoprotein lipase (LPL) is one of the key enzymes in lipid transport in the body, and malfunction of LPL results in decreased clearance of lipoproteins from the blood. In human beings, mutations of the LPL gene have been shown to be associated with hyperlipidemia with concurrent pancreatitis. These include specific mutations at Tyr73, Arg75, Trp86, Gln106, and a region located 6 bp upstream the splicing acceptor region of intron 3. This study evaluates this site in the LPL gene of Miniature Schnauzers with pancreatitis.

Four Miniature Schnauzers were selected on the basis of a clinical history of pancreatitis. Inclusion criteria were: 1) a pancreatic biopsy showing pancreatic inflammation, 2) clinical signs compatible with pancreatitis (such as vomiting or abdominal pain) and a 5-fold elevation of serum lipase activity, or 3) clinical signs compatible with pancreatitis and ultrasonographic evidence of pancreatitis (pancreatic edema, peripancreatic ascites, and changes in echogenicity of the pancreas). A clinically normal Miniature Schnauzer and 3 clinically normal dogs of other breeds were selected as controls. DNA was extracted from white blood cells or buccal mucosal cells using a commercially available kit (Purgene, Gentra Systems). Primers were designed and optimized to amplify exon 3, intron 3, and the intron 3/exon 4 boundary region. PCR was performed, and the quality of PCR-products was assessed by agarose gel electrophoresis. After staining with ethidium bromide DNA was extracted from the gel using a gel extraction kit (QUIAquick, Qiagen). Adenine overhangs were added and DNA fragments were cloned into a pCR4-TOPO vector (TOPO TA cloning kit, Invitrogen). Recombinants were transformed into DH5a-T1 cells. Transformants were cultured and plasmid DNA was isolated (Perfectprep, Eppendorf). Digestion with EcoRI was performed to verify successful cloning. Sequencing was performed on an automated sequencer.

All 4 Miniature Schnauzers with pancreatitis showed the same DNA sequence in the evaluated region as did the healthy control Miniature Schnauzer and the 3 control dogs belonging to other breeds.

We conclude that pancreatitis in these Miniature Schnauzers was not associated with mutations in exon 3, intron 3, or the intron 3/exon 4 boundary.

#### ABSTRACT #155

CHOLECYSTOENTEROSTOMY IN CATS - ETIOLOGY & PROGNOSIS: 22 CASES (1994-2003). Nicole J Buote; Cynthia RL Webster; Lisa Freeman; Dominique Penninck; Susan L Mitchell, Tufts University School of Veterinary Medicine, North Grafton, MA.

Feline extrahepatic biliary tract obstruction (EHBTO) is a diagnostic and therapeutic challenge. In most cases, surgical decompression of the biliary tree is indicated. This retrospective study describes the etiology, clinical presentation, and surgical management of feline EHBTO.

Twenty-two cats with surgically confirmed EHBTO that underwent biliary diversion surgery were included. Records were evaluated for signalment, history, initial complaint, clinical signs, physical exam findings, blood chemistry values, complete blood count, findings on diagnostic imaging, surgical treatment, and clinical outcome. Follow up was obtained via questionnaire.

Median age was 10.1 years old (range 3 to 17 years). Most common clinical signs were vomiting (14; 67%), anorexia (13; 59%), icterus (14; 64%), lethargy/weakness (8; 38%) and weight loss (6; 27%). Mean time from onset of clinical signs to presentation was 29 days (range 1 to 120). Abdominal radiographs in 10 cats showed nonspecific signs while abdominal ultrasound confirmed EHBTO in 21 cats (95%). Surgery consisted of either a cholecystoduodenostomy (13), cholecystojejunostomy (8) or choledochoduodenotomy (1).

During anesthesia 73% (11/15) of cats had episodes of intra-operative hypotension (systolic < 80 mmHg), nine (60%) severe enough to require vasopressor treatment, six (40%) required mechanical ventilation and five (33%) required intra-operative blood products. Intensive postoperative care including blood transfusions, vasopressors, and parenteral nutrition was required in 71% (15/21). EHBTO was due to either neoplasia (9/22:41%) or inflammatory disease (13/22:59%). Neoplastic causes included biliary adenocarcinoma (55%), lymphoma (22%), squamous cell carcinoma (11%), and exocrine pancreatic adenocarcinoma (11%). Inflammatory causes included cholangiohepatitis (31%), lymphoplasmacytic (LPC) enteritis (7.6%), chronic LPC/fibrosing pancreatitis (23%), LC hepatitis (15%), and cholecystitis (23%). Three cats were lost to follow up. Six cats (32%) survived to 6 months postoperatively, all diagnosed with inflammatory disease. Median survival time for all cats was 31 days, range 0-1909. Median survival of all inflammatory cases (10) was 196 days, range 4-1909; neoplasia cases (9) was 21 days, range 0-733. The median duration of clinical signs before presentation was 12 days for inflammatory disease, range 1 to 98; neoplasia had a median of 31 days, range 7-137. Serum biochemical parameters, anesthetic complications, postoperative care, age, and length of clinical signs were not statistical indicators of response to surgery.

This study suggests that: 1) cats with EHBTO that undergo biliary diversion have a 6-month survival rate of 32% (6/19), 2) cats with neoplasia had a tendency for a more prolonged clinical course and shorter median survival time than cats with inflammatory disease and 3) most cats undergoing biliary diversion surgery for EHBTO require intensive anesthetic and postoperative management.

#### **ABSTRACT #156**

BRONCHOSCOPIC OPTICAL BIOPSY: REAL-TIME NON-INVASIVE AIRWAY EVALUATION UTILIZING OPTICAL COHERENCE TOMOGRAPHY (OCT). Walton R.S, Dick E.J, Brenner M\*, Chen Z.\*, Peavy G.M.\*, Nelson J.S. \* U.S. Army Institute of Surgical Research, San Antonio, Texas, USA. \*Beckman Laser Institute, School of Medicine, University of California, Irvine, California, USA.

Accurate bronchoscopic diagnosis depends on visual assessment of gross tissue appearance to determine where biopsies are to be taken. The optimal location for biopsy sampling is not always readily apparent. An accurate non-invasive assessment of tissue architecture would help determine the best location. Many non-invasive modalities have been evaluated including, the use of vital dyes, indocyanine green dye fluorescence, fluorescein fluorometry, laser doppler flowmetry, and ultrasound. Due to various limiting factors, none of these technologies has greatly improved the ability to assess tissue beyond simple observation. Biopsy and histopathology remain the gold standard for tissue evaluation. Polarization-Sensitive Optical Coherence Tomography (PS-OCT) and Optical Coherence Tomography/ Doppler Tomography (OCT/ODT) devices have recently been developed that have the potential to assess tissue non-invasively in real-time. This novel optical technology is analogous to ultrasound except that the images are created with echoes of light rather than sound. OCT images are capable of a 10 fold increase in resolution compared to best ultrasound images. PS-OCT uses coherence gating to image tissue birefringence with a high degree of spatial resolution. When the polarization state of light reflects from various depths in a tissue, PS-OCT can determine lesion depth based on condition of collagen and structural proteins. Optical Doppler Tomography (ODT) essentially combines Laser Doppler Flowmetry (LDF) with optical coherence tomography (OCT) to produce high-resolution tomographic images of static and moving constituents in biologic tissues. OCT/ODT technology has the ability to non-invasively image *in vivo* blood flow with high spatial resolution. By using a Michelson interferometer with a low coherence light source,

ODT can measure the amplitude and frequency of the interference fringe intensity generated to form a combined structural and velocity image. The image resolution currently approaches 10 microns with this device but this technology has produced images with resolutions of 1-2 microns using alternate light sources. The optical imaging fiber is approximately 2mm in diameter and is easily placed in the biopsy channel of a bronchoscope. Using inhalation and ventilator induced airway injury models we compared the results of this instrument to standard histopathology and frozen section techniques in both *ex vivo* and *in vivo* preparations. The OCT measurement had excellent correlation with standard histology techniques. The OCT imaging technique allows the clinician to rapidly scan large anatomic areas searching for the best area to biopsy. It also allows the clinician to the ability serially follow suspicious lesions.

#### **ABSTRACT #157**

8-ISOPROSTAGLANDIN F2a IN BAL IS A DIRECT MARKER OF FREE RADICAL INDUCED LIPID PEROXIDATION IN FELINE ASTHMA. R.A. Hirt,\* A. Guetl,\* F. Delvaux, P. Gustin, N. Kirschvink<sup>o</sup>. \*1<sup>st</sup> Medical Clinic, Veterinary University Vienna, Austria, <sup>o</sup>Institute of Pharmacology, Veterinary University Liege, Belgium.

Feline asthma is characterized by reversible bronchoconstriction and chronic airway inflammation. Reactive oxygen species (ROS) are thought to contribute not only to the pathogenesis, but also to the perpetuation of disease, and may lead to lipid peroxidation of cell membranes. Isoprostanes are a family of eicosanoids produced by random oxidation of tissue phospholipids. In the present study 8-Isoprostaglandin F2a was determined as a direct marker of oxidative stress and lipid peroxidation in bronchoalveolar lavage fluid (BALF) from 6 cats with spontaneous feline asthma. Diagnosis was established based on history and clinical signs, thoracic radiographs, BALF cytology and detection of bronchoconstriction during an asthmatic attack by use of conscious unrestrained barometric whole body plethysmography. Subsequently, the cats underwent a carbachol challenge to test for the presence of airway hyperresponsiveness. 8-Isoprostaglandin F2a was measured by an enzyme immunoassay (Cayman). 6 healthy cats served as controls.

The mean percentage of eosinophils was 62.8±34.4 in asthmatics, as compared to 3.5±4.55 in controls (p<0.002), whereas macrophages accounted for 33.0±31.9% in asthma cats and 88.3±8.1 in controls (p<0.002). Mean PENH, a unitless variable reflecting the degree of bronchoconstriction, was 3.48±1.59 in the asthmatic cats during the asthmatic attack and 0.57±0.23 in controls. The mean provocative carbachol concentration increasing PENH by 300% (PC PENH300) was 0.032% in asthmatics as compared to a mean PCPENH300 of 0.179% in a group of healthy age matched cats.

The mean 8-Isoprostaglandin F2a concentration in cats with asthma was 34.9±36.6 pg/ml versus 0.74±0.32pg/ml in controls (p<0.045). Regression analysis revealed a good correlation between the percentage of BALF eosinophils and 8-Isoprostaglandin F2a concentrations (R<sup>2</sup>=0.744, p<0.0003).

Our data suggest that in asthmatic cats increased oxidative stress plays a significant role, leading to lipid peroxidation, and possibly participating in disease progression. 8-Isoprostaglandin F2a may contribute to objective monitoring of therapeutic effects beyond resolution of clinical signs. It might serve as a surrogate marker for the control of the inflammatory process in the airways of asthmatic cats.

## ABSTRACT #158

MOLECULAR DETECTION OF MICROBES IN NASAL BIOPSIES OF DOGS WITH LYMPHOPLASMACYTIC RHINITIS. Rebecca C. Windsor, Lynelle R. Johnson, Jane E. Sykes, Christian M Leutenegger, Hilde E.V. DeCock. University of California, Davis, CA.

Lymphoplasmacytic rhinitis (LPR) is a common histologic finding in dogs with chronic nasal disease. To the authors' knowledge, the etiology of this disorder has not been defined, however positive bacterial or fungal growth from a nasal swab is common and dogs often display a transient response to antibiotics. We investigated the hypotheses that specific microbes are found in nasal tissue of dogs with LPR and that greater gene transcription of organisms is detected in biopsies of dogs with nonspecific nasal inflammation.

Paraffin-embedded nasal biopsies were obtained from 15 dogs with LPR. For comparison, biopsy samples were also retrieved from 10 dogs with nasal neoplasia and 10 with nasal aspergillosis. Tissue sections (25 µm) were placed in 1.5 ml RNase-free Eppendorf tubes for DNA/RNA extraction using Qiagen DNeasy. Bilateral nasal biopsies from 5 healthy dogs were used as controls. TaqMan PCR was employed for detection of target genes for universal bacteria, universal fungi, Canine Adenovirus 2 (CAV-2), Parainfluenza virus 3 (PI-3), *Chlamydomphila*, and *Bartonella*. GAPDH was used as a housekeeping gene. Statistical analysis was performed using the Mann-Whitney U test for non-parametric data. Significance was set at  $P < 0.05$ .

Gene transcription for CAV-2, PI-3, *Bartonella*, and *Chlamydomphila* was not detected in any nasal biopsy, however universal bacteria and fungi were present in all. Detection of fungal gene transcription in nasal biopsies was higher in dogs with aspergillosis than in dogs with LPR ( $P < 0.05$ ), however nasal biopsies of LPR dogs also displayed higher fungal gene transcription than samples from dogs with nasal neoplasia ( $P < 0.05$ ). Transcription of bacterial RNA in dogs with LPR did not differ between tissue samples from dogs with aspergillosis or neoplasia ( $P = NS$ ).

In nasal biopsies examined here, dogs with LPR displayed higher transcription of fungal genes than did dogs with neoplasia, but less than dogs with aspergillosis. Whether fungal organisms detected using molecular techniques are causally related to the inflammation observed or result from entrapment or accumulation in the nasal cavity requires further investigation.

## ABSTRACT #159

THE EFFECT OF FIROCOXIB, CARPROFEN AND VEDAPROFEN IN A SODIUM URATE CRYSTAL INDUCED SYNOVITIS MODEL OF ARTHRITIS IN DOGS. P.D. Hanson<sup>1</sup>, H.A.W. Hazewinkel<sup>2</sup>, W.E. van den Brom<sup>2</sup>, L.F.H. Theyse<sup>2</sup>, M. Pollmeier<sup>3</sup>, C. Fleishman<sup>1</sup>. <sup>1</sup>Merial Limited, Duluth, GA, USA. <sup>2</sup>Utrecht University, The Netherlands. <sup>3</sup>Merial GmbH, Rohrdorf, Germany.

Firocoxib is a highly selective inhibitor of the inducible isoform of cyclooxygenase (COX-2) and is a member of the coxib class of nonsteroidal anti-inflammatory drugs developed specifically for veterinary use. A randomized, placebo controlled, four-period crossover study was conducted under laboratory conditions involving eight dogs to confirm the effective dose of firocoxib in a sodium urate crystal induced synovitis model of arthritis. The efficacy of firocoxib was compared with placebo vehicle, vedaprofen, and carprofen. Treatments were administered orally once each period and, based on available tablets or oral gel, corresponded to dose levels of at least 5 mg firocoxib/kg body weight (bw), 0.5 mg vedaprofen/kg bw, and 2.0 mg carprofen/kg bw. The washout period between treatments was seven days. One hour prior to each treatment, 19 mg sodium urate suspended in 0.9% saline was injected in the left or right stifle joint to induce synovitis. The injected joint was alternated between

left and right in successive periods. On the day prior to injection and treatment, and at 3 and 7 hours post-treatment, each dog was walked over a force plate to measure the peak vertical force. Six measurements were recorded at each time point and adjusted for animal body weight. Peak vertical force was transformed to the percentage of baseline (%bl, prior to urate) for each dog and time point in each period. Clinical lameness was scored based on a five point scale at the same time as peak vertical force. Analysis of variance for a crossover design was performed for the 3 and 7 hour post-treatment time points using the MIXED procedure of SAS. The model included sequence, period and treatment as fixed effects, and replicate and replicate-by-sequence interaction as random effects. Pairwise contrasts were used to compare firocoxib with each of the other treatment groups. All statistical testing was two-sided and performed at the 5% significance level.

All dogs were free of lameness prior to each urate injection and treatment. At 3 hours after treatment, peak vertical force with firocoxib (72.0±12.3 %bl, ±sem) was significantly better than placebo (40.6±13.3 %bl) ( $p < 0.05$ ). At 7 hours, peak vertical force with firocoxib (99.3±2.7 %bl) was significantly better than placebo (69.6±7.4 %bl) and carprofen (84.6±5.2 %bl) ( $p < 0.05$ ). At 3 hours after treatment, clinical lameness was significantly less in firocoxib treated dogs (1.4±0.5) compared with placebo (2.8±0.4) and carprofen (2.4±0.5) ( $p < 0.05$ ). At 7 hours, clinical lameness was significantly less in firocoxib treated dogs (0.1±0.1) compared with placebo (1.8±0.3) and carprofen (1.0±0.3) ( $p < 0.05$ ). Although not significantly different, scores at 7 hours were better for firocoxib than vedaprofen.

Results of this study confirmed the efficacy of 5.0 mg firocoxib/kg bw in an arthritis model. The level of improvement after treatment with firocoxib was comparable to or greater than that observed with carprofen and vedaprofen.

## ABSTRACT #160

HEALTH EVENTS RECORDED FROM 575 DOGS TREATED FOR OSTEOARTHRITIS WITH FIROCOXIB, CARPROFEN OR ETODOLAC. P.D. Hanson, D. Romano, C. Fleishman, M. Drag, M. Pollmeier, R. Gogolewski, C. Toulemonde, R. Alva. Merial Limited, Duluth, GA.

Firocoxib is a highly selective inhibitor of the inducible isoform of cyclooxygenase (COX-2) and is a member of the coxib class of nonsteroidal anti-inflammatory drugs developed specifically for veterinary use. Studies were conducted in six countries under field conditions to evaluate efficacy and safety of firocoxib and two commonly used NSAIDs, carprofen and etodolac, for the control of pain and inflammation associated with osteoarthritis. Each of the studies was conducted as a positive-control, double-blind, randomized block design, where blocks were replicates of two dogs formed at each location based on order of presentation. Within replicates one dog was randomly allocated to treatment with the positive control (carprofen at 4 mg/kg/day or etodolac at 10-15 mg/kg/day, depending on study location) and the other to treatment with firocoxib at 5 mg/kg/day. Treatments were administered orally for approximately 30 days. Owners recorded health events on a daily basis. The attending veterinarian classified these events based on the relationship to treatment: yes, no, or unknown. A single veterinarian codified all events based on the Veterinary Medical Dictionary for Drug Regulatory Authorities (VEDDRA). For each treatment, irrespective of the relationship to treatment, the overall incidence of at least one event and the incidence of each specific event were tabulated and treatments compared using the Pearson Chi-Square exact test. The tabulations were further assessed according to relationship to treatment. Dogs that dropped out of the study were recorded and classified as due to an adverse event, lack of effect, or other. Dropouts were also compared using the Pearson Chi-Square

exact test. All statistical testing was two-sided and performed at the 0.05 significance level.

The study included 575 dogs of various breeds and sex, with a mean age of 8.0 years (range, 7 months to 20 years) and weight of 32.6 kg (range, 2.9 to 86 kg). There were 292 dogs treated with firocoxib, 132 with carprofen, and 151 with etodolac. Irrespective of the relationship to treatment, fewer dogs were reported to have at least one health event with firocoxib than with carprofen ( $p < 0.05$ ). When considered by specific event, fewer dogs experienced diarrhea with firocoxib (3.1%) than with etodolac (8.6%) and carprofen (6.8%) ( $p < 0.05$ ). Fewer dogs experienced melena with firocoxib (0.3%) than with etodolac (3.3%) ( $p < 0.05$ ).

The incidence of dogs with at least one treatment related health event was significantly less for firocoxib (1.0%) than for carprofen (6.1%) and etodolac (4.0%) ( $p < 0.05$ ). When considered by specific event, fewer dogs experienced diarrhea related to treatment with firocoxib (0%) than with etodolac (2.6%) and carprofen (1.5%) ( $p < 0.05$ ). A total of 41 dogs (7.1%) dropped out of the studies for various reasons. There were significantly fewer dropouts due to an adverse event with firocoxib (0.7%) than with etodolac (4.0%) ( $p < 0.05$ ).

Treatment with firocoxib for 30 days under field use conditions for the control of pain and inflammation associated with osteoarthritis was tolerated well and associated with fewer health events than similar treatment with carprofen or etodolac.

#### **ABSTRACT #161**

ESTABLISHMENT AND VALIDATION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE QUANTIFICATION OF IMMUNOGLOBULIN A IN FECAL SAMPLES FROM DOGS. U Tress, JM Steiner, CG Ruaux, and DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Mucosal surfaces, such as the gastrointestinal mucosa, can be the point of entry for a variety of pathogens. Secretory IgA is the predominant immunoglobulin subtype in mucosal secretions and is believed to protect mucosal membranes from the entry of pathogens. In human beings IgA deficiency is the most common primary immunodeficiency and is associated with frequent infections of the gastrointestinal, respiratory, and urogenital tracts. A comparable disorder has been reported in certain dog breeds, such as German Shepherd Dogs. In human beings IgA deficiency is defined by the presence of a low serum IgA concentration. In dogs, however, it is reported that serum IgA concentrations do not reflect local intestinal IgA production (German *et al*, Vet Immunol. Immunopathol. 76:25-43) and measurement of fecal IgA concentrations might be more meaningful. Therefore, the goal of this study was to establish and validate a method for measurement of IgA concentrations in fecal samples from dogs.

Immunoglobulin A was extracted from canine fecal samples, taken from the first bowel movement of the day. A sandwich ELISA was established for the measurement of IgA concentrations in these extracts. The assay was validated by determination of sensitivity, linearity, accuracy, precision, and reproducibility. Commercially available goat anti-dog IgA antibodies served as primary and secondary antibodies, with the secondary antibody being biotinylated. Commercially available dog IgA reference serum of known IgA concentration was used to prepare a range of standards. A reference range was determined from the mean IgA concentration of four fecal samples from 18 clinically healthy dogs by calculating the central 95<sup>th</sup> percentile.

The sensitivity of the assay was 0.06 mg/g feces. Observed to expected ratios for dilutional parallelism of four fecal samples ranged from 109.7 - 119.8%. Observed to expected ratios for spiking recovery of the same samples ranged from 55.6 - 99.3%. The coefficients of variation for intra-assay and inter-assay variability

ranged from 3.0 to 7.7% and 7.6 to 14.3%, respectively. The reference range for fecal IgA determined in 18 healthy dogs was 0.22 to 3.24 mg/g.

The observed to expected ratio for spiking recovery for one of the samples was 55.6% when the highest amount of IgA was added, indicating that the accuracy of the assay may be decreased at higher IgA concentrations. Since the purpose of this assay is to detect dogs with IgA deficiency, this should not diminish its usefulness, and it was concluded that the method for assay of IgA in feces from dogs described here is sufficiently sensitive, reproducible, accurate, and precise to measure fecal IgA concentrations in dogs and identify dogs with decreased fecal IgA concentrations.

#### **ABSTRACT #162**

INTRA-INDIVIDUAL VARIATION IN FECAL IgA CONCENTRATION OF DOGS. U Tress, JM Steiner, MD Miller, ZM Wright, CG Ruaux, and DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Secretory IgA is the major immunoglobulin subtype secreted by the intestinal mucosa. Polymeric IgA is synthesized by plasma cells located in the intestinal lamina propria, either organized in Peyer's patches or disseminated throughout the intestinal epithelium. Most intestinal IgA synthesis is induced locally by lumenally derived antigens. Secretory IgA is formed during the transport of polymeric IgA through the intestinal epithelial cell. Since antigenic stimulation of the gastrointestinal tract varies, it can be expected that fecal IgA concentrations in any one individual will show some degree of variation. The goal of this study was to assess intra-individual variability of fecal IgA concentrations within the same bowel movement and over time, in order to develop a practical sampling strategy that would reliably identify dogs with consistently low fecal IgA concentrations.

Intra-individual variation within the same bowel movement was assessed by collection of five fecal samples from different locations in the same bowel movement from five different dogs. Intra-individual variations over time were evaluated by collection of 15 separate fecal samples from each of 18 healthy dogs of differing ages and breeds. Samples were taken in five sampling periods on days 0, 1, and 2, 14, 15, and 16, 28, 29, and 30, 42, 43, and 44, and days 56, 57, and 58. Immunoglobulin A was extracted from all fecal samples and IgA was quantified with a previously validated in-house ELISA. Variability of fecal IgA concentrations was assessed for each of the dogs by calculation of the coefficients of variation.

Coefficients of variation of IgA concentrations in five different extracts from the same bowel movement of five dogs ranged from 18.5 to 74.2% with a mean $\pm$ SD of 38.1 $\pm$ 21.9%. Coefficients of variation of the five mean IgA concentrations for each of the 18 dogs within each of the five sampling periods ranged from 2.4 to 131.6% with a mean $\pm$ SD of 47.2 $\pm$ 29.2%. Coefficients of variation of the mean IgA concentrations for each of the 18 dogs from days 0, 1, 28 and 29, days 15, 16, 43 and 44, days 14, 15, 42 and 43, days 28, 29, 56 and 57, days 1, 2, 29 and 30 and days 29, 30 57 and 58 ranged from 5.4 to 137% with a mean $\pm$ SD of 61.8 $\pm$ 30.8%. Variation of fecal IgA concentrations within the same bowel movement was lower than the variation in fecal IgA of the same dog on different days. There was no significant difference between the coefficients of variation of the mean calculated from three consecutive day samples compared to the mean of four fecal samples taken two times on two consecutive days with 28 days in between.

We conclude that a total of four fecal samples, two collected on two consecutive days and two collected on two consecutive days 28 days later are sufficient to reliably identify dogs with consistently decreased fecal IgA concentrations.

### ABSTRACT #163

DO ENVIRONMENTAL FACTORS INFLUENCE THE OUTCOME OF SEROLOGIC ALLERGY TESTING IN HORSES? G. Kolm and R. Wagner, Clinic for Obstetrics, Gynecology and Andrology, University of Veterinary Science, Vienna, Austria.

Until now serologic allergy testing (SAT) in horses is not consistent with manifestation of hypersensitivity or intradermal skin testing (IDST) results. Recent studies give evidence that circulating allergen-specific IgE levels, apart from genetic factors, are significantly influenced by environmental factors. The purpose of the study reported here was to examine in a population of 37 healthy and 40 Icelandic horses suffering from sweet itch (categories 0,1,2,3), whether the level of agreement between insect-specific IgE values as measured by a commercially available polyclonal anti-horse IgE-based ELISA and manifestation of disease or IDST was improved by statistical control for potentially influencing variables such as farm, sex, and age.

The study was conducted during fall, when affected horses showed acute clinical signs. IDST was performed as standard procedure using extracts of black ant, mosquito, horsefly, deerfly, housefly, and *Culicoides variipennis* (Greer Laboratories, Inc, North Carolina, USA). Insect-specific IgE levels in horse sera were measured by using Equine ELISA (Bio-Medical Services, Austin, TX) and subjected to statistical analysis as primary performance data.

Despite controlling for environmental factors, circulating allergen-specific IgE levels were not significantly different regarding the incidence and size of positive skin reactions to tested insects. The incidence and category of sweet itch had a significant effect on housefly-specific IgE levels, only ( $p < 0.01$ ). The distribution of housefly-specific levels was not appropriate to discriminate between horses with or without sweet itch, as slightly and severely affected horses exhibited higher specific IgE levels compared with moderately affected and clinically healthy horses. Sex significantly effected mosquito-specific IgE levels ( $p < 0.05$ ). Significant interactions on specific IgE levels to housefly ( $p < 0.01$ ), mosquito ( $p < 0.05$ ), and horsefly ( $p < 0.01$ ) were observed between farm and categories of sweet itch. A 2-way interaction between farm and sex was documented for mosquito ( $p < 0.05$ ) and horsefly ( $p < 0.01$ ). Mosquito specific IgE levels significantly differed by the interaction of farm and IDST results ( $p < 0.05$ ). Age did not correlate with IgE levels despite controlling for other influencing parameters. No significant effect of tested variables on IgE levels specific to black ant, deerfly, and *Culicoides variipennis* was observed. Insect specific IgE levels showed linear association among each other.

Our results demonstrate that current SAT reveals significant influences of environmental factors on circulating allergen-specific IgE levels in horses. However, even controlling of these factors in statistical analyses does not result in higher agreement between measured specific IgE concentration and clinical data.

### ABSTRACT #164

COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* COLONIZATION IN HORSES AND HORSE PERSONNEL. J. Scott Weese and Joyce Rousseau, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen in human medicine. Initially regarded as strictly a nosocomial pathogen, there are increasing reports of community-acquired MRSA (CA-MRSA) in humans. Recent evidence indicates that MRSA may be emerging as an equine pathogen. This study evaluated CA-MRSA in horses and humans that work with horses. Nasal swabs were collected from horses and horse personnel from farms with (targeted) or without (non-targeted) a history of equine MRSA infection or colonization and from equine veterinarians. Direct and enrichment culture were performed and isolates were typed using PFGE.

972 horses from 44 farms were enrolled. One to 177 horses were evaluated per farm (mean 22.1, SD 32.3). Methicillin-resistant *S. aureus* was isolated from 46/972 (4.7%) horses: 0/581 from farms without a previous history of MRSA infection or colonization and 46/391 (12%) from farms with previously identified MRSA infection or colonization. The prevalence of MRSA colonization in horses on individual farms ranged from 0-45%. All equine isolates were identified as subtypes of Canadian epidemic MRSA-5 (CMRSA-5). No colonized horses had been hospitalized within the preceding 3 months. Residing on a breeding farm or a farm with previously identified MRSA colonization were the only identified risk factors identified using multiple regression.

Methicillin-resistant *S. aureus* was isolated from 14/107 (13%) humans: 12/66 (18%) from targeted surveillance and 2/41 (5%) from non-targeted surveillances. Colonization of at least one human with an indistinguishable strain was identified in all farms with colonized horses. The number of colonized personnel per farm ranged from zero to six. Six were farm labourers, three were veterinarians in private practice, two were farm managers, two were farm veterinarians and one was a horse owner. Twelve of 14 (86%) colonized humans had recent contact with horses colonized with an indistinguishable subtype. All human isolates were subtypes of CMRSA-5, an uncommon isolate in humans Ontario. Recent contact with greater than 20 horses, and employment as a farm veterinarian were risk factors. No colonized humans reported recent hospitalization of themselves or a family member. If a positive result using either technique is considered to be the Gold Standard, the sensitivity for direct and enrichment techniques for equine samples were 43 and 100%, respectively ( $P < 0.0001$ ). For human samples, the sensitivity for both direct and enrichment culture was 79% ( $P = 1.0$ ).

Community-acquired MRSA colonization occurs in horses. This study further supports the suspicion that CMRSA-5 is adapted for survival in horses, and that transmission between horses and humans can occur. Identification of MRSA in horses should trigger an investigation of colonization of humans and horse personnel.

### ABSTRACT #165

DOES THE CHEMICAL FORM AND MOLECULAR CONFIGURATION OF RETINOIDS INFLUENCE IL-4, IL-10 AND IL-13 MRNA EXPRESSION IN EQUINE LYMPHOCYTES AND MACROPHAGES? C.P. Coyne, G. Haygood, M. Lawrence. Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University.

The investigation objective was to discover if different retinoid analogs can modulate mechanisms that regulate the transcription of IL-4, IL-10 and IL-13 Type Th2 interleukins in equine monocyte and lymphocyte cell populations. The research hypothesis proposed that the variables of chemical form and molecular configuration of retinoid analogs directly influences mechanisms that regulate the genetic expression of mRNA sequences for equine IL-4, IL-10 and IL-13 Type Th2 interleukins. The rationale guiding the investigations was based on the premise that the molecular pathogenesis of intense septic inflammatory reactions in horses can potentially be disrupted by modifying the genetic expression of IL-4, IL-10 and IL-13 Type Th2 interleukins.

Equine lymphocytes and monocyte-derived macrophages were isolated from fresh whole-blood using a combination of Percoll density-gradient media and differential adhesion. Cell populations were then exposed to three different chemical forms and three different molecular isomers of retinoid analogs [ $n = 8$ ; final concentration =  $10^{-5}$  M, (96 hrs; 37°C; CO<sub>2</sub> 5.0%)]. Cell populations were then disrupted to facilitate the harvest of total RNA followed by purification of mRNA fractions by poly (dT) affinity chromatography. Equine IL-4, IL-10 and IL-13 primer sequences were then applied to detect alterations in mRNA expression applying a conventional PCR schedule ( $n = 25$  to 30 cycles; 95°C for 1 min;

60°C for 1 min; 72°C for 2 min; PCR termination at 72°C for 5 min). Products from PCR reactions were then developed by electrophoresis in agarose gel containing a nucleotide fluorescent dye (1.5%; 100 volts; 3.5 hrs). Individual cDNA bands were visualized under ultraviolet light. Equine beta-actin, G3DPH and 18s rRNA were used as reference control gene sequences.

Equine lymphocytes and macrophages can both be induced to express IL-4, IL-10 and IL-13 Type Th2 interleukin mRNA sequences. Two of the three retinoid chemical forms evaluated altered equine IL-4, IL-10 and IL-13 mRNA expression. Similarly, two of three retinoid molecular isomers evaluated modified equine IL-4, IL-10 and IL-13 mRNA expression. Retinoid analogs were identified that can either enhance or suppress IL-1, IL-10 and IL-13 Type Th2 interleukin mRNA expression. One retinoid chemical form and one isomer type were superior in this regard.

Specific chemical forms and molecular isomers of retinoid analogs can modulate mechanisms that regulate equine IL-4, IL-10 and IL-13 mRNA transcription. Due to the immunological properties of these Type Th2 interleukins, retinoid analogs can potentially modify endogenous protective mechanisms and disrupt the molecular pathogenesis of intense septic inflammatory reactions independent of the species of 1<sup>o</sup> or 2<sup>o</sup> bacterial pathogens. Residual Benefit [i] potential discovery of genetic traits corresponding to septic disease resistance.

#### **ABSTRACT #166**

**DUODENAL MUCOSAL BIOPSY BY ENDOSCOPY IN HORSES WITH MALABSORPTION SYNDROME AND IN NORMAL HORSES.** D. Jean, J.P. Lavoie, P. Hélie, Faculté de Médecine Vétérinaire, Université de Montréal.

Malabsorption may be caused by several conditions in horses and most of them involve the small intestine. Histopathology of small intestinal biopsies performed under laparotomy are usually required to achieve diagnosis in most cases. The purpose of this study was to describe the results of duodenal mucosal biopsy obtained by digestive endoscopy in horses with malabsorption syndrome and in normal horses.

Six horses were considered normal on the basis of history, clinical examination, and normal oral glucose absorption test. Eight horses were considered to have malabsorption syndrome on the basis of weight loss, hypoproteinemia and/or an abnormal result of an oral glucose absorption test. Hypoalbuminemia were present in 6/8 (75%) horses. Results of the oral glucose absorption tests were abnormal in 8/8 horses (range between 20 to 80%). The duodenal and rectal mucosal biopsies including mucosal and submucosal (4-5 samples per horse) were performed in standing horses using a videoendoscope. The histologic diagnosis in horses with malabsorption syndrome included lymphocytic-plasmacytic enteritis (3 cases), non-specific infiltrative enteritis (2 cases), lymphocytic-plasmacytic-eosinophilic enteritis (2 case) and granulomatous enteritis (1 case). Histological results of rectal mucosal biopsies were different from duodenal mucosal biopsies in 4/6 horses; histologic examinations were considered normal in 4 horses that had an infiltrative enteritis diagnosed by duodenal mucosal biopsies. Duodenal biopsies from normal horses showed variable numbers of lymphocytes, plasma cells and/or eosinophils in the lamina propria and occasionally in the submucosa. Two of the normal horses had mucosal leucocytic infiltration that was compatible with a diagnosis of lymphocytic-plasmacytic enteritis. This procedure was well tolerated by horses except for occasional transient abdominal discomfort which was controlled by analgesics. In conclusion, duodenal mucosal biopsies performed under endoscopic guidance is well tolerated by horses. As normal horses may have a slight-moderate lymphocytic-plasmacytic infiltration of duodenal mucosal, results of duodenal biopsies in horses with malabsorption syndrome must be interpreted in light of the clinical findings. Despite this

limitation, duodenal biopsy may be an adjunctive diagnostic technique in cases of equine malabsorption syndrome. This research project was support by the GREMEQ.

#### **ABSTRACT #167**

Abstract Withdrawn

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#### **ABSTRACT #168**

**ADENOSINE MEDIATED RELAXATION OF THE EQUINE AORTIC VALVE *IN-VITRO*.** Bowen IM<sup>1</sup>, Marr CM<sup>1</sup>, Chester AH<sup>2</sup>, Wheeler-Jones CPD<sup>1</sup>, Elliott J<sup>1</sup>. <sup>1</sup>Royal Veterinary College, University of London. Hawkshead Lane, Hatfield. UK. <sup>2</sup>National Heart and Lung Institute, Imperial College London, Harefield. UK.

Aortic valve regurgitation is a common echocardiographic finding in the horse, even in the absence of structural valve pathology and is termed 'physiological regurgitation.' However the mechanisms that lead to physiological regurgitation and its subsequent association

with aortic valve disease are unknown. The aortic valve is a dynamic structure capable of contraction *in-vitro*. Although the significance of contraction has not yet been fully elucidated, it may contribute to diastolic competence *in-vivo*. While the ability of the aortic valve to relax has not been previously investigated, mechanisms that cause relaxation of the valve may be a factor in physiological regurgitation. The aim of this study was to investigate the ability of adenosine to cause relaxation of the normal equine aortic valve *in-vitro* and to identify the receptors involved in this response.

Equine aortic valves were harvested from horses killed at an abattoir that had no evidence of aortic valve disease at post-mortem examination. Segments of the right coronary cusp of the valve were suspended between wires attached to isometric force transducers, and bathed in a modified Krebs Henseleit solution (KHS) maintained at 37°C, aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Following equilibration, viability of the valves was assessed by contraction with a potassium containing solution. Valves were then washed in KHS and all re-equilibrated. Following pre-contraction with norepinephrine (10<sup>-6</sup>M), the cumulative relaxant effects of adenosine were assessed in parallel with a saline control. Cumulative relaxant effects of either the A<sub>2</sub> receptor selective agonist N-ethylcarboxamido adenosine (NECA; 10<sup>-11</sup>M to 10<sup>-7</sup>M) or the A<sub>1</sub> receptor selective agonist cyclopentyladenosine (CPA; 10<sup>-9</sup>M to 10<sup>-6</sup>M) were assessed in paired valve segments. Relaxant responses were expressed as a percentage of the contractile force generated by norepinephrine. Cumulative response curves were fitted using non-linear regression. Data are presented as arithmetic mean ± sem (maximum response) or geometric mean with 95% CI (EC<sub>50</sub> values).

Adenosine caused significant relaxation of the equine aortic valve (P=0.001) with a mean relaxation of 66% (±13.59). NECA was significantly more potent than CPA with EC<sub>50</sub> values of 1.4x10<sup>-9</sup> (1.2x10<sup>-10</sup> to 3.5x10<sup>-9</sup>) vs 1.3x10<sup>-7</sup> (6.3x10<sup>-8</sup> to 12.4x10<sup>-7</sup>) respectively (p<0.01). The maximum relaxant responses to NECA and CPA did not differ significantly (87.7 ±17.4 vs 103.6 ±74.6) respectively.

These data show for the first time that valvular tissue possess receptors mediating relaxation in addition to those mediating contraction. Adenosine causes relaxation of the valve cusps through valvular A<sub>2</sub> receptors. The characterisation of adenosine mediated relaxation of the equine aortic valve contributes to our knowledge of valve function in the horse. Further studies are necessary to evaluate differences between normal and diseased valves and those with physiological regurgitation.

#### ABSTRACT #169

ELECTROCARDIOGRAPHIC AND HEMODYNAMIC EFFECTS OF THE CALCIUM-CHANNEL BLOCKER DILTIAZEM IN HORSES. C.C. Schwarzwald, J.D. Bonagura, V. Luis Fuentes. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Quinidine is an effective treatment for atrial fibrillation (AF) in horses, but often accelerates ventricular response rate. Diltiazem controls heart rate response to AF in other species, but has not been studied in horses. This investigation examined the effects of diltiazem on cardiac rate and rhythm, systolic and diastolic left ventricular (LV) function, central hemodynamics, and peripheral blood flow in normal, standing, non-sedated horses instrumented under local anesthesia.

Eight healthy horses were treated with IV diltiazem every 30 minutes to achieve cumulative dosages of 0 mg/kg (saline control), 1 mg/kg, 1.5 mg/kg, and 2 mg/kg. Doses were based on results of a previous dose-finding study. Plasma diltiazem concentration, ECG (HR, rhythm, PR interval), LV function (±dp/dt<sub>max</sub>, LVEDP, *Tau*), central hemodynamics (RAP, PAP, aortic pressure, CO by thermodilution, SVR), LV dimensions (by echocardiography), and forelimb blood flow (by duplex Doppler sonography) were measured or calculated during each treatment period. Data were analyzed using

one-way ANOVA for repeated measures. When mean values were statistically different (p <.05), Dunnett's test was used to compare treatment effects to baseline.

Diltiazem plasma concentrations between 390 and 910 ng/ml were achieved, with considerable variation among horses. Diltiazem increased mean ventricular rate insignificantly and increased atrial rate significantly. Variable degrees of sinoatrial and atrioventricular blocks were observed. The PR interval during conducted beats was prolonged significantly. Systemic blood pressure decreased, while right atrial, pulmonary arterial, and end-diastolic LV pressures increased significantly. Significant decreases in LV fractional shortening, ±dp/dt<sub>max</sub> and -dp/dt<sub>max</sub>, and a small but significant increase in *Tau* were measured. Cardiac output did not change, but stroke volume declined non-significantly (p=.506) at the highest dose range. Systemic vascular resistance decreased significantly at all treatment periods. Significant increase in diameter of the brachial artery and decrease in the resistive index of blood flow to the forelimb were demonstrated. Two horses developed high-grade sinus arrest with clinically significant systemic hypotension.

*Cardiac effects* of diltiazem, at 1 to 2 mg/kg IV, included mild impairment of systolic and diastolic LV function and intermittent depression of the sinus and AV nodes. *Vascular effects* of diltiazem included arterial vasodilation, increased limb blood flow, and decreased systemic vascular resistance. The fall in ABP seemingly invoked the baroreceptor reflex, causing sympathetic activation that increased sinus node rate, and presumably blunted the depressive effects of diltiazem on myocardial and nodal tissues. Diltiazem appears relatively safe in healthy horses, but dose is critical, and use may be limited by hypotension from vasodilation and direct suppression of sinus node discharge. Further studies are required to determine the pharmacokinetic profile of diltiazem, the potential frequency-dependence of diltiazem effects on nodal tissues, and the effects and safety of combined treatment with diltiazem and quinidine in horses with AF.

#### ABSTRACT #170

DETERMINATION OF GLUTATHIONE AND LIPID PEROXIDATION IN FELINE PERIPHERAL BLOOD CELLS USING FLOW CYTOMETRY. C. Webb, S Dow, M Lappin, A Guth, D Twedt, Colorado State University, Fort Collins, CO.

Reduced glutathione (GSH) plays a critical role in maintaining intracellular oxidative balance and neutralizing potentially harmful free radicals. Cell membrane lipid peroxidation (LPO) is a common and deleterious consequence of oxidative imbalance and may result in loss of cell function, cell death, or hemolysis in the case of erythrocytes. There are very few clinically accessible assays for either of these important components of oxidative stress in samples from feline patients. This study investigates the use of flow cytometry to measure these parameters.

Sixty-nine clinical samples of feline peripheral blood were analyzed for GSH levels and susceptibility to lipid peroxidation. Monochlorobimane (mBCl) (Molecular Probes™) was used to determine relative intracellular GSH levels. mBCl conjugates with intracellular reduced glutathione to form a fluorescent product whose mean intensity is linearly correlated with GSH concentration. The fluorescence of the BODIPY581/591 (Molecular Probes™) fluorophore shifts from red to green upon peroxidation (stimulated by cumene hydroperoxide), allowing for the ratiometric measurement of lipid oxidation in live cells. The fluorescence of these molecular probe reactions is captured and analyzed using a DakoCytomation™ 3-laser 9-color Cyan flow cytometer.

The distinct scatter pattern and appropriate gating paradigm allowed for the separate analysis of neutrophils, monocytes, and lymphocytes from a sample in which the RBCs had been lysed (NH<sub>4</sub>Cl). Cell identity was confirmed using fluorescent antibodies directed towards specific cell surface markers. The relative amount



of GSH was significantly greater in neutrophils than in monocytes, and both cell types had significantly greater GSH levels than lymphocytes. The samples were then grouped according to diagnosis and included healthy controls, cats undergoing  $I^{131}$  treatment, anemic animals, cats with neoplasia, and 'other disorders'. The results suggest that diseased cats in general have significantly greater levels of intracellular leukocyte GSH than healthy animals.

Applying the BODIPY assay to 13 cats experimentally infected with hemobartonella (separate study) revealed that as the infection progressed two distinct populations of erythrocytes became apparent. Those RBCs with low levels of GSH had high levels of peroxidation, while those cells with high levels of GSH had lower levels of peroxidation.

Flow cytometry revealed distinct differences in important parameters of oxidative stress both between cell types (WBCs) and within a single cell type (RBCs). Furthering our understanding of the role of oxidative stress in feline diseases may help direct future intervention (i.e. GSH supplementation in cats with RBC parasitemia).

#### ABSTRACT #171

LONG-TERM COMBINED ANTI-RETROVIRAL THERAPY (CART) IN FELINE IMMUNODEFICIENCY VIRUS INFECTED CATS: A CASE REPORT. J. Huebner<sup>1</sup>, D. Klein<sup>2</sup>, E. Müller<sup>1</sup>, T.W. Vahlenkamp<sup>3</sup>, I. Langbein<sup>1</sup>. LABOKLIN, Bad Kissingen, Germany <sup>1</sup>, Institute for Virology, University of Veterinary Medicine Vienna, Austria<sup>2</sup>, Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA<sup>3</sup>.

This case report describes the long-term treatment of a feline immunodeficiency virus (FIV)-infected cat with a combination of different anti-retroviral drugs. The cat showed clinical signs similar to the *AIDS Related Complex* (ARC) stage of the infection using the classification for human immunodeficiency virus (HIV) infection of the Center of Disease Control (CDC). The *combined anti-retroviral therapy* (CART) consisted of the simultaneous application of ABC (ABACAVIR<sup>TM</sup>), PMPA [9-(2-phosphonylmethoxyethyl)adenine] (VIREAD<sup>TM</sup>), and 3TC (LAMIVUDIN<sup>TM</sup>), drugs also used in *highly active anti-retroviral therapy* (HAART) treatment regimen in HIV-infected patients. The treatment was supplemented by feline Interferon-omega (IFN $\omega$ ).

Therapy was followed up by routine clinical investigations and by the measurement of several diagnostic parameters. Measurement of the immune status was performed using fluorescence activated cell sorter (FACS) analysis to determine the role of CART on the different lymphocytesubpopulations. The immune status was measured four times during a treatment period of one year. The results were compared with data obtained from 26 FIV-infected and 36 uninfected cats sent to our laboratory during this time period for routine diagnosis. In addition proviral load was determined from the cat receiving CART by real-time PCR.

During the treatment period the general health status of the cat improved, although the improvement was accompanied by intermittent periods of clinical disease. FACS analysis revealed that all T cell populations increased remarkably. The analysis of the CD4<sup>+</sup>/CD8<sup>+</sup>-cell ratio showed that CART resulted in a sustained increase with almost reference range values of normal uninfected cats at the end of the study. In concordance with the improvement of the immune status of the cat the proviral load decreased significantly by more than 2 log.

Previous studies using single drug treatment regimen in FIV-infected cats showed to be of only limited clinical value. To the best of our knowledge this is the first long-term follow up study of a diseased, chronically FIV-infected cat receiving CART. Data reveal that CART results in the improvement of the clinical and immunological status of infected cats and also results in reduced viral

loads. Establishing of flow cytometric analysis in routine diagnostics is a new tool for therapy control of immune-mediated diseases, like FIV infection of cats. It should, however, to be emphasized that CART requires a high degree of commitment to ensure the continuous application of the drugs.

#### ABSTRACT #172

IN VITRO LYMPHOCTE RESPONSE IN VACCINATED DOGS TO CANINE DISTEMPER VIRUS VACCINE. MA Barry<sup>1</sup>, JP Woods<sup>1</sup>, SA Kruth<sup>1</sup>, D Bienzle<sup>2</sup>, P Shewen<sup>2</sup>, <sup>1</sup>Department of Clinical Studies, <sup>2</sup>Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

For many years vaccines have provided an important means of inducing immunity against infectious diseases in domestic animals, however, there is little evidence that annual re-vaccination is required to maintain protective immunity. With the increased recognition of adverse vaccine reactions, vaccine-associated immune-mediated disorders, and post-vaccinal canine distemper encephalitis, veterinarians and owners are questioning the necessity of annual re-vaccinations. Ideally, re-vaccination would be limited to those animals at risk because they no longer have protective immunity, but such animals are difficult to identify at present.

This study was an investigation of the response of lymphocytes from vaccinated dogs to *in vitro* exposure with canine distemper virus (CDV) vaccine and lymphocyte mitogens. The objectives of this study were to develop an *in vitro* method of measuring the response of lymphocytes to CDV vaccine in previously vaccinated dogs to determine which subset(s) of lymphocytes respond to CDV antigens, and to compare this with the response of lymphocytes to non-specific mitogens.

Blood samples were collected from 15 clinically healthy, vaccinated dogs. Peripheral blood mononuclear cells were separated by density gradient centrifugation and lymphocytes were cultured for 72 hours with concanavalin-A (Con A) 7.5  $\mu$ g/ml; pokeweed mitogen (PWM) 7.5  $\mu$ g/ml; canine distemper modified-live virus vaccine (Onderstepoort strain) 1:10 concentration; or no addition (unstimulated controls). Following the culture period, lymphocyte proliferation was measured by Trypan blue exclusion and tritiated thymidine incorporation. Flow cytometry was then used to quantitate specific lymphocyte subsets following incubation with monoclonal antibodies to canine CD4, CD8, and CD21-like molecules. Finally, CDV titers were determined by fluorescent antibody assay.

Significant lymphocyte proliferation ( $P = 0.001$ ) was found following incubation with Con A, PWM, and CDV antigen when compared to unstimulated controls. When lymphocyte subsets were examined by flow cytometry no significant difference in the proportion of cells labeled with CD4 ( $P = 0.6772$ ), CD8 ( $P = 0.829$ ), or CD-21-like ( $P = 0.112$ ) antibodies were found between CDV antigen exposed lymphocytes and unstimulated controls. There were significant differences in subgroups of lymphocytes stimulated with Con A and PWM ( $P < 0.05$ ). When CDV titers were compared to lymphocyte subsets in the CDV stimulated cells no correlation was found ( $P > 0.05$ ).

These findings suggest CDV vaccine is able to stimulate canine lymphocytes to proliferate *in vitro*, but does not produce a detectable change in the proportion of lymphocyte subsets as measured by the monoclonal antibodies used in this study. It is possible that with more specific monoclonal antibodies to detect memory T and B cells or a more sensitive assay a measurable difference in the proportion of cells responding to CDV antigen stimulation could be measured.

## ABSTRACT #173

TREATMENT OF OBESITY IN DOGS THROUGH INCREASING ENERGY EXPENDITURE BY MITOCHONDRIAL UNCOUPLING PROTEINS. K. Ishioka<sup>1</sup>, M. Sagawa<sup>2</sup>, M. Okumura<sup>3</sup>, A. Omachi<sup>1</sup>, H. Shibata<sup>4</sup>, T. Honjoh<sup>4</sup>, K. Kimura<sup>1</sup>, M. Saito<sup>1</sup>. <sup>1</sup>Laboratory of Biochemistry, Department of Biomedical Sciences, and <sup>3</sup>Laboratory of Surgery, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo; <sup>2</sup>Nippon Pet Food Co., Ltd. Research Center, Iwata; <sup>4</sup>Morinaga Institute of Biological Science, Yokohama, Japan.

Obesity is the most common nutritional disorder in small animal practice. Although it is the severe risk factor for a number of diseases in dogs and cats, recent findings based on the molecular biology of obesity have not been fully applied to veterinary medicine. To establish a novel approach to the treatment of obesity in dogs, we focused on the mitochondrial uncoupling protein (UCP). UCP is a proton transporter, which dissipates the electrochemical gradient across the inner mitochondrial membrane, and thereby uncouples oxidative phosphorylation to result in heat production. Thus, UCP is implicated in the regulation of energy expenditure, and expected to be a target molecule for prevention and treatment of obesity. We cloned three isoforms of canine UCPs 1, 2 and 3, and revealed that their structure and tissue distribution were almost identical to those of other species including rodents and humans. We also reported that beta-3 adrenergic receptor agonists increased UCP expression in adipose tissue and thereby reduced body fat content in dogs. Gene expression of UCPs is also regulated by a peroxisome proliferator-activated receptor (PPAR), which is a nuclear receptor using polyunsaturated fatty acids (PUFA) as effective ligands. We investigated the effects of dietary PUFA on adiposity in dogs.

Seven obese male beagles were divided into two groups, and one (14.1±0.5 kg, n=4) was fed on a food rich in fish oil (n-3 PUFA, 4.1%), and the other (13.9±0.7 kg, n=3) on a tallow food (n-3 PUFA, 0.2%). The amount of energy intake was restricted to 620 kcal/day in the both groups. Blood biochemistry including leptin and adiponectin, the cytokines secreted by adipocytes, computed tomography (CT) and UCP mRNA analyses were performed before and after a 14-week period.

Body weight decreased on the both groups, but significantly more on the fish oil diet (10.9±0.3 kg at week 14) than the tallow diet (12.4±0.3 kg at week 14). Total body fat assessed by CT, and serum leptin levels decreased more on the fish oil diet. Serum adiponectin levels tended to increase only on the fish oil diet. Northern blot analysis revealed that the UCP2 mRNA level did not change in adipose tissue and skeletal muscle, but the UCP3 mRNA level in skeletal muscle increased on the fish oil diet compared with that on the tallow diet.

These results harmonize with those reports in mice, and indicate that dietary fish oil has an anti-obesity effect in dogs, probably by increasing UCP expression and energy expenditure. We conclude that UCPs are hopeful targets for treatment of obesity in dogs.

## ABSTRACT #174

THE EFFECTS OF DIETARY REGIME AND HORMONAL STATUS ON GROWTH AND BODY COMPOSITION IN GROWING KITTENS. Calvert EL, Wilkinson J, Callcut TA, Butterwick RF. The WALTHAM Centre for Pet Nutrition, Waltham on the Wolds, Leicestershire, UK.

Diet offered post-weaning might be predictive of growth in later life, with high fat diets leading to increased weight gain<sup>1</sup>. The role of sex hormones on weight gain have also been assessed but the combined effects of post-weaning diet, differing in fat content, and neuter status has not been studied in cats. It was the aim of this study to assess these parameters

Methods: 24 (12 male, 12 female: all neutered) kittens were fed diet 1 (Whiskas® Cocktail Kitten: 12g fat/400 kcal) and 36 kittens (12 male (neutered), 12 female (neutered), 12 female (entire) were fed diet 2 (Whiskas® Advance Growth 20g fat/400kcal) diets from wk 10-52. Cats were neutered at 19 wks. The following parameters were measured: energy intake (daily), body weight (wkly), DXA, body condition score (BCS), biochemistry & haematology (at 10, 18, 30 & 52 weeks).

Results: Diet 1 vs diet 2 comparisons: At 10 weeks, energy intake (kcal/day) was higher on the diet 2; mean (95%CI): 198 (185,212) vs diet 1 153 (140,166) diet; p<0.001. This was not significant at any other time point (18 wk 283 (266,301) vs 270 (253,288); 30 wk 334 (307,360) vs 341 (315,368), 52 wk 235 (209,260) vs 262 (237,288). Bone mineral density was higher in the cats fed diet 2 from 18 wks. BCS and blood parameters were not different in cats fed diet 1 or 2.

Entire vs neutered comparisons: Energy intake and body weight was significantly higher in neutered vs entire cats at wk 52 [Intake Kcal/day; mean (95%CI) 10 wk: neutered 188 (166,210) vs entire 192 (170,214); 18 wk 230 (207,253) vs 237 (214,260); 30 wk 295 (272,319) vs 216 (192-240); 52 wk 216 (192,241) vs 184 (160,209) p<0.001]. [Body weight (g) 10 wk: neutered 916 (823,1009) vs entire 964 (871,1056); 18 wk 1930 (1761,2098) vs 2034 (1866,2202); 30 wk 3161 (2863,3460) vs 2982 (2683-3280); 52 wk 4118 (3697,4540) vs 3380 (2959,3802) p=0.018]. Bone mineral density, % body fat & BCS were significantly higher in neutered cats at wk 52 but there were no differences in blood parameters.

Conclusions: Kittens were able to regulate their kcal intake on diets of different fat content from an early age, however neutering at week 19 had a significant effect on intake and weight gain, as previously described<sup>2</sup>.

<sup>1</sup>Thorsdottir 2003, IJO 27:S9. <sup>2</sup>Harper et al, JSAP 42: 433

## ABSTRACT #175

PHARMACOKINETICS OF DEXTROMETHORPHAN AND ITS ACTIVE METABOLITE DEXTROPHAN FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF DEXTROMETHORPHAN. Butch KuKanich and Mark Papich, North Carolina State University, Raleigh, NC.

Dextromethorphan is an N-methyl-d-aspartate noncompetitive (NMDA) antagonist, which has been used as an antitussive, analgesic adjunct, probe drug, experimentally to attenuate acute opiate and ethanol withdrawal, and as an anticonvulsant. A metabolite of dextromethorphan, dextrophan, has been shown to behave pharmacodynamically in a similar manor to the parent compound.

The purpose of the study was to evaluate the pharmacokinetics of dextromethorphan and dextrophan in 6 healthy beagle dogs following intravenous and oral administration of dextromethorphan. Dogs were administered a single dose of dextromethorphan (2.2 mg/kg IV and 5 mg/kg PO) in a randomized crossover design. Intravenous injections were made through an indwelling catheter over a period of 3 minutes, which was then flushed with 10 cc of saline. Oral dextromethorphan was administered in gelatin capsules, followed by 30 cc of water to insure the capsule was swallowed. Dextromethorphan was analyzed via high pressure liquid chromatography with fluorescence detection. Intravenous plasma samples were analyzed by compartmental and noncompartmental analysis with commercially available software.

Dextromethorphan behaved in a similar manor to other NMDA antagonists upon intravenous injection causing muscle rigidity, ataxia to recumbence, sedation, urination, and ptialism, all of which resolved within 90 minutes. One dog consistently vomited following repeated oral administration. Mean ± SD values for half-life, volume of distribution, and clearance after IV dextromethorphan administration were 1.76 ± 0.33 hours, 5.01 ± 1.69 L/kg, and 34.36 ± 16.66 ml/min/kg. Oral bioavailability was 12.3 ± 8.1%. Free dextrophan was not detected in any plasma sample.

Pharmacokinetic variables could not be calculated for dextromethorphan following oral administration due to its poor oral bioavailability and erratic plasma profile. Dextrophan glucuronides were present in the plasma. Dextromethorphan's short half-life, rapid clearance, and poor bioavailability of limit its potential use as a chronic orally administered therapeutic.

#### **ABSTRACT #176**

PHARMACOKINETICS OF TRAMADOL AND ITS ACTIVE METABOLITE O-DESMETHYLTRAMADOL FOLLOWING INTRAVENOUS AND ORAL ADMINISTRATION OF TRAMADOL AND INTRAVENOUS O-DESMETHYLTRAMADOL. Butch KuKanich and Mark Papich, North Carolina State University, Raleigh, NC.

Tramadol is an analgesic and antitussive agent that is metabolized to O-desmethyltramadol (M1) which is also active. Tramadol and M1 exert their mode of action through complex interactions between opiate, adrenergic, and serotonin receptors. Tramadol, and subsequently, M1 exist as a 50:50 racemic mixture, with each racemate exerting different pharmacodynamic effects. However studies have previously shown the majority of analgesia present from tramadol administration is due to the activity of M1.

The purpose of the study was to evaluate the pharmacokinetics of tramadol and M1 following intravenous and oral tramadol administration to 6 healthy dogs. Tramadol HCl was administered intravenously (4.4 mg/kg) and orally (11.2 ± 2.0 mg/kg) in a randomized crossover design. Additionally, M1 (1 mg/kg) was administered intravenously to 3 randomly chosen dogs. Only 3 dogs were dosed with M1 due to limited supply. Intravenous dosing was performed through an indwelling cephalic catheter, which was flushed with 10 cc of saline following drug administration. Tablets were administered orally followed by 30 cc of water. Plasma samples, urine samples, stability of the injection solution, and stability of an oral suspension were assessed via high pressure liquid chromatography with fluorescence detection.

Intravenous administration of tramadol best fit a 2 compartment model in 5/6 dogs with the remaining dog best fit to a one compartment model. Oral tramadol administration and M1 plasma profiles following oral and intravenous tramadol administration best fit a 1 compartment model with a first order input. Intravenous and oral tramadol along with M1 profiles following tramadol administration were also modeled with noncompartmental analysis. Intravenous M1 administration was modeled with noncompartmental analysis. Tramadol and M1 were both detected in the urine of dogs administered tramadol orally and intravenously. The calculated parameters for half-life, volume of distribution, and total body clearance for tramadol were 0.80 ± 0.12 hr, 3.79 ± 0.93 L/kg, and 54.63 ± 8.19 mL/kg/min respectively (mean ± standard deviation) following intravenous tramadol administration. The systemic availability was 65 ± 38% and half-life 1.71 ± 0.12 hr following oral tramadol. M1 had a half-life of 1.69 ± 0.45 and 2.18 ± 0.55 hr following intravenous and oral administration of tramadol, respectively. Following intravenous M1 administration the half-life, volume of distribution, and clearance of M1 were 0.94 ± 0.09 hr, 2.80 ± 0.15 L/kg, and 34.93 ± 5.53 mL/kg/min respectively. Tramadol oral suspension and solutions for injection were >90% stable at 92 days when refrigerated. Simulated oral dosing regimens in dogs at 5 mg/kg every 6 hours and 2.5 mg/kg every 4 hours predict tramadol and M1 plasma concentrations consistent with analgesia in humans, however efficacy studies need to be performed.

#### **ABSTRACT #177**

MARGIN OF SAFETY OF THE ANESTHETIC ALFAXAN<sup>®</sup>-CD RTU IN DOGS AT 0, 1, 3 AND 5X THE INTRAVENOUS DOSE OF 2 MG/KG. Schnell M, Weiss C, Heit M, \*Whittem T and \*Pasloske K. Provident Preclinical Inc., Doylestown, PA; \*Jurox, Rutherford, Australia.

Neuroactive steroids produce hypnosis and muscle relaxation by enhancing the inhibitory effect of gamma-amino butyric acid (GABA) on the GABA<sub>A</sub> receptor chloride channel complex. Alfaxalone (Alfaxan<sup>®</sup>-CD RTU, Jurox, Australia) is a steroid hypnotic drug developed for both induction and maintenance of anesthesia in dogs. The primary objective of this study was to investigate the gross and microscopic pathology and clinical side effects caused by Alfaxan<sup>®</sup>-CD RTU at clinical and exaggerated doses. Twenty-four (24) healthy beagle dogs (12M/12F) were randomly assigned to four (4) treatment groups of eight animals (4M/4F) each. Dogs were intravenously administered either 0.9% saline (0x), or Alfaxan<sup>®</sup>-CD RTU at 2 mg/kg, 6 mg/kg, or 10 mg/kg (1, 3 and 5X the label dose) q 48 hr for three (3) treatments. Study parameters included the following; clinical observations, mortality, body weight (kg), physical examination, body temperature (°C), clinical pathology (hematology, chemistry, coagulation, urine analysis and fecal analysis), anesthetic examinations (heart rate and rhythm [beats/min; lead II ECG], pulse [/min], direct blood pressure [mm Hg], hemoglobin saturation [%SpO<sub>2</sub>] and respiratory rate [breaths/min]), anesthetic event times and injection site observations. Forty-eight (48) hr after the last treatment, animals were euthanized, necropsied and tissues were submitted for histopathology. Kaplan-Meier curves were used for comparison of anesthetic event data across treatment groups using the log rank test. Other quantifiable parameters were analyzed using an ANCOVA model with baseline values as covariates. A P < 0.05 was considered significant.

All animals appeared clinically healthy throughout the study. Dogs consumed normal quantities of food, had stable body weights and normal physical examinations and injection sites throughout the study. There were no unscheduled deaths. Blood chemistry, hematology, coagulation, urine, fecal and ECG analyses showed no effects attributable to the test article. Statistical examination of anesthetic event times showed a dose proportional treatment effect for time to onset of recumbency, duration of non-responsiveness to stimulus, duration of anesthesia and duration of recumbency amongst the Alfaxan<sup>®</sup>-CD RTU treatment groups. There were no significant treatment-related changes in body temperature or respiratory rate. At 10 and 20 min after Alfaxan<sup>®</sup>-CD RTU dose administration, heart rate and pulse rate significantly increased as dose increased. At 10 min post Alfaxan<sup>®</sup>-CD RTU treatment, systolic (also at 30 min), diastolic and mean blood pressure all decreased as dose increased. A dose-dependent decrease in hemoglobin saturation was seen at 2 and 4 min post Alfaxan<sup>®</sup>-CD RTU administration. All animals recovered from anesthesia uneventfully and without need for artificial support. Blinded gross and histological examination of tissues showed a no observed effect level (NOEL) of at least 10 mg/kg. The intravenous anesthetic Alfaxan<sup>®</sup>-CD RTU has at least a 5X margin of safety (i.e., 10 mg/kg) in dogs both clinically and pathologically.

#### **ABSTRACT #178**

SAFETY AND EFFICACY OF ALFAXAN<sup>®</sup>-CD RTU ADMINISTERED ONCE TO CATS SUBCUTANEOUSLY AT 10 MG/KG. Heit M, Schnell M, \*Whittem T and \*Pasloske K. Provident Preclinical Inc., Doylestown, PA; \*Jurox Pty. Ltd., Rutherford, Australia.

Alfaxalone was first introduced as a veterinary injectable anesthetic agent in combination with alfadalone as Saffan<sup>®</sup> in 1971. Because the solubilizing agent, Cremaphor-EL<sup>®</sup>, causes histamine release, Jurox Pty. Ltd. has developed a water-soluble formulation using 2-hydroxypropyl-beta-cyclodextrin (CD). The anesthetic drug

formulation is marketed in Australia as Alfaxan<sup>®</sup>-CD RTU for intravenous (2-5 mg/kg) and intramuscular (5-10 mg/kg) use for induction and maintenance of anesthesia in cats. The objective of this study was to determine the safety and efficacy of Alfaxan<sup>®</sup>-CD RTU when administered to six cats (3M/3F) subcutaneously at 10 mg/kg. Animals were administered acepromazine (1.1 mg/kg IM) 25 minutes prior to subcutaneous injection of Alfaxan<sup>®</sup>-CD RTU in the right scapular area. Animals also received an equivalent volume of 0.9% NaCl in the left scapular region. Study parameters included clinical observations, body weight, mortality, physical examination, hematology and serum chemistry, anesthetic event times (onset and duration of recumbency, anesthesia, and non-responsiveness to noxious stimuli), overall anesthetic score, injection site evaluation and gross examination of the injection site. Where appropriate, means and standard deviations were calculated and compared.

All animals remained healthy throughout the study and had stable body weights and physical examination parameters. There were no changes in hematology during the study period. Increases in AST (~2X) and CPK (~6X) were observed after acepromazine and Alfaxan<sup>®</sup>-CD RTU administration. Animals became recumbent approximately 30 minutes (28 ± 7 min) after subcutaneous injection. Recumbency lasted 2 h, on average but was variable (2.0 ± 1.28 h). While recumbent, animals were immobile and relaxed, however, they never became anesthetized enough to be intubated. Jaw tension, gag reflex and response to noise and touch were maintained throughout the period of recumbency. All animals recovered uneventfully. The injection sites were normal twenty four hours following dose administration and upon gross necropsy examination. Overall anesthetic score was not calculated since animals never became fully anesthetized.

Alfaxan<sup>®</sup>-CD RTU, when administered subcutaneously to cats at 10 mg/kg, provided safe, deep sedation. However, the variability of the duration of recumbency (range 0.9 to 3.8 h) and inability to intubate animals show that subcutaneous Alfaxan<sup>®</sup>-CD RTU (10 mg/kg) is unsuitable for induction of anesthesia when combined with intramuscular acepromazine (1.1 mg/kg).

#### ABSTRACT #179

PLASMA PHARMACOKINETICS OF ALFAXALONE IN CATS AFTER ADMINISTRATION AT 5 AND 25 MG/KG AS AN INTRAVENOUS BOLUS OF ALFAXAN<sup>®</sup>-CD RTU. Heit M\*, Pasloske K#, Whittem T#, Ranasinghe MG#, Li Q#. \*Provident Preclinical, Doylestown, PA; #Jurox Pty Ltd, Rutherford, Australia.

Alfaxalone was first introduced into veterinary medicine in 1971 in combination with alfadalone as Saffan<sup>®</sup>, an intravenous (IV) anesthetic agent with the excipient Cremophor-EL<sup>®</sup>. However, adverse effects reported in the dog and cat were attributed to the release of histamine associated with the excipient, Cremophor-EL<sup>®</sup>. Alfaxalone has recently been reformulated (Alfaxan<sup>®</sup>-CD RTU, Jurox Pty Ltd, Rutherford, Australia) using only the alfaxalone active and with the much safer excipient, 2-hydroxypropyl-beta cyclodextrin. This study was undertaken to determine the pharmacokinetic parameters of alfaxalone in cats at clinical and super-clinical doses and to test for dose or period effects.

Eight, adult, healthy domestic short haired cats were included in the study. Alfaxan<sup>®</sup>-CD RTU was administered to un-premedicated cats, IV at 5 and 25 mg alfaxalone/kg bodyweight in a two period balanced randomized crossover design. Plasma concentrations of alfaxalone were assayed at various times using LC/MS and the resulting data were analyzed by compartmental pharmacokinetic analysis. Comparisons used ANOVA or paired Student's T tests with P < 0.05 considered significant.

The mean durations of anesthesia (lateral recumbency to head lift) and recumbency (lateral recumbency to standing) were 44 ± 17, 68 ± 11 min and 172 ± 16, 204 ± 35 min for the 5 and 25 mg/kg doses respectively. Alfaxalone plasma concentrations for all cats at both

doses were adequately described by a 2 compartment open model. The mean terminal plasma elimination half-lives ( $t_{1/2}$ ) were 52 ± 17 and 74 ± 27 min for the 5 and 25 mg alfaxalone/kg doses, respectively (p = 0.008). The volumes of distribution (V<sub>d</sub>) for the 5 and 25 mg/kg doses were 0.589 ± 0.231 and 0.325 ± 0.123 L/kg, respectively (p = 0.009). The mean plasma clearances for the 5 and 25 mg/kg doses were 28.4 ± 9.2 and 16.1 ± 1.4 mL/kg/min respectively (p = 0.003). At 5 but not 25 mg/kg plasma clearance increased in the second period by 65% (p = 0.011).

Alfaxan<sup>®</sup>-CD RTU administered IV to cats provided effective and convenient anesthesia. Although alfaxalone clearance appears to be dose-dependent, total clearance is rapid at both dose rates tested. At the intended labeled dose rate there may be an induction of clearance mechanisms. Both the volume of distribution and terminal elimination half life also differed between 1x and 5x the intended label dose.

#### ABSTRACT #180

PHARMACOKINETICS OF INTRAVENOUS LIDOCAINE AND ITS ACTIVE METABOLITE, MONOETHYLGLYCINEXYLIDIDE, IN CATS ANESTHETIZED WITH ISOFLURANE. S.M. Thomasy<sup>1</sup>, B. Pypendop<sup>2</sup>, S. Stanley<sup>2</sup>, J. Ilkiw<sup>1</sup>. <sup>1</sup>KL Maddy Equine Analytical Chemistry Laboratory, <sup>2</sup>Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA.

Lidocaine is most commonly used in small animal medicine as a local anesthetic or an anti-arrhythmic agent. However, lidocaine also has analgesic properties and has been reported to decrease the requirement for both inhalant and injectable anesthetic agents. This study investigated the pharmacokinetics of intravenously administered lidocaine and its active metabolite, monoethylglycinexylidide (MEGX), in nine healthy adult cats anesthetized with isoflurane.

Anesthesia was induced with isoflurane in oxygen by use of an induction box and a face mask. Following intubation with a cuffed endotracheal tube, anesthesia was maintained with isoflurane in oxygen via a Bain circuit using a fresh gas flow rate of 500 ml/kg/min. The minimum alveolar concentration (MAC) of isoflurane was determined in each cat and end-tidal isoflurane concentration was then maintained at 0.75 times the individual's MAC. After a 15 min equilibration period, a bolus of 2 mg/kg of lidocaine was administered intravenously (IV). Venous blood samples were collected immediately prior to and 1, 2, 4, 8, 16, 30, 60, 90, 120, 150, 180, 210, and 240 minutes after lidocaine administration. Plasma lidocaine and MEGX concentrations were determined using liquid chromatography/mass spectrometry. A three compartment model best described the decline in plasma lidocaine concentrations, while plasma MEGX concentrations were analyzed noncompartmentally.

The MAC of isoflurane was 2.31% ± 0.21%. Following IV lidocaine administration, the mean (± SD) extrapolated peak lidocaine plasma concentration at time zero was 10.3 ± 6.6 µg/ml, the volume of distribution (V<sub>d</sub>) of the central compartment was 0.25 ± 0.11 L/kg, V<sub>d</sub> at steady state was 1.31 ± 0.43 L/kg, total body clearance was 19.8 ± 6.0 ml/min·kg, and the elimination half-life ( $t_{1/2}$ ) was 58.1 ± 19.6 min. The MEGX area under the concentration-time curve (AUC) to lidocaine AUC ratio was 0.11 ± 0.06. A peak plasma MEGX concentration of 95.3 ± 32.6 ng/ml occurred 18.9 ± 9.0 min after IV lidocaine administration. The MEGX elimination  $t_{1/2}$  of 74.3 ± 25.9 min was significantly longer than the lidocaine elimination  $t_{1/2}$ .

The three-compartment model and elimination  $t_{1/2}$  of lidocaine were consistent with those reported in cats anesthetized with halothane and nitrous oxide (N<sub>2</sub>O). However, the V<sub>d</sub> of the central compartment was higher in the present study than in the halothane/N<sub>2</sub>O study and may be due to the different anesthetic agents used. These pharmacokinetic

parameters provide information necessary for determination of suitable lidocaine loading and infusion doses in isoflurane anesthetized cats and will be used in future analgesic and anesthetic studies of lidocaine.

#### **ABSTRACT #181**

**INTRACELLULAR ACCUMULATION OF THE ACTIVE CYTOTOXIC METABOLITE OF GEMCITABINE IN DOGS.** Martin-Jimenez, T., Freise, K.J.. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Gemcitabine (dFdC) is a relatively new chemotherapeutic agent in humans and its use in canine oncology is being actively explored with mixed responses. Results in humans and other species indicate improved therapeutic outcome and increased intracellular accumulation of the active cytotoxic metabolite, gemcitabine triphosphate (dFdCTP) when doses are given over a long infusion relative to a short bolus dose. Additionally, it appears that plasma and extracellular dFdC concentrations exceeding a certain threshold actually cause a decrease in intracellular dFdCTP concentrations. It is unknown in dogs if dFdC infusions give increased intracellular dFdCTP concentrations and if inhibition of dFdCTP accumulation occurs with increasing dFdC levels. Without this information, it is difficult to design and adjust doses of dFdC in clinical oncology.

Five intact female dogs in an incomplete block crossover design were administered 3, 10, and 30 mg/kg dFdC as an IV bolus in phase I. In phase II using the same design the dogs were administered 10, 30, and 60 mg/kg doses as an IV bolus with a 30 minute CRI so that plasma dFdC concentrations remained constant the entire infusion period. Serial blood samples were collected after each dose, the plasma fraction being analyzed for dFdC by HPLC and the peripheral blood mononuclear cells (PBMCs) being isolated. The PBMCs were counted via flow cytometry and analyzed for intracellular dFdCTP by LC/MS. The resulting dFdCTP concentrations, normalized by cell number, were compared for the different doses and administration routes. Additionally, an *in vitro* study was conducted examining the intracellular accumulation of dFdCTP in canine melanoma cells administered 5 doses for either 2 or 4 hours.

In phase I the 10 mg/kg dose of dFdC had a higher PBMC intracellular accumulation of dFdCTP in selected animals than the 30 mg/kg dose. In phase II results were mixed, with some animals showing the 10 mg/kg dose had a higher dFdCTP accumulation than the 60 mg/kg dose while other animals saw a slight increase in PBMC intracellular accumulation with increasing dose, though not proportional to the dose. The *in vitro* study showed that dose ( $p < 0.0001$ ), time ( $p < 0.0001$ ), and the time by dose interaction ( $p = 0.0485$ ) all had significant effects on intracellular dFdCTP accumulation and a simple  $E_{max}$  model best fit the data. Increased dFdC exposure did not cause a corresponding increase in intracellular dFdCTP accumulation when the higher exposure occurred by administration of a high concentration over 2 hours compared to a lower concentration administered over 4 hours.

The phase I *in vivo* results suggest an inhibition model best describes the intracellular metabolism of dFdC to dFdCTP, while in phase II a saturation model appeared most appropriate. A saturation model clearly best fit the data for the *in vitro* experiment. Based on these results and clinical reports our recommendation for the most appropriate dFdC dose to maximize therapeutic potential is 10 mg/kg administered IV over 30 minutes or longer.

#### **ABSTRACT #182**

**CORRELATION OF AGE AND INCIDENCE OF PANCREATIC EXOCRINE NODULAR HYPERPLASIA IN THE DOG.** Shelley J Newman<sup>1</sup>, Jörg M. Steiner<sup>2</sup>, Kristen Woosley<sup>1</sup>, Linda Barton,<sup>1</sup> and David A. Williams<sup>2</sup>. <sup>1</sup>The Animal Medical Center, New York, NY, <sup>2</sup>Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Nodular hyperplasia (NH) of the exocrine pancreas has been described previously, but the incidence of this lesion has not yet been evaluated. The purpose of this study was to determine the incidence of NH and its relation to age in the dog.

A total of 101 consecutive dogs presented for necropsy examination to the Department of Pathology at the Animal Medical Center, New York, had the pancreas removed in its entirety within six hours of death and fixed in neutral buffered formalin. Starting at the right limb of the pancreas, serial 2 cm sections of the organ were obtained in each dog. Hyperplastic foci were characterized as nodular to compressive masses of variable size, composed of histologically normal appearing exocrine pancreatic epithelial cells or less commonly ductular epithelial cells. Hyperplastic lesions were judged as being present and were also scored by a single pathologist (S.J.N). Dogs were given a score of zero (no evidence of NH), one (less than 10 % of the section showing NH), two (10-40 % of the section showing NH) or three (greater than 40 % of the section showing NH). A mean nodular hyperplasia score was calculated by the sum of all scores of all sections divided by the total number of sections. Age was recorded from the medical records for all 101 dogs and was compared between dogs with NH and those that had no evidence of this lesion in any of the sections. Correlation of age and presence of hyperplastic lesions was evaluated in all 101 dogs and also in a subgroup of 30 dogs that did not have any evidence of pancreatic inflammation and/or necrosis. In addition, correlation of age and the mean nodular hyperplasia score was evaluated in both populations. A  $p$ -value  $< 0.05$  was considered statistically significant.

Of the 101 dogs that underwent necropsy, 80 (79.2%) had evidence of NH. Thirty dogs did not have any evidence of pancreatic inflammation and/or necrosis, 22 (73.3%) of which had evidence of NH. Mean ( $\pm$ SD) age of all 101 dogs was 8.3 ( $\pm$ 4.2) years. Mean ( $\pm$ SD) age in dogs with NH was significantly higher when compared to dogs without NH (9.6 ( $\pm$ 3.4) years vs. 3.3 ( $\pm$ 3.3);  $p$ -value  $< 0.0001$ ). There was a positive correlation of age and presence of nodular hyperplasia in all 101 dogs (Spearman  $r = 0.5550$ ;  $p$ -value  $< 0.0001$ ) and in the 30 dogs without any evidence of pancreatic inflammation and/or necrosis (Spearman  $r = 0.6604$ ;  $p$ -value  $< 0.0001$ ). Furthermore, there was a positive correlation between age and mean nodular hyperplasia score in both groups of dogs (Pearson  $r = 0.6160$  and  $0.6436$ ;  $p$ -value  $< 0.0001$  and  $= 0.0001$ , respectively).

We conclude that nodular hyperplasia is a common pathologic lesion in dogs. Furthermore, dogs with nodular hyperplasia are older than those without this finding. Finally, mean nodular hyperplasia shows a positive correlation with age regardless of the presence or absence of pancreatic inflammation and/or necrosis.

#### **ABSTRACT #183**

**DEWORMING PROTOCOLS, PREVALENCE, AND VETERINARIAN-PERCEIVED ZOONOTIC POTENTIAL OF SMALL ANIMAL ENDOPARASITES IN WESTERN CANADA.** Stull J<sup>1</sup>, Carr A<sup>2</sup>, Chomel B<sup>1</sup>. <sup>1</sup>University of California, Davis, CA; <sup>2</sup>Western College of Veterinary Medicine, Saskatoon, SK Canada.

Disease secondary to endoparasitism is a frequent clinical or subclinical concern in small animal veterinary medicine. Additionally, many endoparasites are zoonotic, causing disease in humans. Despite well-documented deworming recommendations, previous practitioner surveys have noted significant deviations from these guidelines. This study attempted to establish current small animal deworming protocols in Western Canada, an area unstudied

previously. Data were also collected on veterinarian-perceived prevalence and zoonotic risk of several endoparasites to determine if associations existed between deworming protocols, client education, and veterinarian perceptions.

An inclusive sample was taken from the Canadian Veterinary Medicine Association database. All small or mixed animal practitioners residing in British Columbia, Alberta, Saskatchewan, or Manitoba, (n = 2145) were surveyed through a mailed questionnaire. Surveys were returned by 545 veterinarians (25%). Fourteen percent of veterinarians recommended prophylactic deworming of puppies starting at  $\leq 3$  weeks of age, while the majority (74%) recommended commencing deworming at the time of vaccination ( $\geq 6$  weeks of age). Veterinarian practice type (small or mixed animal) and number of years in practice were independent of recommended age at first deworming (chi square;  $P > 0.05$ ). Over 96% of veterinarians recommended fecal examination/deworming at least once in young adults (5-12 months) and annually in adults ( $> 12$  months). Seventy-two percent of veterinarians recommended routine deworming of nursing bitches/queens. Sixty-eight percent of veterinarians revealed the existence of an established hospital deworming protocol, although only 80% of these followed the protocol. Forty-four percent of surveyed veterinarians stated they discussed with all clients the zoonotic risk of small animal-derived endoparasites, whereas the remainder only discussed it with clients "at risk" or under particular circumstances (i.e. diagnosis of endoparasites, etc.). Several parasites differed regarding veterinarian-perceived prevalence and/or zoonotic concern (mean  $\pm$  95% CI), with *Toxocara canis* ranking highest in prevalence in young animals and of greatest zoonotic concern. Veterinarian-perceived high zoonotic risk of *T. canis* was associated with recommending first deworming at  $\leq 3$  weeks of age.

Current data supports the hypothesis that early small animal deworming protocols in Western Canada begin too late to inhibit endoparasite shedding and environmental contamination. Further education of veterinarians regarding endoparasite prevalence and zoonotic risk, appropriate deworming protocols, and consequent client education, is warranted.

#### **ABSTRACT #184**

PHYSIOLOGICAL STRESS AND GASTROINTESTINAL MUCOSAL DYSFUNCTION DURING SUSTAINED STRENUOUS EXERCISE. Christopher M. Royer<sup>1</sup>, Katherine K. Williamson<sup>1</sup>, Michael D. Willard<sup>2</sup>, Jörg M. Steiner<sup>2</sup>, David A. Williams<sup>2</sup>, and Michael S. Davis<sup>1</sup>. <sup>1</sup>Oklahoma State University, Stillwater, OK. <sup>2</sup>Texas A&M University, College Station, TX.

Sustained strenuous exercise such as occurs during marathons and triathlons has been reported to cause gastrointestinal dysfunction in human beings. However, the extent of dysfunction and the underlying pathophysiology have not been determined. Racing sled dogs are also reported to suffer from exercise-induced gastrointestinal dysfunction, and can thus serve as a model of the analogous human condition. In this study, we used 3 elite sled dog teams competing in the 2003 Iditarod sled dog race to characterize the extent of the gastrointestinal dysfunction and determine whether the dysfunction is related to physiological and/or oxidative stress that occurs during the race.

Dogs were examined 1 week prior to the race and again at the conclusion of the race (if the dog completed the race). Each examination consisted of blood samples for determination of serum cortisol (physiological stress marker) and isoprostane (oxidative stress marker) concentrations, gastric endoscopy, and determination of intestinal permeability by use of inert sugar markers administered by orogastric gavage and collected in urine. Of the 54 dogs examined before the race, 18 were examined after completing the race.

Serum cortisol, but not serum isoprostane concentration, was significantly increased in post-race blood samples. Furthermore, pre-

race cortisol concentrations in dogs that ultimately failed to complete the race were higher than those in dogs completing the race. No dogs had visible gastric lesions during the pre-race gastroscopy, but 61% of finishers had visible gastric lesions. Post-race intestinal permeability was significantly increased when compared to pre-race measurements, but did not correlate with endoscopic findings or post race serum cortisol concentrations.

Although sustained strenuous exercise leads to hypercortisolemia, gastric ulceration, and increased intestinal permeability, our data do not support the hypothesis that the severity of exercise-induced gastrointestinal disease is related to concurrent hypercortisolemia. Furthermore, in this group of dogs, gastrointestinal permeability as assessed by urinary lactulose to rhamnose ratio was a poor predictor of the presence of gastric ulcers, and thus cannot be used as a substitute for gastric endoscopy. The underlying pathophysiology of exercise-induced gastrointestinal disease remains unknown.

#### **ABSTRACT #185**

COMPARISON OF BREATH HYDROGEN CONCENTRATION IN DOGS AFTER ADMINISTRATION OF A 5 AND A 4 SUGAR SOLUTION. JS Suchodolski, JM Steiner, CG Ruaux, G Aste, SR Teague, and DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

The hydrogen breath test measures the hydrogen concentration in breath produced through microbial metabolism of orally administered carbohydrates in the intestine. Under physiologic conditions most orally administered carbohydrates are digested in the small intestine and do not reach the bacterial flora in the large intestine. Malabsorbed carbohydrates reach the colon and hydrogen is produced by mostly anaerobic bacteria. Previous studies report that the hydrogen breath test using a mixture of 4 sugars (xylose, lactulose, rhamnose, and 3-0-methylglucose) may be useful in the diagnosis of small intestinal bacterial overgrowth in dogs. Recently, serum and urinary recovery of 5 sugars (4 sugar solution plus sucrose) has been used as a marker for gastrointestinal permeability and intestinal absorptive function. It would be useful if both, permeability and breath hydrogen testing could be performed simultaneously. Therefore, the purpose of this study was to evaluate if addition of sucrose has an effect on breath hydrogen concentration compared to the previously used 4-sugar solution.

Seven healthy dogs were enrolled in this study. Two different isotonic sugar solutions were compared: a 4-sugar solution (4S: 4 g xylose, 4 g lactulose, 4 g rhamnose, 2 g 3-0-methylglucose in 240 ml of deionized water) and a 5-sugar solution (5S: 4-sugar solution plus 16 g sucrose in a total of 400 ml of deionized water). Food was withheld for at least 17 hours before each study. Exhaled breath samples were collected with a close-fitting anesthesia face mask attached to a breath collection bag through a unidirectional valve. After collection of a baseline breath sample, the sugar solution was administered using a gastric feeding tube. Additional breath samples were collected 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 195, 210, 225, 240, 270, 300, 330, 360, 390, 420, 450, and 480 minutes after administration of the sugar solution. Breath hydrogen concentration was measured by gas chromatography. The effect of the different sugar solutions on exhaled hydrogen over time were analyzed by two-way ANOVA.

The mean peak of breath hydrogen was higher (mean $\pm$ SD: 15.9 $\pm$ 8.5 ppm) and later (mean $\pm$ SD: 147.9 $\pm$ 22.0 min) for 5S compared to 4S (mean $\pm$ SD: 11.2 $\pm$ 3.2 ppm of hydrogen; 115.7 $\pm$ 25.6 min). There was a significant effect of sugars on breath hydrogen concentration after 120 minutes ( $p=0.0215$ ).

The addition of sucrose to the sugar solution when performing a breath hydrogen test leads to increased hydrogen breath concentration. However, this increase in peak occurs late and at time-points that have previously been shown to be past the normal orocolonic transit time. Thus, these differences are most likely to be

caused by colonic bacteria and thus should not affect diagnostic evaluation of the small intestine.

#### **ABSTRACT #186**

DEVELOPMENT OF A  $^{13}\text{C}$ -GLYCOCHOLIC ACID BLOOD TEST FOR ASSESSMENT OF THE SMALL INTESTINAL MICROFLORA IN DOGS. JS Suchodolski, CG Ruaux, JM Steiner, K Fetz, N Berghoff, SR Teague, and DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

Quantitative bacterial culture of duodenal juice is considered the gold standard for assessment of the small intestinal microflora. The technical difficulty of this procedure, however, limits its use in clinical practice. There is a need for a sensitive, specific, and non-invasive test that reflects the microflora of the small intestine in dogs. The  $^{13}\text{C}$ -glycocholic acid (GCA) test relies on the deconjugation of the bile acid glycocholic acid by intestinal bacteria, with removal and metabolism of  $^{13}\text{C}$ -glycine to  $^{13}\text{CO}_2$ , which enters the blood stream of the animal by passive diffusion. The aim of this study was to establish optimal doses and sampling times for the use of the GCA test as a marker for small intestinal bacterial biomass and metabolic activity in dogs.

Eight dogs were enrolled in this study. Two doses of GCA, 1 mg/kg and 2 mg/kg body weight, were evaluated. For each study period dogs were given the same dose, with a 14-day rest period between study periods. Food was withheld for 17 hours before each study. Indwelling jugular catheters were placed in the morning. After collection of a 1 ml baseline blood sample, GCA dissolved in 50 ml of deionized  $\text{H}_2\text{O}$  was administered using a gastric feeding tube. Additional 1 ml blood samples were collected at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, and 480 min after GCA administration. Blood samples were immediately transferred into vacutainer tubes containing 2 ml of hydrochloric acid. The percent dose/min of  $^{13}\text{C}$  administered as GCA (PCD) and cumulative PCD (CUMPCD) were determined by fractional mass spectrometry. PCDs and CUMPCDs were compared for the different doses and sampling times using 1-way ANOVA and a Student's *t*-test. Correlation between the highest PCD and the CUMPCD was calculated using Pearson's correlation coefficient.

No clinically obvious side effects were noted during the course of the study. Both doses led to a significant increase in PCD and CUMPCD over time ( $p < 0.001$ ). There was a sudden increase in the rate of accumulation of CUMPCD after 210 min for the 1 mg dose, suggesting a change in deconjugation kinetics possibly due to deconjugation of GCA by colonic bacteria. The time-point for the peak PCD showed a high degree of variation between dogs and doses. There was a significant correlation between the highest PCD and the CUMPCD at 180 min for the 2 mg/kg dose ( $r = 0.769$ ,  $p = 0.025$ ). The mean CUMPCD at 180 min was lower for the 2 mg/kg dose, suggesting saturation of the capacity of the intestinal microflora to deconjugate administered GCA. This difference, however, did not reach statistical significance ( $p = 0.264$ ).

We conclude that oral administration of 1 and 2 mg/kg of  $^{13}\text{C}$ -glycocholic acid lead to a measurable increase in PCD and CUMPCD over time. Administration of 2 mg/kg of  $^{13}\text{C}$ -glycocholic acid and determination of CUMPCD at 180 min showed the lowest degree of variation between dogs and is thus suggested for future clinical studies.

#### **ABSTRACT #187**

SERUM PEPSINOGEN A CONCENTRATIONS IN CATS WITH INDUCED RENAL FAILURE. U Tress<sup>1</sup>, JM Steiner<sup>1</sup>, D Finco<sup>2</sup>, CG Ruaux<sup>1</sup>, and DA Williams<sup>1</sup>. <sup>1</sup>Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX. <sup>2</sup>University of Georgia, Athens, GA.

Pepsinogen (PG) is the zymogen of the major proteolytic enzyme in gastric juice, pepsin. In human beings serum PG A is used as a marker for the diagnosis of specific disorders of the stomach. Recently, a radioimmunoassay for the measurement of feline pepsinogen A (fPG A) in serum has been developed and validated. Almost two thirds of serum PG A is cleared from the circulation by the kidneys. Thus, the objective of this study was to examine the influence of experimentally-induced chronic renal failure (CRF) on serum fPG A concentrations in cats.

Serum fPG A concentrations were measured in serum samples from 20 cats with experimentally induced chronic renal failure. The renal failure was induced by subtotal (15/16) nephrectomy. Serum fPG A concentrations in cats with CRF were compared with those from 25 clinically healthy cats using a two-sided *t*-test (GraphPad Prism 4.0). Statistical significance was defined as a *p*-value  $< 0.05$ .

Mean $\pm$ SD serum fPG A concentration in 20 cats with CRF was 224.8 $\pm$ 73.8  $\mu\text{g/L}$ , with a range of 125.2 to 360.0  $\mu\text{g/L}$ . The mean serum fPG A concentration in cats with chronic renal failure was significantly higher than that in clinically healthy cats (mean $\pm$ SD; 125.2 $\pm$ 58.9  $\mu\text{g/L}$ ; range: 35.0 to 263.0  $\mu\text{g/L}$ ;  $p < 0.0001$ ). Serum fPG A concentrations in cats with CRF were above the upper limit of the reference range (260.0  $\mu\text{g/L}$ ) in 6 of 20 cats (30%).

We conclude that decreased renal function has a significant effect on serum fPG A concentrations in cats. Thus, renal function will need to be considered when assessing serum pepsinogen concentrations A in feline patients.

#### **ABSTRACT #188**

CULTURED CANINE COLONIC EPITHELIAL CELLS EXPRESS PATTERN RECOGNITION RECEPTORS. M. Swerdlow, D. Kennedy, D. Clayton, P. Felsburg, and R. Washabau. Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania.

The gut maintains a delicate balance between the downregulation of inflammatory reactions to the commensal bacteria and food antigens and the capacity to respond to pathogens with vigorous cellular and humoral immune responses. Intestinal epithelial cells (IECs) play an important role in sensing the external environment and communicating this information to the local immune system to effect appropriate responses. IECs possess many properties of cells of the innate immune system, in particular their ability to recognize and respond to microbial antigens. IEC recognition of microorganisms is based upon recognition of signature molecules on microorganisms called microbe-associated molecular patterns (MAMPs). MAMPs are shared by large groups of microorganisms, including peptidoglycans (PGs) found in most bacteria, and lipopolysaccharides (LPS) of Gram-negative bacteria. The response of IECs to microbial MAMPs is mediated by pattern recognition receptor (PRR) families of which two types have been identified: membrane Toll-like receptors (TLRs) and cytosolic Nod1/CARD4 and Nod2 that represent the intracellular counterparts of TLRs. The purpose of the studies reported here was to develop a method for the isolation and culture of a purified population of canine colonic epithelial cells which are capable of expressing PRRs.

IECs were obtained by serial digestion of intestinal mucosa in a Hank's buffered salt solution containing 0.05 mM dithiothreitol, 3 mM EDTA, 15 mM HEPES, and Fungibact. Cells were plated into tissue culture-grade plasticware coated with a 1:1 solution of Matrigel/DMEM at 37 °C in a  $\text{CO}_2$  incubator. Cells were evaluated and media was changed every 24 hours. Total cellular RNA was isolated from IEC using TRIZOL reagent (Invitrogen). TLR4, TLR2, and Nod2 mRNA expression were determined by RT-PCR using canine specific primers.

Isolated IECs were viable and could be maintained and studied *in vitro*. TLR4, TLR2, and Nod2 were constitutively expressed by IECs under these conditions. The expression of PRRs suggest that these

cells retain functionality under cell culture conditions, and that this cell system may be used to study the up-regulation of PRRs following stimulation with tumor necrosis factor (TNF), interleukin-1, or LPS. We conclude that canine colonic epithelial cells may be cultured *in vitro* and may serve as a useful model for the study of IECs in the evolution of canine inflammatory bowel disease.

#### **ABSTRACT #189**

PREVALENCE OF ENTEROPATHY IN THE NORTH AMERICAN POPULATION OF THE NORWEGIAN LUNDEHUND. N. Berghoff, JM Steiner, CG Ruaux, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Dogs of the Norwegian Lundehund breed commonly have gastroenteropathy that is associated with protein loss (PLE). The aim of this study was to estimate the prevalence of PLE in this breed in the USA and Canada. Samples were obtained from 53 Norwegian Lundehunds. This represents approximately one quarter of the North American population of Norwegian Lundehunds.

Owners of Norwegian Lundehunds in the US and Canada were contacted and encouraged to enroll their dog or dogs regardless of presence of signs of gastrointestinal disease. The diagnosis of PLE was based on an increased concentration of canine  $\alpha_1$ -proteinase inhibitor in fecal samples (cF $\alpha_1$ -PI). Canine F $\alpha_1$ -PI was considered increased when either the mean of three samples from one sampling period exceeded 9.4  $\mu\text{g/g}$  fecal material or a single one of three samples had a value higher than 15.0  $\mu\text{g/g}$  fecal material. Serum cobalamin and folate concentrations were measured using a modified chemiluminescent immunoassay. Serum trypsin-like immunoreactivity (TLI) was measured using a radioimmunoassay. Serum canine C-Reactive Protein (CRP) concentrations were measured in 37 Lundehunds with a commercially available ELISA and compared to those in 25 healthy dogs using a Mann-Whitney test. Correlation of serum CRP concentrations with mean cF $\alpha_1$ -PI from a 3 day collection was evaluated by Spearman rank correlation analysis.

Twenty-six of 53 dogs (49 %) tested had increased cF $\alpha_1$ -PI concentrations ranging up to 125.9  $\mu\text{g/g}$  fecal material. Serum cobalamin concentrations were below the lower limit of the reference range (<249 ng/L) in 4 dogs (7.5 %). Serum folate concentrations were increased (>11.5  $\mu\text{g/L}$ ) in 19 dogs (35.8 %) and decreased (<6.5  $\mu\text{g/L}$ ) in 9 dogs (16.9 %). Two Lundehunds had a combination of low cobalamin and high folate concentrations. Serum TLI concentrations were within the reference range for all dogs. Serum CRP concentrations with a median of 5.7 mg/L were significantly higher in the Lundehunds when compared to healthy control dogs (0.03 mg/L; p-value=0.0003). No correlation was found between serum CRP and cF $\alpha_1$ -PI concentrations (Spearman  $r=0.0528$ ; p-value=0.7561).

We conclude that approximately 50 % of Norwegian Lundehunds in North America are affected by PLE. Decreased serum cobalamin and folate concentrations would indicate the presence of mucosal dysfunction in at least some of these dogs. Also, increased serum CRP concentrations in many of these dogs indicate the presence of an inflammatory process.

#### **ABSTRACT #190**

DEVELOPMENTAL CHANGES IN THE CANINE COLON. K. Meltzer, D. Kennedy, Mattie Hendrick, and R. Washabau. School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

Previous studies have shown that intestinal digestive, absorptive, and motor functions continue to develop after birth. Our laboratory has shown, for example, that the cholinergic and substance P responsiveness of the post-natal feline intestine continues to develop

well into the ninth month of life. Developmentally immature digestive, absorptive, and motor function may predispose some young animals to gastrointestinal pathophysiologic states. The present study was performed to elucidate the relationship between stages of development and contractility in the canine colon.

Colonic tissue was obtained from healthy puppies ranging in age from 4-6 weeks, 2-4 months, and 4-6 months. Colonic smooth muscle strips were suspended in physiologic (HEPES) buffer solution in the longitudinal or circular orientation, attached to isometric force transducers, and set to optimal muscle length ( $L_0$ ) with acetylcholine (ACh;  $10^{-4}$  M). Muscle strips were contracted with ACh ( $10^{-8}$  to  $10^{-4}$  M) or substance P (SP;  $10^{-10}$  to  $10^{-7}$  M), and maximal force output ( $P_{\text{max}}$ ) was normalized for cross-sectional area ( $N = x \cdot 10^4$  Newtons/m<sup>2</sup>) or muscle thickness (microns).

Cholinergic responses were observed in all muscle sites and in all age groups, although significant differences ( $P<0.05$ ) were noted in the peak active isometric stress between the three age groups. In general, the 4-6 week age group exhibited the least active isometric stress ( $P_{\text{max}} = 0.3\text{-}0.7$  N), while the 2-4 month age group was intermediate in responsiveness ( $P_{\text{max}} = 3.3\text{-}5.2$  N) between the 4-6 week and 4-6 month ( $P_{\text{max}} = 5.9\text{-}10.8$  N) age groups. These differences were observed in longitudinal and circular smooth muscle from proximal and distal colon. Differences in developmental stages were observed whether force output was normalized for whole tissue cross sectional area or muscle thickness. Similar results and trends were observed with substance P responses.

Cholinergic and substance P receptors are present and functional on canine colonic smooth muscle post-natally, and neurotransmitter responsiveness appears to improve with further development. Developmental maturation of the canine colon appears to significantly differ from that of the feline colon in two respects: (1) Substance P responsiveness appears at an earlier development stage in the canine colon, and (2) overall contractility matures at an earlier developmental stage (6 vs. 9 months) in the dog.

#### **ABSTRACT #191**

UTILITY OF DIAGNOSTIC TESTS USED FOR DIAGNOSIS OF EXPERIMENTALLY INFECTED DOGS WITH *LEISHMANIA INFANTUM* VT-1 STRAIN. Rosypal AC, Troy GC, Zajac AM, Gogal R, Frank G, Lindsay D. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Eight female beagles were injected with promastigotes of the *L. infantum* VT – 1 strain grown in cell cultures and isolated from naturally infected Foxhounds from Virginia. Dogs were injected IV with  $2 \times 10^8$  (N=4; high dose) or  $1 \times 10^7$  promastigotes (N=4, low dose) in 1 ml of HBSS. Two female beagles were inoculated with 1 ml HBSS and served as negative controls. Two male beagles chronically infected (> 3 years) with *L. infantum chagasi* served as positive controls. Bone marrow and lymph node aspirates were collected every 6 – 8 weeks for cytologic evaluation, parasite culture (30% v/v fetal bovine serum, 1% penicillin/streptomycin, 2% v/v human urine, in Grace's Insect Media at 25°C) and PCR. Serum samples were collected monthly (n=17) for determination of serologic responses by IFAT, and to K 39 antigen.

Slides of aspirates of bone marrow (BM) and lymph nodes (LN) slides were stained with DifQuik® stain and examined at 1000X. Two hundred fields/BM sample and 100 fields/LN aspirates were examined from each dog on multiple occasions (N=8 for LD, N=7 HD).

Cultures of BM and LN aspirates, and cytologic evaluation of BM and LN aspirates in the 2 positive controls dogs were positive at every sampling period (N=8). BM and LN cultures, and cytologic evaluation of aspirates on negative controls dogs were negative on all occasions (N=8). Amastigotes were present on cytological examination of BM aspirates in 2 dogs (1 dog -LD; 1 dog-HD) on two different samples. Cultures of LN aspirates were positive on 22



samples from all dogs (N=12). BM cultures were positive on 12 samples for all dogs (N=12)

Positive control dogs had IFAT > 1:400 for 17 months. IFA test results were negative on all samples of negative control dogs, except one sample (1:25). The LD and HD groups developed serological responses (> 1:100) at approximately 2 months post-infection. Dogs in the LD group developed serologic titers that persisted for approximately 3 months, but dropped to levels (<1:100) over 12 months. IFA titers in the HD group increased to greater than >1:100 by 2 months and persisted > 1: 400 in 2 dogs in this group. PCR and K 39 antigen test results are incomplete at this time but will be described at the time of the presentation. Three of four low dose dogs were culture positive on LN/BM samples at the termination of the experiment.

Cytologic examination of BM and LN samples were not efficient tests in identifying infected dogs. Cultures of LN were more likely to be positive than BM cultures. Serologic response > 1:100 is suggestive of infection with LIVT – 1 strain in experimentally infected dogs. Supported in part by grant D01CA-16 from the Morris Animal Foundation.

#### ABSTRACT #192

TREATMENT OF *MYCOPLASMA HAEMOFELIS* INFECTED CATS WITH IMIDOCARB DIPROPIONATE. JE Woods, M Brewer, SV Radecki, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, Colorado.

*Mycoplasma haemofelis* and '*Candidatus M. haemominutum*' are associated with hemolytic anemia in some cats. A variety of drugs have been used in the treatment of hemoplasmosis; doxycycline, enrofloxacin, and azithromycin have been evaluated most recently. Azithromycin was unsuccessful in one study and while 14 day treatment protocols with enrofloxacin and doxycycline are usually successful in ameliorating clinical abnormalities, infection is not eliminated in most cats. Tetracyclines and fluoroquinolones have potential for toxicities and are generally given orally when used for extended durations which can be difficult for owners. Imidocarb dipropionate can be given by SQ or IM injection, was shown to be safe in cats with chronic *M. haemofelis* infection, and was effective in resolving the clinical abnormalities associated with hemoplasmosis in some naturally-infected cats that were resistant to tetracyclines and quinolones. The goal of this study was to assess the effect of imidocarb dipropionate administered IM to cats experimentally infected with *M. haemofelis*.

Young adult mixed-sexed cats were used. One cat was used to amplify *M. haemofelis* infection after IV inoculation from a chronic carrier. The remaining 12 cats were administered 2 ml of heparinized blood from this cat when it was shown to be strongly positive for infection by a PCR assay on day 21. A clinical score that included attitude, mucus membrane coloration, and body temperature was determined for all cats daily. A CBC and PCR assay was performed weekly. The 12 cats were randomly assigned to either a treatment or control group. Treatment with imidocarb dipropionate was administered at 5 mg/kg, IM, q 2 weeks for four injections when cats in the treatment group showed signs of clinical illness (fever, pale mucus membranes or depression) and hematocrit < 25%.

Three cats were treated on PI days 15, 28, 63, and 78 and three cats were treated on PI days 21, 35, 63 and 78. Control cats received only supportive care as needed. Statistical analysis revealed no significant differences when clinical scores or hematocrit values were compared between treated and control cats.

Treatment with imidocarb dipropionate as described was not effective for the treatment of this *M. haemofelis* strain. Further studies will be required to determine if imidocarb dipropionate is effective for the treatment of other *M. haemofelis* strains.

#### ABSTRACT #193

DETECTION OF FELINE CALICIVIRUS IN URINE BY RT-PCR: A COMPARISON OF RNA ISOLATION METHODS. BA Scansen<sup>1</sup>, JM Kruger<sup>1</sup>, AG Wise<sup>2</sup>, PJ Venta<sup>1,2</sup>, P Bartlett<sup>3</sup>, RK Maes<sup>2</sup>. Departments of Small Animal Clinical Sciences<sup>1</sup>, Microbiology and Molecular Genetics<sup>2</sup>, and Population Medicine<sup>3</sup>, Michigan State University, East Lansing, MI.

Investigating the role of feline calicivirus (FCV) in the pathogenesis of idiopathic cystitis has been hindered by lack of sensitive and efficient means of detecting FCV urinary tract infections. We have developed a FCV p30 gene-based real-time reverse transcriptase PCR (RT-PCR) assay that would facilitate rapid detection of FCV urinary tract infections. However, pilot studies indicate that feline urine contains substances that may degrade target RNA or inhibit RT-PCR amplification. The purpose of this study was to evaluate 4 RNA isolation methods for their ability to recover FCV RNA from feline urine for amplification with RT-PCR.

RNA isolation methods included polyethylene glycol precipitation (PEG), oligo(dT)<sub>25</sub>-coated magnetic beads (Dynabead mRNA direct kit<sup>®</sup>), and two silica gel-based extraction columns (RNeasy mini kit<sup>®</sup> and QIAamp viral RNA mini kit<sup>®</sup>). Urine and blood were obtained from 6 specific-pathogen-free cats. Unaltered and centrifuged urine specimens, and urine specimens with added whole or hemolyzed blood, from each cat were spiked with FCV, serially diluted in urine, and extracted with each RNA isolation method. Isolated RNA was amplified in duplicate with the FCV RT-PCR assay. Tissue culture media spiked with FCV served as a positive control; noninfected tissue culture medium served as a negative control. The log<sub>2</sub> of the mean RT-PCR detection threshold cycle (C<sub>t</sub>) value for each RNA preparation method and for each urine specimen modification and positive control were compared with a mixed-effects model ANOVA.

Overall, the lower detection limits for the assay (expressed as TCID<sub>50</sub>/sample) were 1,950 for PEG, 104 for oligo(dT)<sub>25</sub>-coated magnetic beads, 11 for RNeasy<sup>®</sup>, and 7 for QIAamp viral RNA<sup>®</sup>. The mean RT-PCR C<sub>t</sub> values for the two silica gel-based extraction column methods were not significantly different from each other, but both were significantly lower than those for the oligo(dT)<sub>25</sub>-coated magnetic bead and PEG methods (p<0.001). Modification of urine did not appear to substantially affect performance.

In conclusion, there are notable differences between RNA isolation methods for recovery of FCV nucleic acids from feline urine. The FCV p30 gene-based RT-PCR assay performed significantly better when amplifying RNA isolated from feline urine with either of the two silica gel-based extraction column methods. This effect may be due to either more efficient removal of inhibitors or higher RNA yields.

#### ABSTRACT #194

SEROPREVALENCE OF LEPTOSPIROSIS IN MIDWEST DOGS. CJ Baldwin<sup>1</sup>, CA Schreiner<sup>2</sup>, E Zhou<sup>1</sup>, RB Evans<sup>1</sup>. 1: College of Veterinary Medicine Iowa State University, Ames Iowa;2: Issaquah, WA.

Canine leptospirosis has been diagnosed more often in North America over the past twenty years. The realization of increased incidence of clinical disease and the emergence of disease from non-traditional *Leptospira* serovar has led to more comprehensive testing and vaccine development. The purpose of this study was to determine the overall seropositivity of healthy dogs in the Midwest to *Leptospira*.

Nine cooperating veterinary clinics or animal shelters cooperated by submitting serum from 482 healthy dogs. Seven facilities were in Iowa, one in Nebraska, and one in Minnesota. Information collected on the individual dogs included breed, age, gender, date and type [2-way (*canicola* and *icterohemorrhagiae*) or 4-way (*canicola* and *icterohemorrhagiae* plus *pomona* and *grippityphosa*)] of last vaccination, and housing. Antibody titers to *bratislava*, *canicola*,

*grippytyphosa*, *hardjo*, *icterohemorrhagiae*, and *pomona* were measured using the microscopic agglutination test. A positive titer was defined as one that reacted at the second dilution (1:200) or higher (up to  $\geq 3200$ ).

Sixty-eight of 482 dogs (14%) were positive to one or more serovars. The lowest % positive from the individual practices/shelters was 4% and the highest was 33%. The mean number of positive serovar per dog was 1.9. Distribution of the positive serovar was 0/124 *hardjo*, 10% (12/124) *icterohemorrhagiae*, 12% (15/124) *canicola*, 19% (24/124) *pomona*, 27% (33/124) *bratislava*, and 32% (40/124) *grippytyphosa*. Two dogs had titers  $\geq 3200$ . Age was unknown or not recorded for 103 dogs. Of these, 10% (10/103) had positive titers. In dogs < 1 year old, 20% (9/46) had positive titers. In dogs 1 – 4 years of age, 15% (26/173) had positive titers. In dogs 5 – 9 years of age, 12% (11/93) had positive titers. In dogs  $\geq 10$  years of age, 18% (12/67) had positive titers. Vaccination status was known in 41% (28/68) of dogs; 21 had received the 2-way vaccine and 7 had received the 4-way. Of the 28 dogs with vaccination history, 14 had been vaccinated within the preceding 6 months. Only one (4-way) had a titer  $\geq 200$  for the combination of *canicola* and *icterohemorrhagiae*, without seropositivity to the non-vaccinate serovars. Seropositivity to *bratislava*, not included in any of the vaccines available, was present in 10 dogs. Of the seronegative dogs, 20% (81/414) are known to have been previously vaccinated.

This study demonstrates a seroprevalence of 14% for *Leptospira* in Midwest dogs. Most commonly detected were *grippytyphosa* and *bratislava*, which correlates with data from another investigation. Antibodies were detected in all age groups. Vaccination status was known in 41% of the seropositive dogs. Patterns of titers in vaccinates were difficult to interpret due to either lack of antibody response to some components of the vaccine, or to antibodies against non-vaccinate serovars.

#### ABSTRACT #195

ATTEMPTED TRANSMISSION OF *MYCOPLASMA HAEMOFELIS* BY INGESTION OF *M. HAEMOFELIS*-INFECTED FLEAS. JE Woods, MR Lappin, N. Wisniewski. Department of Clinical Sciences (Woods, Lappin), Colorado State University, Fort Collins, CO and Heska Corporation (Wisniewski), Fort Collins CO.

Feline infectious anemia is caused by at least two *Mycoplasma* species; *Mycoplasma haemofelis* and 'Candidatus *M. haemominutum*'. Although experimentally the organisms have been successfully transferred by a variety of routes, all natural modes of transmission for these organisms have yet to be elucidated. Blood-sucking arthropods have long been incriminated in the natural transmission of the disease, and recently, our experiments have shown that the cat flea, *Ctenocephalides felis*, can transfer *M. haemofelis* between cats during hematophagous activity. In those studies, the cats were unable to groom and ingest the fleas or flea excrement, a significant activity of cats with natural flea infestations. We hypothesize that the cat flea may transmit *M. haemofelis* when infected fleas or flea by-products are ingested, a transmission route known in other infectious diseases (e.g. *Dipylidium caninum*). The goal of this study was to determine whether *M. haemofelis* infection of previously naïve cats could be initiated by the ingestion of infected fleas.

Four, young-adult, mixed-sexed cats were used. Two cats were known chronic carriers of *M. haemofelis*. The other two cats were shown to be negative for hemoplasmosis by a PCR assay that amplifies the DNA of both *Mycoplasma* species. One flea chamber containing 100 *C. felis* fleas was attached to each of the chronic carrier cats and left in place for a period of five days during which time the fleas could feed. At chamber removal, a random sample of fleas was analyzed by PCR assay and shown to be positive for *M. haemofelis* DNA. The remaining fleas and flea by-products were then fed to each of the two *M. haemofelis*-naïve cats. One cat was fed 91

viable fleas (37 female, 54 male) and 0.2 gram of flea by-products, including feces, larvae and eggs. The other cat was fed 93 viable fleas (39 female, 54 male) and 0.163 gram of flea by-products. These items were mixed into 71 grams of a commercially available beef-based human baby food to facilitate feeding.

A CBC and PCR assay performed weekly failed to document infection during the first six weeks post-oral inoculation. Results of this study suggest that ingestion of *M. haemofelis*-infected fleas is not be a route of transmission, an inadequate quantity of fleas was fed, the timing of flea feeding was inappropriate for transmission, or the observation time was inadequate.

#### ABSTRACT #196

DISCRIMINANT ANALYSIS OF EXTENDED UROVIRULENCE GENOTYPES DISTINGUISHES HUMAN, CANINE, AND FELINE URINARY *ESCHERICHIA COLI* ISOLATES FROM NEW ZEALAND. T. Freitag, R.A. Squires, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, NZ.

Numerous genes of *E. coli* encode proteins putatively important to urovirulence, for example, adhesins and protectins. Human, canine, and feline urinary *E. coli* isolates have been characterized on the basis of their extended urovirulence genotypes in studies that typically test for the presence or absence of about 25 of these genes. It has been reported recently that extended urovirulence genotypes of canine and feline urinary *E. coli* isolates overlap with, and are essentially indistinguishable from, those of human strains that cause serious extraintestinal infections. On the basis of these and other phylogenetic findings, concern has been expressed that some canine and feline uropathogenic *E. coli* strains pose a significant human health hazard. However, very few canine isolates and even fewer feline isolates have been adequately studied to date.

We investigated whether discriminant analysis could accurately classify a urinary *E. coli* isolate as originating from a dog, cat, or human based on its extended urovirulence genotype. This genotype was obtained by multiplex PCR analysis of 25 putative urovirulence genes in each isolate. 45 canine and 22 feline urinary *E. coli* isolates were acquired from a large network of veterinary clinical pathology laboratories in New Zealand. 11 human isolates were obtained from the clinical microbiology laboratory of a local hospital. All isolates were acquired between November 2001 and November 2003, originating from cases undergoing clinical investigation of suspected urinary tract inflammation and / or infection. Statistical analysis was carried out using the DISCRIMINANT function of SPSS ver. 11.5, with cross-validation, using a stepwise procedure based on minimization of Wilk's lambda.

When the genotypes of the feline and human isolates were subjected to discriminant analysis, 21/22 (96%) of the feline isolates and 10/11 (91%) of the human isolates were correctly classified. Analysis of genotypes from canine and human isolates resulted in a correct categorization of 43/45 (96%) of the canine isolates and 7/11 (64%) of the human isolates. In a combined analysis of all three populations' genotypes only 4/45 (9%) of the canine isolates and 1/22 (5%) of the feline isolates were misclassified as being of human origin. When analyzing the genotypes of human isolates versus a combined set of companion animal genotypes, 63/67 (94%) of the non-human isolates and 8/11 (73%) human isolates were correctly classified.

Contrary to previous findings, these preliminary results suggest that it may be possible to differentiate canine and feline urinary *E. coli* isolates from human isolates with reasonable accuracy using discriminant analysis of urovirulence genotypes. These results will be of interest to researchers studying comparative aspects of *E. coli* urovirulence and others seeking to determine which, if any, canine and feline urinary *E. coli* genotypes are of particular zoonotic concern.

### ABSTRACT #197

PREVALENCE OF FHV-1, *MYCOPLASMA* SPP., AND AEROBIC BACTERIA IN SHELTER CATS WITH ACUTE UPPER RESPIRATORY TRACT DISEASE. JK Veir,<sup>a</sup> R Ruch-Gallie<sup>a</sup>, ME Spindel,<sup>b</sup> and MR Lappin.<sup>a</sup> From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University<sup>a</sup> and Larimer Humane Society, Fort Collins, CO.<sup>b</sup>

Infectious causes of upper respiratory disease in cats are common in humane societies. Feline herpesvirus 1 (FHV-1) is thought to be the most common infection. With the exception of *Bordetella bronchiseptica* and *Chlamydomphila felis*, most bacterial infections are thought to be secondary to other primary diseases. *Mycoplasma* spp. are known to be normal oropharyngeal flora in some cats but have also been proposed to be associated with feline upper respiratory tract disease. The purpose of this study was to describe the isolation rates of FHV-1, aerobic bacteria, and *Mycoplasma* spp. from cats with acute clinical upper respiratory disease in a humane society in North Central Colorado.

Between January 24, 2003 and December 4, 2003, 61 cats with clinical evidence of acute upper respiratory disease had swabs collected from the right nares and right pharyngeal region. The first swab was placed in transport media and submitted within 4 hours for aerobic bacterial and *Mycoplasma* spp. culture. A second swab for DNA extraction from each site was placed in sterile saline and stored at -80°C between 2 and 3 hours after collection until batch analysis. Fluorogenic PCR targeting FHV-1 was performed on the DNA extracts.

The majority of cats with positive results were positive on both nasal and pharyngeal swabs and so results from the 2 sites were combined. Of the 61 cats, DNA of FHV-1 was amplified from 52 cats, aerobic bacteria were cultured from 57 cats, and *Mycoplasma* spp. were cultured from 35 cats. The distribution of positive results were as follows: FHV-1 alone (4 cats); FHV-1 and aerobes (18 cats); FHV-1 and *Mycoplasma* spp. (0 cats); FHV-1, aerobes and *Mycoplasma* spp. (30 cats), aerobes alone (4 cats), aerobes and *Mycoplasma* spp. (5 cats), and *Mycoplasma* spp. alone (0 cats). *Bordetella bronchiseptica* was isolated from 3 cats; 1 cat was coinfecting with other aerobes, FHV-1 and *Mycoplasma* spp., 1 cat was coinfecting with FHV-1 and *Mycoplasma* spp., and 1 cat was coinfecting with other aerobes only.

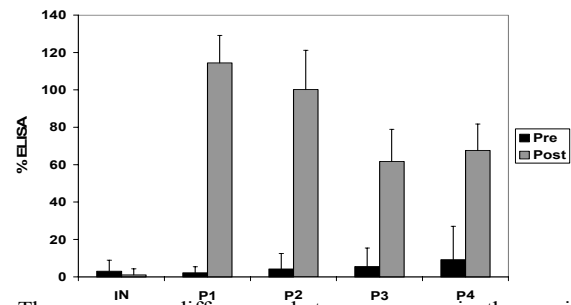
In cats of this study, coinfections of FHV-1 with aerobic bacterial or *Mycoplasma* spp. were most common (78.7%). *Bordetella bronchiseptica* infections were uncommon (4.9%). Because FHV-1, aerobic bacteria, and *Mycoplasma* spp. are commonly isolated from normal and clinically ill cats, results of these tests do not correlate to clinical disease in individual cats. Further studies will be required to determine the pathogenic potential of *Mycoplasma* spp. isolated from cats with upper respiratory tract disease.

### ABSTRACT #198

MAGNITUDE OF CRANDELL REESE FELINE KIDNEY CELL LINE ANTIBODY RESPONSES OF CATS ADMINISTERED FVRCP VACCINES. MR Lappin, RW Sebring, WA Jensen, GR Frank, SV Radecki. From Colorado State University, Fort Collins, CO (Lappin), and Heska Corporation, Fort Collins CO (Sebring, Jensen, Radecki).

Feline herpesvirus 1, calicivirus, and panleukopenia virus for use in feline vaccines (FVRCP) are commonly grown in Crandall-Reese Feline Kidney (CRFK) cells. We previously showed that SQ, but not intranasal (IN) FVRCP vaccines that contain CRFK proteins induce CRFK antibody levels detectable by ELISA. However, only two cats per group were assessed precluding statistical comparisons between groups. The objectives of this study were to determine and compare the magnitude of CRFK antibody responses of cats inoculated with commercially available FVRCP vaccines for IN or SQ administration that are known to contain CRFK proteins.

Fifty unvaccinated kittens were divided into five groups of 10. One of the following was administered to a group of kittens on days 0, 28, and 56: the FVRCP vaccine for IN administration or one of four FVRCP vaccines for SQ administration (P1; P2; P3; P4). Pre-inoculation and day 67 post-inoculation sera were assayed for CRFK antibodies by ELISA and vaccine group mean and standard deviation %ELISA values calculated and compared between groups.



There were no differences between groups in the pre-inoculation samples. When the pre-inoculation results were used as a covariate, a significant group effect post-inoculation was detected ( $P < 0.05$ ) with the IN group values being lower than each SQ group value ( $P < 0.05$ ). When the difference in %ELISAs from pre-inoculation to post-inoculation was compared within groups, the change was not significant in the IN group but increased significantly in each of the SQ groups.

CRFK protein-containing FVRCP vaccines administered SQ induce greater CRFK antibody responses than a CRFK protein-containing FVRCP vaccine administered IN.

### ABSTRACT #199

EFFICACY OF AMOXICILLIN AND AZITHROMYCIN FOR THE EMPIRICAL TREATMENT OF CATS WITH UPPER RESPIRATORY INFECTIONS. R Ruch-Gallie,<sup>a</sup> ME Spindel,<sup>b</sup> JK Veir,<sup>a</sup> and MR Lappin.<sup>a</sup> From the College of Veterinary Medicine and Biomedical Sciences, Colorado State University<sup>a</sup> and Larimer Humane Society, Fort Collins, CO.<sup>b</sup>

Feline upper respiratory infections (URI) continue to be a major cause of morbidity and mortality in U.S. animal shelters. Limited resources rarely allow for determining the definitive causative agent(s). Feline herpesvirus 1 (FHV-1), with or without secondary bacterial infections, is considered the most common cause of disease. *Bordetella bronchiseptica*, *Chlamydomphila felis*, and *Mycoplasma* spp. may induce disease primarily in some cats. Amoxicillin has classically been used for the treatment of cats with URI in shelters. Recently, azithromycin has been used in some cats because of its broad spectrum, presumed efficacy against primary pathogens, and extended biological activity enabling administration every third day. This purpose of this study is to assess the comparative efficacy of amoxicillin and azithromycin in cats with clinical URI from a humane society in north-central Colorado.

Between January 24 and December 4, 2003, 31 cats with URI and a suspected bacterial component were selected for participation by the humane society veterinarian. Samples were collected for aerobic bacterial and *Mycoplasma* spp. culture, calicivirus RT-PCR, and FHV-1 PCR. A standardized clinical score was calculated for each cat every 3<sup>rd</sup> day by a person blinded to the treatment groups. Cats were randomly assigned to receive either amoxicillin at 22 mg/kg, q12hr, PO for nine days or azithromycin at 15 mg/kg q72hr, PO for nine days. Cats with persistent clinical signs after nine days were crossed over to the other antibiotic for nine additional days.

Of 31 cats that have completed the study to date, 21 were initially administered amoxicillin and 10 were administered azithromycin. There were no differences between the initial clinical scores between antibiotic groups (Student's t-test;  $p = 0.33$ ). Of the 21 cats initially

administered amoxicillin, eight had resolution of clinical signs, 10 were switched to azithromycin, and three were removed from the study. Of the 10 cats initially administered azithromycin, three had resolution of clinical signs, six were switched to amoxicillin and one was removed from the study. There were no differences in outcome between groups (Chi-square test of outcomes between groups;  $p = 0.8$ ). There were no differences in clinical scores between groups at the time of crossover (Student's  $t$ -test;  $p = 0.16$ ). Of the 10 cats switched from amoxicillin to azithromycin, five resolved. Of the six cats switched from azithromycin to amoxicillin, three resolved. There were no differences in the crossover outcome between groups (Chi-square test of outcomes between groups;  $p = 0.71$ ).

Based on clinical scores and treatment outcomes, there were no statistically significant differences between the empirical use of amoxicillin or azithromycin using these protocols in these cats.

#### **ABSTRACT #200**

FELINE SERUM ANTIBODY RESPONSES TO CRANDALL REESE FELINE KIDNEY CELL LINE INOCULATIONS AND CHARACTERIZATION OF TARGET ANTIGENS. JC Whittemore, WA Jensen, JR Hawley, MR Lappin. From the Department of Clinical Sciences Whittemore, Hawley, Lappin), Colorado State University, Fort Collins, CO and Heska Corporation (Jensen), Fort Collins, CO.

Feline viruses for use in FVRCP vaccine production are sometimes grown on the Crandall Reese Feline Kidney (CRFK) cell line. Some vaccinated cats develop antibodies against CRFK cell line antigens as determined by ELISA, but the immunodominant target antigens are unknown. The objective of this study was to validate a Western blot immunoassay to characterize the immunodominant CRFK antigens recognized by feline serum antibodies for use in further studies.

Previously, 14 age-matched, mixed-sex, unvaccinated kittens were divided into seven groups and were given one of the following: 10 $\mu$ g of CRFK lysate SQ; 50 $\mu$ g of CRFK lysate SQ; 50 $\mu$ g of CRFK lysate plus an aluminum adjuvant SQ; a FVRCP vaccine for IN administration, or one of three FVRCP vaccines for SQ administration. The CRFK lysate concentrations used were comparable to those detected in the vaccines. Kittens given CRFK lysates were inoculated every two to four weeks with the last of the 11 injections being given on week 50. Kittens given vaccines were inoculated on weeks 0, 3, 6, and 50.

The optimized western blot immunoassay for detection of CRFK antibodies in feline serum used pre-cast mini-gels (NuPage 4-12% gel, Bis-Tris discontinuous buffer system; Invitrogen, Carlsbad, CA), 65 $\mu$ g CRFK lysate per lane, a 1:10 serum dilution, and a 1:250 goat  $\alpha$ -cat IgG ( $\gamma$  only; Kirkegaard and Perry Laboratories, Gaithersburg, MD) secondary antibody concentration. Sera collected from the kittens prior to inoculation and at weeks 4, 8, 16, 24, 50, and 56 were assayed. A similar western blot immunoassay was optimized for use with rabbit serum and applied to samples collected from a rabbit that was hypersensitized with the CRFK lysate.

Immunodominant antigens recognized by the CRFK hypersensitized kittens and the rabbit were in the following molecular mass groupings: 46-49 kD, 41-43 kD, 37-39 kD, and 35-37 kD. Sera from 4 cats collected prior to inoculation recognized one of the immunodominant antigens (faint bands). Sera from 13 of the 14 kittens collected after inoculation recognized at least one of the immunodominant antigens; one SQ vaccinated kitten failed to develop detectable bands. For most kittens, both the number of bands recognized and the intensity of the bands were highest in samples collected between weeks 50 through 56.

Future studies of the association between feline clinical syndromes and CRFK inoculation should include assessment of the immunodominant CRFK proteins detected in this study.

#### **ABSTRACT #201**

COMPARISON OF THE MODIFIED SELDINGER AND THROUGH-THE-NEEDLE JUGULAR CATHETER PLACEMENT TECHNIQUES IN THE DOG. EM Portillo, AJ Mackin, PK Hendrix, CR Boyle, L Chrestman. Mississippi State University, Starkville, MS.

Jugular intravenous catheters, although extremely useful for rapid fluid delivery, collection of multiple blood samples, measurement of central venous pressures and administration of hyperosmolar fluids, are underutilized by many veterinarians. Two established methods for the percutaneous insertion of jugular catheters are the through-the-needle technique and the modified Seldinger technique. The objectives of our study were to evaluate the teachability of these two jugular catheter placement techniques in dogs, and to demonstrate whether the adoption of one or both of these central catheter placement procedures was feasible for practitioners with limited or no experience with either technique.

Junior and senior veterinary students ( $n = 35$ ) were taught both the through-the-needle and the modified Seldinger catheter placement technique on a synthetic jugular venipuncture model. The students then attempted both techniques (one method for each jugular vein) in anesthetized dogs. The students scored the two techniques for ease of placement in comparison to cephalic vein catheterization, and also subjectively reported which placement technique seemed less difficult. Catheter placements were timed from the time of initial venipuncture until the time the catheter was fixed in place. Placement was considered to be a failure if the catheter could not be placed during the original venipuncture attempt. Immediately after successful catheter placement, the catheters were evaluated for ease of blood sample collection, and for ease of fluid flow. A limited necropsy was then performed to determine the degree of trauma at the insertion site.

Jugular catheter placement was successful for 27/35 (77.1%) first attempts on a live dog with the modified Seldinger technique, and for 26/35 (74.3%) first attempts with the through-the-needle technique. The modified Seldinger technique took significantly longer (mean 452 seconds) than the through-the-needle technique (mean 273 seconds). There was no significant difference in the time taken to collect 5 milliliters of blood through catheters placed by either technique. The time to flow 25 milliliters of fluid through the catheter was slightly shorter for the through-the-needle technique (mean 113 seconds) than for the modified Seldinger technique (mean 133 seconds). Post-mortem hematomas were detected at 26/26 (100%) of through-the-needle insertion sites and at 24/27 (89%) of modified Seldinger insertion sites. Hematoma diameter at the modified Seldinger insertion site (mean 1.1 centimeters) was significantly less than at the through-the-needle insertion site (mean 2.2 centimeters). Student assessments of the comparative difficulties of the two jugular catheter placement techniques were not significantly different, and both techniques were assessed to be only slightly more difficult than placement of a cephalic catheter.

Jugular catheter placement by either the modified Seldinger or through-the-needle technique can both be relatively easily mastered by entry level veterinarians. The teaching of jugular catheter placement techniques is facilitated by the use of a synthetic jugular venipuncture model.

#### **ABSTRACT #202**

DIFFERENCES BETWEEN HUMAN AND OTHER MAMMALIAN ALBUMINS RAISES CONCERNS OVER THE USE OF HUMAN SERUM ALBUMIN IN THE DOG. Juliene L. Throop<sup>1</sup>, Susan Bingaman<sup>2</sup>, Virginia Huxley<sup>2</sup>. <sup>1</sup>School of Veterinary Medicine, <sup>2</sup>Dept. of Med. Pharmacology and Physiology, MA 415 MSB, University of Missouri, Columbia, MO.

Human serum albumin is occasionally used in hypoalbuminemic canine patients to increase albumin levels and hence colloid oncotic

pressure. The assumption is that since there is significant amino acid homology among mammalian albumins, there is similar homology with regard to biochemical and physiologic functions. To test this hypothesis, selected characteristics (molecular weight, relative charge, and isoelectric point) from several mammalian species (human, dog, horse, pig, cow, rat and mouse) were determined using gel electrophoresis. Molecular weights were very similar with the exception of the canine albumin which was approximately 2 kDa greater than the other albumins examined. Differences existed between species with regard to relative charge and isoelectric point, especially in the case of the dog albumin which was relatively anionic. These biochemical differences despite high sequence homologies raise concern over the use of human serum albumin in canine patients. Further research investigating the potential antigenicity and efficacy of human albumin in the canine patient needs to be performed before this product can be safely recommended for routine use.

#### ABSTRACT #203

PREVENTIVE USE OF BRONCHODILATORS REDUCES BRONCHOSCOPY-INDUCED AIRFLOW LIMITATION IN CATS. Kirschvink Nathalie<sup>1</sup>, Leemans Jérôme<sup>1</sup>, Delvaux François<sup>1</sup>, Billen Frédéric<sup>2</sup>, Clercx Cécile<sup>2</sup>, Gustin Pascal<sup>1</sup>. <sup>1</sup>Department for functional sciences B41, <sup>2</sup>Department for clinical sciences B44, Faculty of veterinary medicine, University of Liège, Belgium.

Bronchoscopy and bronchoalveolar lavage (BAL) have been shown to induce transient but significant airflow obstruction in healthy cats, which might be assessed by barometric whole body plethysmography (BWBP). Especially Penh (enhanced pause), an index of bronchoconstriction, is increased immediately after BAL in healthy cats. The purpose of this study was to test whether preventive administration of bronchodilators prior bronchoscopy in healthy and allergen-sensitised cats allowed to decrease post-BAL airflow obstruction.

Six healthy cats (Controls) and six ovalbumin-sensitised cats (OVA) were investigated twice at a 2-months interval by bronchoscopy, BAL and BWBP. OVA-cats were challenged twice weekly by 5-min nebulisations of allergen, leading to a persistent neutrophilic airway inflammation. Penh was recorded after medetomidine-induced sedation, after propofol-induced anesthesia and immediately after BAL. Cats remained once untreated and received once prior sedation two puffs of salbutamol (200 µg) using a metered dose inhaler and a spacing chamber connected to a facemask. BAL was performed using a pediatric endoscope (5 mm OD) and 10 ml of heated saline for each lung side. BAL fluid was analysed cytologically.

Without bronchodilator treatment, Penh-values of control and OVA cats recorded prior sedation ( $1.07 \pm 0.13$  vs  $0.94 \pm 0.09$ , NS), after sedation ( $0.63 \pm 0.06$  vs  $0.72 \pm 0.05$ , NS) and after anesthesia induction ( $1.05 \pm 0.16$  vs  $1.16 \pm 0.16$ , NS) were similar. Post-BAL values were significantly higher in OVA cats than in control cats ( $1.87 \pm 0.16$  vs  $1.36 \pm 0.17$ ,  $P < 0.05$ ), as well as BAL neutrophil (PMN) percentage ( $17 \pm 7$  vs  $8 \pm 3$ ,  $P < 0.05$ ). After salbutamol inhalation, Penh values recorded after sedation and anesthesia induction were similar in both groups and were similar to the preceding results. After BAL, Penh records in OVA cats were significantly decreased ( $1.42 \pm 0.11$  vs  $1.87 \pm 0.16$ ,  $P < 0.05$ ), whereas no significant decrease was noted in control cats ( $1.32 \pm 0.12$  vs  $1.36 \pm 0.17$ , NS). BAL cytology of both groups was similar to the preceding dataset. Correlation between Post-BAL Penh and BAL PMN percentage was significant when cats were untreated prior bronchoscopy ( $r = 0.54$ ,  $P < 0.05$ ), which was no more the case after preventive salbutamol treatment ( $r = 0.05$ , NS).

In conclusion, these results show that 1) bronchoscopy followed by BAL induces bronchoconstriction in healthy and allergen-sensitised cats, 2) the intensity of bronchoconstriction is independent on Penh-

values recorded in conscious cats, 3) the degree of bronchoconstriction is increased if lower airway inflammation is present and 4) preventive use of a short-acting bronchodilator significantly decreases BAL-induced bronchospasm.  
Funded by: RW DGTRE & ULg, Belgium.

#### ABSTRACT #204

WHOLE-BODY BAROMETRIC PLETHYSMOGRAPHY IN HEALTHY DOGS: REPEATABILITY AND MEASUREMENT OF AIRWAY RESPONSIVENESS. J. Talavera<sup>1</sup>, F. Billen<sup>1</sup>, P. Gustin<sup>2</sup>, N. Kirschvink<sup>2</sup>, C. Clercx<sup>1</sup>. <sup>1</sup>Department for clinical sciences B44, <sup>2</sup>Department for functional sciences B41, Faculty of veterinary medicine, University of Liège, Liège, Belgium.

Barometric whole-body plethysmography (BWBP) is a non-invasive technique able to monitor airway responses to induced bronchoconstriction, as shown in rodents and cats. So far this technique has not yet been used in dogs. The aims of this study were to assess the validity of BWBP in healthy dogs and to standardize a simple bronchoconstriction test to investigate airway responsiveness in healthy dogs using BWBP.

Six healthy beagle dogs (14 to 18 kg; 6 to 8 years) were used. The chamber of BWBP (Model PLY 3220, Buxco Electronics Incorporated, Sharon, CT, USA) included a test chamber (inner volume of 299,5 L) and a reference chamber, a differential pressure transducer attached to both chambers, a preamplifier and a computing system for recording, digitalizing and calculating box pressure derived parameters. Continuous bias flow (8 L/min) was introduced by controlled suction (Bias Flow Regulator PLY 1050, Buxco Electronics Inc, Sharon, Conn). Values calculated from the box signal included frequency, tidal volume, inspiratory and expiratory times, peak inspiratory and expiratory flows, pause and enhanced pause (Penh). Penh was used as an index of bronchoconstriction. In order to evaluate the repeatability of the BWBP measurements and the influence of the time passed into the chamber, respiratory variables were collected during two morning and two evening sessions of 10.5 minutes each. Data from each 10.5 minutes session were then divided in three 3.5 minutes periods. For the bronchoprovocative challenge, the respiratory variables were recorded during 5-minutes period before and after 3-minute of aerosol administration (jet nebulizer, AIGLON, Impec) of saline solution and each increasing concentrations of histamine (0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4%) directly into the chamber. When mean value for Penh of 10 consecutive breaths exceeded 300% baseline, additional aerosol administration was not performed. A two-way ANOVA was used to test for differences between sessions and periods, the influence of histamine concentration on respiratory parameters as well as to test for the repeatability of bronchoconstrictive challenge ( $p < 0.05$ ).

Basal values of Penh were  $0.74 \pm 0.19$  (0.45-1.02). There were no significant effects of the session or periods in the measurements, but there was a significant dog effect. Bronchoprovocative challenge with histamine was safe, highly reproducible and induced a significant increase in Penh.

In conclusion: (1) BWBP is a safe, non-invasive and valid method for monitoring respiratory function in dogs, (2) Penh is a sensitive index for bronchoconstriction and (3) challenge with increasing concentrations of histamine is a reproducible and safe method of investigation of bronchoreactivity in dogs.

## ABSTRACT #205

EVALUATION OF THE AUTOMATED d-ROMs TEST FOR DETECTION OF HYDROPEROXIDES IN EXHALED BREATH CONDENSATE OF HEALTHY CATS. K.Vondrakova<sup>o</sup>, I. Schwedenwein\*, R.van den Hoven\*, R.A. Hirt\*. \*1<sup>st</sup> Medical Clinic, Veterinary University Vienna, Austria, <sup>o</sup>Small Animal Clinic, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic.

Analysis of inflammatory markers such as hydroperoxides in exhaled breath condensate (EBC) represents a new diagnostic tool for lower airway diseases. In this study an automated spectrophotometric assay (Diacron Reactive Oxygen Metabolites test, d-ROMs test) for detection of reactive oxygen metabolites in EBC was compared with a manually performed standard spectrophotometric method (MS) of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) measurement in EBC.

Ten clinically healthy European shorthair cats (age 3-5y) were included in the study. Each cat was placed in a plexiglass box connected to a cooled glass tube for collection of EBC; movement of the expired air through the system was supported by active suction. EBC samples were analysed immediately after collection by d-ROMs test and MS (A). In addition, respiratory rate (RR), tidal volume (VT) and enhanced pause (Penh) were measured by use of barometric whole body plethysmography at baseline and after nebulisation of increasing concentrations of carbachol. Endpoint was the concentration of carbachol which increased Penh to 300% of the value obtained after saline nebulisation (PCPenh300). In order to assess the inter-day repeatability of results the second EBC collection and hydroperoxides measurement were performed (B). Statistical analysis was performed using ANOVA. Data in the table are shown as mean ± SD.

Sample	V <sub>EBC</sub> (ml)/30min	H <sub>2</sub> O <sub>2</sub> (μmol/ml)	dROMs(U.Carr)	dROMs(μmol <sub>H2O2</sub> /ml)
A	1.16±0.67	9.57±13.80	22.8±2.14	0.54±0.05
B	0.55±0.52	12.56±14.23	29.9±13.61	0.70±0.32
	RR	TV	Penh	PCPenh300
	107±53	25±16	0.65±0.22	0.11±0.05

There was significant inter-day difference in volume of collected EBC but not in results of both methods of hydroperoxides measurement. No good correlation was found between results of MS and d-ROMs test (r=0.58).

The data suggest a high variability in EBC production in cats and various possibly influencing factors require further investigation. Because of the lack of good correlation between both methods of hydroperoxide measurement, the easy practicable D-ROMs test cannot be considered a replacement of MS in detection of hydroperoxides in EBC.

## ABSTRACT #206

CLINICAL EFFICACY AND SAFETY OF RECOMBINANT FELINE ERYTHROPOIETIN IN CATS WITH CHRONIC KIDNEY DISEASE AND CATS WITH HUMAN ERYTHROPOIETIN-INDUCED RED CELL APLASIA. J.F. Randolph, J.M. Scarlett, T. Stokol, J.N. MacLeod. College of Veterinary Medicine, Cornell University. Ithaca, NY.

Efficacy and safety of recombinant feline erythropoietin (rEPO) was evaluated in 19 cats with the anemia of chronic kidney disease (Group 1) and 7 cats with chronic kidney disease and recombinant human erythropoietin-induced red cell aplasia (Group 2). The rEPO was synthesized using Chinese Hamster Ovary (CHO) cells transfected with feline erythropoietin cDNA (GenBank accession number U00685). Hematocrit (Hct) and absolute reticulocyte count (ARC) were monitored weekly for the first 8 weeks, CBC including ARC and serum iron profiles were done monthly, and serum biochemical analyses were performed every 2 months during a 6 (Group 2) to 12 (Group 1) month course of rEPO therapy. Clinically relevant changes in median hematologic and biochemical values compared to baseline values were evaluated for statistical significance (p≤0.05) using the Wilcoxon signed rank test.

In both groups of cats, median Hct and ARC increased significantly during the first 3 weeks of rEPO treatment. Eighty nine % (17/19) of Group 1 and 71% (5/7) of Group 2 cats responded (≥50% increase above baseline Hct) to rEPO treatment. Improvements in appetite and energy coincided with increased hematocrit, which generally could be maintained within a target range of 30%-40% with periodic rEPO dose adjustments. Unexpectedly, five Group 1 cats and three Group 2 cats that initially responded to rEPO therapy re-developed anemia after 12 to 38 (median, 14.5) weeks of rEPO therapy that was refractory to further rEPO treatments even at higher doses. This rEPO non-responsive anemia was associated with profound reticulocytopenia and was ultimately more severe than on entry into the study. Bone marrow aspirate cytology completed on 4 of these 8 affected cats confirmed red cell aplasia.

In conclusion, rEPO can re-establish active erythropoiesis in most cats with chronic kidney disease, even those suffering from rEPO-induced red cell aplasia, but some patients become refractory to therapy. Unfortunately, development of red cell aplasia during treatment with CHO-derived recombinant erythropoietin proteins was not eliminated as a serious safety concern even with this feline-specific preparation.

## ABSTRACT #207

D-DIMERS AND OPTIMIZED PROTHROMBIN TEST FOR DIAGNOSIS OF D.I.C. IN DOGS. Johannes Hirschberger<sup>1</sup>, Anke Regel<sup>1</sup>, Stefan Krieger<sup>2</sup>, Helmut Küchenhoff<sup>2</sup>; <sup>1</sup>Medizinische Kleintierklinik, University of Munich, Germany, <sup>2</sup>Statistisches Beratungslabor, Institute for Statistics, University of Munich, Germany.

For the diagnosis of D.I.C. many coagulation tests are used and the sum of results is interpreted. We evaluated the diagnostic sensitivity and specificity for D.I.C. of the usual coagulation tests plus the d-dimer test (D-D), and an optimized prothrombin test (oPT) (Mischke R, Nolte I, 1999).

Blood samples from 304 diseased dogs (anemia, Cushing's syndrome, gastrointestinal, cardiac, hepatic, renal, pulmonary, neoplastic disease or pancreatitis) and 50 healthy dogs were tested for D-dimers, optimized prothrombin time, conventional prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TZ), fibrinogen degradation products (FDP), AT III activity (ATIII), fibrinogen concentration (Fibr.), platelet count (PC), and schistocytes (Schist). Neoplastin™ Plus (Roche Diagnostics, Mannheim, Germany) was used according to the official manufacturer's instructions for the estimation of oPT and the modifications by Mischke and Nolte (1999). D-D were measured by use of the latex agglutination test Accuclot™ D-Dimere (Sigma Diagnostics, Munich, Germany). In order to standardize the diagnosis of D.I.C. statistically, the following definition was used: all dogs with abnormal coagulation values for at least 5 out of 10 tested laboratory parameters were assumed to have D.I.C.. One hundred seven of 304 mostly severely diseased dogs had at least 5 out of 10 parameters, which were out of the reference range, and were regarded as having D.I.C.. The method of binary logistic regression was used to analyse, which laboratory parameters are most efficient in the diagnosis of D.I.C.

The analysis showed that regarding sensitivity and specificity the oPT and the D-D were by far superior to the other tests. Sensitivity of the oPT was 82.9 % and the specificity was 97.2%; the D-D test had a higher sensitivity (94.8%) but a lower specificity (92.8%).

	oPT	D-D	PT	APTT	TZ	FDP	FC	ATIII	PC	Schist.
Sensitivity	82.9%	94.8%	4.8%	9.8%	9.8%	68.3%	0.8%	69.9%	52.0%	6.5%
Specificity	97.2%	92.8%	99.4%	97.8%	99.4%	83.4%	100%	84.5%	82.3%	100%

Table 1: Diagnostic sensitivity and specificity of coagulation tests and parameters for D.I.C.

Measurement of D-dimer concentration and optimized prothrombin test have proven to be highly sensitive and specific tests for D.I.C. in

dogs. Thanks to their simple usability these tests are suitable for intensive care units as well as for veterinary practitioners.

Mischke R, Nolte I. Hämostasediagnostik beim Hund: Prinzip, Technik und Referenzbereich verschiedener Untersuchungsverfahren. *Prakt Tierarzt* 1999;80:836-855.

#### **ABSTRACT #208**

AZATHIOPRINE AND ULTRA-LOW-DOSE ASPIRIN THERAPY FOR CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA. TK Weinkle, SA Center, JF Randolph, SC Barr, HN Erb. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Ten years ago we prioritized azathioprine (AZA, 1-2 mg/kg PO EOD) and glucocorticoids (Glc) as preferred treatment for canine immune-mediated hemolytic anemia (IMHA) in our clinic. We hypothesized that ultra-low dose aspirin (ASA, 0.5 mg/kg/day) may provide better thromboprophylaxis than mixed-molecular weight heparin (Hep, 75-125 U/kg, sc, q 6-8 hrs) after whole blood platelet impedance aggregometry (11 healthy dogs treated with this ASA regimen) confirmed reduced platelet aggregation. A retrospective study of patients treated during this interval was undertaken to characterize: population, prognostic markers, influence of AZA & Glc +/- ultra-low dose ASA or mixed-molecular weight Hep, and transfusion therapy on case outcome.

Dogs (n=151) were stratified into groups based on: presence or absence of 1)spherocytes, 2)regeneration, 3)agglutination; treatments with 4)AZA, 5)AZA & ASA, and 6) AZA & Hep (hospitalization only); and 7) survival vs non-survival categories. Nonparametric analyses were applied to physical and clinicopathologic data in IMHA dogs, platelet aggregation data in healthy dogs; IMHA groups were analyzed for bias variables. Parametric analyses were applied to signalment and breed data versus the general hospital population. Kaplan-Meier survival curves and two-sample survival analyses compared treatment responses; two by two tables compared outcome to collated published information for canine IMHA. An  $\alpha$  of 0.05 was applied.

Female, neutered, and Cocker spaniel dogs and presentation during warm months were over-represented; 30% of dogs with vaccination histories presented within 2 months of immunization. Overall features: mean age 6.6 +/- 2.9 yrs, median PCV 15% (4-35%), spherocytes 89%, regeneration 71%, autoagglutination 78%, transfusion therapy 70% [21% receiving bovine hemoglobin-based oxygen carrier (HBOC)], AZA 27%, AZA & ASA 68%, AZA & Hep 27%. Dogs given AZA & Hep had markers of more severe consumptive coagulopathy. Factors associated with survival included: younger age, slower respiratory rate, lower mature and band neutrophil and higher platelet counts, higher total protein, albumin and potassium, lower bilirubin, ALT, and CK, and shorter APTT. Except for age, platelet count and ALT, these factors did not bias AZA vs AZA & ASA treatments. Transfusion therapy and HBOC did not negatively impact survival. Survival to hospital discharge (1), at 30 days (2), 60 days (3), and 365 days (4) for AZA, AZA & ASA, and AZA & Hep and collective published survival data\* were: (1) 58%, 84%, 54%, 59%\* (2) 43%, 78%, 49%, 58%\*, (3) 38%, 71%, 49%, not available\*, (4) 34%, 67%, 46%, 34%\*. Treatment with AZA & ASA significantly improved survival compared to AZA alone, AZA & Hep, and previously reported treatment regimens at all time intervals. Ultra-low-dose ASA significantly reduced platelet aggregation in healthy dogs and presumably provides similar benefit in dogs with IMHA, although other ASA effects may have influenced our findings.

#### **ABSTRACT #209**

SUCCESSFUL THERAPY WITH A VITAMIN K2 ANALOG (MENATETRENONE) IN FELINE MYELODYSPLASTIC SYNDROMES. Hisasue M, Neo S, Tuchiya R, Yamada T. Azabu University, College of Veterinary Medicine. Kanagawa, Japan.

Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoiesis characterized by multi-lineage cytopenia and bone marrow dysplasia, and the prognosis was poor. Recently, it was described that menatetrenone, a vitamin K2 analog, could develop differentiation of leukemic cell lines and apoptosis of immature blasts in human myelodysplastic syndromes (MDS). To determine a possible role of this agent in treatment of feline MDS, we conducted a prospective trial to assess efficacy of menatetrenone. The 6 cats diagnosed as MDS were received administration of menatetrenone (2mg/kg), 4 cats (67%) showed marked improvements of hematological abnormalities. Improvement of anemia was found in 4 cats (67%). Additionally, increase of neutrophils was seen in 3 of 5 cats (60%) with neutopenia, and thrombocytopenia was resolved in 2 of 4 cats (50%). Of the cats treated with menatetrenone, median of survival duration (166 days) was longer than that of previous study (63 days). Furthermore, none of complication was shown during study. In the present study, we suggested that menatetrenone differentiation-inducing therapy might be effective and safe to treat feline MDS.

#### **ABSTRACT #210**

BIOLOGIC BEHAVIOR OF CALCIUM OXALATE UROLITHS IN BICHON FRISE DOGS. J\_Lulich, C. Osborne, B. Daubs, T. Hill. Minnesota Urolith Center, University of Minnesota, St Paul, MN.

Epidemiological studies have revealed that the risk of calcium oxalate (CaOx) uroliths is approximately 7 times greater in Bichon Frise dogs admitted to veterinary hospitals than dogs admitted without urinary tract disease. However, the frequency and time of recurrence of CaOx uroliths in Bichons has not been evaluated in a systematic fashion. This information is needed to determine whether therapy designed to prevent recurrence of CaOx uroliths is warranted.

Records of 50 Bichons (34 males and 16 females) with CaOx uroliths admitted to our hospital between 1990 and 2002 were reviewed. Mean age of Bichons at initial urolith detection was 7.6±2.4 years. Urinalyses (n=27) revealed that specific gravity was 1.027±1.012 and pH was 7.3±0.8; only 37% had CaOx crystalluria.

Thirty-three Bichons were evaluated following surgery. CaOx recurrence occurred in 24. Recurrence rate increased with length of time that Bichons were evaluated. After 1 year, 37% had their first recurrence; after 2 years, 57% had their first recurrence and 7% had their second recurrence; after 3 years, 73% had their first recurrence, 16% had their second recurrence, and 3% had their third recurrence. Urolith recurrence was detected in 100% of dogs evaluated ≥ 4 years. At first recurrence; neither age, gender, urine specific gravity, urine pH, CaOx crystalluria, nor serum calcium concentration was associated with the propensity for urolith formation.

These observations indicate that following urolith removal CaOx recurrence is imminent. Therefore, long-term clinical trials are needed to evaluate the efficacy of therapy to minimize urolith recurrence.

#### **ABSTRACT #211**

COYRNEBACTERIUM UREALYTICUM CYSTITIS IN DOGS AND CATS. NL Bailiff, JL Westropp, SS Jang. School of Veterinary Medicine, University of California, Davis, CA.

*Corynebacterium urealyticum* (formerly known as *Corynebacterium* group D2) is a non-hemolytic Gram positive, aerobic, nonspore-bearing bacillus, which is a rare bacterial cause of urinary tract infections (UTIs) in small animals. *C. urealyticum*

infection in the bladder often causes an encrusting cystitis characterized by an ulcerative necrotic mucosa, and whitish adherent plaques that can be visualized cystoscopically in humans. Isolated cases of *C. urealyticum* have been described in the dog. The purpose of this study was to characterize the morphologic features of *C. urealyticum* cystitis in small animals and to evaluate treatment regimens.

Medical records of dogs and cats from 1996-2003 from the UC Davis VMTH with positive urine cultures for *C. urealyticum* were reviewed. Prior to 1996 no *Corynebacterium* isolates (22 canine cases) were subclassified specifically as *urealyticum* (or group D2.) Five dogs and 3 cats were included for review. Three dogs were spayed females and 2 were castrated males (ages 6 months – 14.5 years). Four of the 5 dogs were large mixed breeds with an average weight of 25.5 kg and one was a Bichon Frise. Cats were male, castrated domestic shorthairs. All but one animal had lower urinary tract signs; 4/5 dogs had gross hematuria. At the time of the positive urine culture, all dogs had micturition disorders including pelvic fractures with urethral injury, multifocal myelopathy, recurrent UTIs with repeated catheterizations to aid in urine sampling, ectopic ureter, and vaginal carcinoma. Two cats had previous urethral obstructions, one of which had a perineal urethrostomy.

Urine samples from all 8 patients were collected by cystocentesis. The pH ranged from 6.0-9.0 (median, 8.0). WBC and bacteria were variably seen upon sedimentation. Growth of *C. urealyticum* was slow, often requiring 2-3 days before identification. Growth during MIC testing occurred in only 4/5 dog samples. *C. urealyticum* was frequently reported susceptible to only chloramphenicol and tetracycline, variable susceptibility to trimethoprim sulfa and enrofloxacin, and resistant to ampicillin, cephalixin, and amoxicillin/clavulanic acid. Ultrasound was available on all 5 dogs. All dogs had thickened bladder walls, three had sediment visualized, two of which contained marked echogenic material. In two dogs unilateral ureteral dilation was seen as well. Three dogs had findings on urinary endoscopy compatible with encrusting urethritis/cystitis.

Two dogs were cleared of *C. urealyticum* with surgical intervention for plaque debridement and antibiotic therapy. One dog had recurrent UTIs over 2 years until its death due to an unrelated cause. The dog with vaginal carcinoma was euthanized, and one dog was lost to follow up. One cat was treated successfully with antibiotics, one was euthanized, and the third had recurrent UTIs.

*C. urealyticum* is a rare but serious cause of UTIs in small animals due to its multi-drug resistance patterns and capability to form adherent plaques to the bladder mucosa (“encrusting” cystitis). Animals with micturition disorders appear predisposed and warrant extended urine culture period (72 hours) to improve detection of this bacteria.

#### ABSTRACT #212

ACCURATE LIGHT MICROSCOPIC DETECTION OF BACTERIURIA IN CATS. Swenson CL, Kruger JM, Gibbons-Burgener SN, Boisvert AM. Michigan State University, East Lansing, MI.

This study compared the diagnostic performance of light microscopic examination of unstained wet mount and air-dried modified Wright-stained urine sediment preparations with quantitative aerobic bacterial culture as the gold standard for detection of bacteriuria in cats.

Complete urinalysis and quantitative aerobic bacterial culture were performed on 476 feline urine samples collected by cystocentesis. Routine unstained wet mount and air-dried modified Wright-stained (Diff Quick®) urine sediment preparations were examined by light microscopy for the presence of bacteria. The total number of bacteria in 20 oil immersion fields (oif, X1000) on Wright-stained slides was recorded and a ROC curve analysis was employed to determine the optimum lower limit for reporting bacteriuria. Quantitative aerobic

bacterial urine culture was performed within 12 hours of urine collection.

Results of routine unstained urine sediment examination compared with quantitative urine culture had sensitivity, specificity and positive and negative predictive values of 76.7%, 57.2%, 10.7% and 97.3%, respectively. Overall test efficiency of unstained urine sediment examination was 58.4%. Results of Wright-stained urine sediment examinations (following ROC curve analysis) compared with quantitative urine culture, showed sensitivity, specificity and positive and negative predictive values of 80.0%, 97.8%, 70.6%, and 98.6%, respectively. Overall test efficiency of Wright-stained urine sediment examination was 96.6%. Specificity, positive predictive value and test efficiency of the Wright-stained method for detecting bacteriuria were significantly improved over the routine unstained urine sediment method.

In conclusion, modified Wright-stained examination of urine sediment was a rapid, cost effective method that substantially improved light microscopic detection of bacteriuria in cats.

#### ABSTRACT #213

ACUTE PANCREATITIS IN TWO DOGS ASSOCIATED WITH SHOCK WAVE LITHOTRIPSY. M.A. Daugherty,<sup>1</sup> L.G. Adams,<sup>1</sup> D.K. Baird,<sup>1</sup> J.J. Siems,<sup>2</sup> J.E. Lingeman.<sup>3</sup> <sup>1</sup>School of Veterinary Medicine, Purdue University, West Lafayette, IN, <sup>2</sup>Inland Empire Veterinary Imaging, Spokane, WA, <sup>3</sup>Methodist Hospital Institute for Kidney Stone Disease, Indianapolis, IN.

Extracorporeal shockwave lithotripsy (ESWL) has been reported to be a safe and efficacious therapy for the treatment of nephroliths and ureteroliths in dogs. Acute pancreatitis as a result of ESWL treatment of nephroliths has been described infrequently in human medical literature; however, this complication has not been reported in veterinary literature. In a previous study, we reported that ultrasonography performed before and after ESWL in 14 dogs failed to demonstrate any evidence of pancreatic injury. Recently, we have documented two dogs with acute pancreatitis following ESWL treatment. In this retrospective study, medical records from all dogs undergoing ESWL for treatment of nephroliths, ureteroliths, and/or urocystoliths at Purdue University School of Veterinary Medicine from November 1992 through June 2003 were evaluated for evidence of acute pancreatitis as a complication of ESWL. The purpose of this study is to describe two cases of acute pancreatitis which developed following ESWL.

ESWL treatments were performed under general anesthesia using an unmodified Dornier HM3 lithotripter. A total of 109 ESWL treatments were administered to 76 dogs. Twenty-three dogs received more than one treatment. Increases in amylase or lipase were noted after 26 of 109 ESWL treatments; 9/26 had an increase in both amylase and lipase. The magnitude of elevation of amylase or lipase ranged from less than 2-fold elevation to a 7-fold increase in one dog with clinical pancreatitis. Six dogs had mildly increased lipase prior to ESWL that decreased after treatment but did not normalize.

Two of the 76 dogs developed clinical evidence of acute pancreatitis following ESWL treatment. Diagnostic evaluation prior to ESWL treatment including abdominal ultrasound and routine biochemical analysis did not reveal any evidence of pancreatitis in either dog. Pancreatitis was confirmed via clinical signs and abdominal ultrasonography in one case, and clinical signs, biochemical analysis, and post-mortem examination in the second case. One of the two dogs died from cardiac infarction and thromboembolism which may have been secondary to consumption of antithrombin from acute pancreatitis.

Mean body weight, mean voltage per shock wave and mean number of shock waves per treatment were similar for dogs with normal pancreatic enzymes post ESWL, dogs with increased pancreatic enzymes post ESWL, and dogs that developed pancreatitis. Both cases of confirmed pancreatitis involved ESWL treatment of the left



and right kidneys and/or proximal ureters. Due to the close anatomical proximity of the right lobe of the pancreas to the right kidney, ESWL treatment of the right kidney or ureter may result in shockwave induced injury to the pancreas.

#### **ABSTRACT #214**

USE OF COMBINED HEMOPERFUSION AND HEMODIALYSIS IN ACCIDENTAL ENROFLOXACIN OVERDOSE. T. Francey, N Benitah, V Pantaleo, VJ Wiebe, LD Cowgill. School of Veterinary Medicine, University of California Davis, CA.

Extracorporeal blood purification techniques including hemoperfusion (HP) and hemodialysis (HD) can be used to remove toxins and drugs from the blood stream in the case of accidental exposure or overdose. Combination of sorbent HP (adsorption) and conventional HD (diffusion and convection) widens the spectrum of removable toxins to larger molecular weight solutes and molecules with different protein-binding characteristics. The efficacy and safety of combined HP/HD for toxin removal is demonstrated in a uremic cat with accidental enrofloxacin (ERF) overdose.

A Siamese cat (6 y MC, 7 kg) with advanced CRF (creatinine, 9.1 mg/dl) was accidentally injected with 25 mg/kg ERF (10x overdose) intravenously. Rapid onset of seizures was initially controlled with diazepam. HP/HD was performed using a neonatal extracorporeal circuit modified to include a 50 ml Clark<sup>®</sup> Biocompatible Hemoperfusion System (Folsom, La.) activated charcoal cartridge upstream to a Cobe 100HG hemodialyzer. The total volume of the modified extracorporeal circuit was 98 ml. ERF and its metabolite, ciprofloxacin (CPF) were measured with HPLC hourly for 4 h during and at 12 and 45 h following HP/HD. The respective plasma clearances for the HP cartridge and the hemodialyzer were computed from the combined clearance of both devices and the clearance of the devices when the dialyzer was in bypass.

Plasma ERF concentration at initiation of treatment (6 h post injection) was 22.7 mcg/ml. The concentration immediately after injection was extrapolated to be 26.2 mcg/ml and the volume of distribution was calculated at 6.7 L (95% of the BW). A total of 3.4 L of blood were processed in 4 h, resulting in a 64% decrease of ERF, compared to 76% for BUN, 78% for creatinine, and 78% for phosphorus. The in vivo elimination half-life of ERF was 29.8 h in this uremic cat, in marked contrast to 3-6 h reported in cats with normal renal function, and to 2.9 h during HP/HD. Single-pass extraction ratios for ERF and CPF were 62% and 43% for HP alone and 93% and >53% (post-device concentration of CPF below the detection limit) with combined HP/HD, respectively. Fractional clearance of ERF was 49.2% for HP alone, 63.8% for HD alone, and 73.1% for combined HP/HD at a blood flow rate of 12.5 ml/min. No sign of saturation of the HP cartridge was observed during the procedure. Clinically, the seizures subsided without further diazepam and no adverse consequences were noted with the combined HP/HD technique. Body temperature and systemic blood pressure remained unchanged. Total white blood cell and neutrophil counts decreased by 43% and 55%, respectively, with evidence of cellular toxicity at the end of the procedure. Platelet count was adequate but could not be counted due to clumping at 4 h.

These results confirm that renal excretion following large doses of ERF represents a major route of elimination in cats and that combined HP/HD is an effective and safe means to treat accidental ERF overdose.

#### **ABSTRACT #215**

EFFECTS OF DERACOXIB AND ASPIRIN ON THYROID FUNCTION TESTS IN HEALTHY DOGS. Panciera DL<sup>1</sup>, Refsal KR<sup>2</sup>, Sennello KA<sup>1</sup>, Ward DL<sup>1</sup>. <sup>1</sup>Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA; <sup>2</sup>Endocrine Section Diagnostic Center for

Population and Animal Health, College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Some nonsteroidal anti-inflammatory drugs have been shown to depress serum thyroxine (T4), 3,5,3'-triiodothyronine (T3), and free T4 (fT4) concentrations, while others have no effect. This study was performed to evaluate the effects of a high dose of aspirin and a therapeutic dose of deracoxib on thyroid function tests in dogs. Twenty-four healthy, adult dogs were randomly assigned to one of three treatment groups consisting of 8 dogs each. Dogs in group 1 received placebo TID; group 2 received aspirin 25 mg/kg TID; group 3 received deracoxib 1-2 mg/kg QD and placebo BID. All treatments were administered for 28 days. This study was carried out in conjunction with a study comparing the gastrointestinal complications of aspirin and deracoxib administration. Dogs were anesthetized for endoscopy on days -7, 6, 14, and 28. Prior to anesthesia on days -7, 14, and 28, and 14 days after cessation of treatment, blood samples were collected for measurement of serum concentrations of T4, T3, fT4 (equilibrium dialysis), and canine TSH. Plasma total protein, albumin, and globulin concentrations were measured on day -7 before treatment, and day 28 of treatment.

Serum T4, fT4 and T3 concentrations decreased significantly from baseline in the aspirin group compared with the placebo group on days 14 and 28 of treatment. Mean and 95% confidence interval serum T3 concentrations at baseline (day -7), 14, and 28 days of treatment in the placebo group were 1.49 (1.28, 1.70), 1.43 (1.23, 1.65), and 1.68 (1.45, 1.78) nmol/L, respectively, and in the aspirin group were 1.50 (1.29, 1.71), 1.06 (0.85, 1.27), and 1.18 (0.84, 1.51) nmol/L, respectively. Mean and 95% CI serum T4 concentrations at baseline, 14, and 28 days of treatment in the placebo group were 29 (22, 36), 29 (24, 34), and 28 (21, 35) nmol/L, respectively, and in the aspirin group were 30 (23, 37), 14 (9, 19), and 13, (5, 20) nmol/L, respectively. Mean and 95% CI serum fT4 concentrations at baseline, 14, and 28 days of treatment in the placebo group were 16.5 (11.8, 21.2), 15.6 (12.8, 18.5), and 14.5 (10.5, 18.5) pmol/L, respectively, and in the aspirin group were 18.0 (13.3, 22.7), 9.4 (6.5, 12.2), and 7.1 (3.1, 11.2) pmol/L, respectively. At 14 days after cessation of treatment, serum T4, T3 and fT4 returned to baseline values in the aspirin group. Plasma total protein, albumin, and globulins decreased significantly on day 28 of treatment in the aspirin group. Significant changes in TSH were not noted at any time during the study. There were no significant changes in any hormone or protein concentration in dogs treated with deracoxib compared with placebo. Administration of aspirin, but not deracoxib, substantially decreases serum concentrations of T4, T3 and fT4. While the significant decrease in plasma proteins may account for a portion of the decrease in T4 and T3, it was not of sufficient magnitude to fully account for these decreases or the change in fT4.

#### **ABSTRACT #216**

INCIDENCE OF HYPOTHYROIDISM IN DOGS WITH CHRONIC HYPERLIPIDEMIA. P.A. Schenck, D. Donovan, K. Refsal, R. Nachreiner, M. Rick, Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI.

Hyperlipidemia refers to the presence of excess lipid in blood. Chronic hyperlipidemia may be due to a primary defect in lipid metabolism or secondary to an underlying systemic disease. Diseases associated with secondary hyperlipidemia include hypothyroidism, hyperadrenocorticism, diabetes mellitus, pancreatitis, cholestasis, and nephrotic syndrome. To determine the association of a history of chronic fasting hyperlipidemia with the occurrence of hypothyroidism, a database survey was conducted over a two-year period. Dogs with any illness (other than hypothyroidism) that could potentially be responsible for chronic hyperlipidemia were excluded. Results from 139,668 dogs not receiving thyroid supplement were reviewed. Of these, 2007 dogs had a history of chronic fasting hyperlipidemia, and 413 (20.6%) were determined to be hypothyroid

based on low thyroid hormone concentrations and an elevation of thyroid stimulating hormone. Of 137,661 dogs with no history of chronic hyperlipidemia, 10,396 (7.6%) were determined to be hypothyroid. Using the Yates' Chi Square fitness test, a history of chronic fasting hyperlipidemia was significantly associated with hypothyroidism ( $p=0$ ). The odds ratio was 3.2, indicating that dogs with chronic hyperlipidemia were 3.2 times more likely to be hypothyroid than those with no history of chronic hyperlipidemia. The presence of thyroglobulin autoantibodies in hypothyroid dogs was not significantly associated with chronic hyperlipidemia, occurring at a similar incidence in hypothyroid dogs with (48%) or without (50%) a history of chronic hyperlipidemia. The possibility of hypothyroidism should be investigated in dogs with a history of chronic hyperlipidemia due to the increased risk of hypothyroidism associated with chronic hyperlipidemia.

#### **ABSTRACT #217**

**CALCIUM METABOLIC HORMONES IN FELINE IDIOPATHIC HYPERCALCEMIA.** P.A. Schenck, D.J. Chew\*, K. Refsal, R. Nachreiner, M. Rick. Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI, \*The Ohio State University, Columbus, OH.

The etiopathogenesis of idiopathic hypercalcemia in cats is unknown, and abnormalities in calcium metabolic hormones may play a role. Excess concentrations of parathyroid hormone (PTH), 25-hydroxyvitamin D or calcitriol (1,25-dihydroxyvitamin D) could potentially result in hypercalcemia in these cats. A total of 427 cases of suspected feline idiopathic hypercalcemia were reviewed. Cats ranged in age from 0.5 to 20 years old (mean  $9.8 \pm 4.6$  yr), and 27% were long-haired cats. A lack of clinical signs was noted in 196 cases (46%). Uroliths or renoliths were observed in 15%, and calcium oxalate stones were specifically noted in 10% of cases. Mild weight loss with no other clinical signs was found in 18% of cats. Chronic constipation was noted in 5% of cats, and inflammatory bowel disease was seen in 6% of cats with idiopathic hypercalcemia. Mean PTH concentration was  $1.1 \pm 0.7$  pmol/L (reference range 0 – 4.0 pmol/L) and mean ionized calcium concentration was  $1.7 \pm 0.2$  mmol/L (reference range 1.0 – 1.4 mmol/L). Parathyroid hormone related protein was analyzed in 301 cases, and was negative in all samples. Ionized magnesium analysis was available in 327 cases, with a mean of  $0.6 \pm 0.1$  mmol/L (reference range 0.43 – 0.70 mmol/L). Concentration of 25-hydroxyvitamin D was determined in 65 cases, with a mean of  $96.2 \pm 38.4$  nmol/L, and ranged from 22 to 198 nmol/L (reference range 65 – 170 nmol/L). Calcitriol concentration was determined in 12 cases, with a mean of  $44.6 \pm 12.4$  pmol/L (reference range 50 – 100 pmol/L). Hypercalcemia is apparently not due to excessive PTH, 25-hydroxyvitamin D or calcitriol concentrations in cats with idiopathic hypercalcemia. Normal concentrations of ionized magnesium indicate that PTH secretion is not being inhibited by alterations in ionized magnesium.

#### **ABSTRACT #218**

**PREVALENCE OF URINARY TRACT INFECTIONS IN DIABETIC CATS.** N Bailiff, R Nelson, S Jang, J Westropp. School of Veterinary Medicine, University of California, Davis, CA.

The prevalence of positive urine cultures was determined in cats with and without diabetes mellitus. Aerobic urine cultures, collected by cystocentesis, from patients at the UC Davis VMTH obtained between 1995 and 2002 were reviewed. Urine specific gravity (USG) was required for inclusion in the study. Cats were excluded if records indicated antibiotic use or urinary catheterization within two weeks of sample collection or a previous urethrostomy surgery. For cats with multiple urine cultures, the first culture was used in the study.

Eight hundred and seventy-nine cats qualified for the study. One hundred forty-one diabetic cats were identified. An additional 738 cats were placed into 3 groups: renal failure (azotemic with USG  $\geq 1.007$  but  $< 1.030$ ;  $n=397$ ), lower urinary tract disease [LUTD] (130), or miscellaneous diseases (211). Miscellaneous diseases included gastrointestinal disease (GI; 52), early renal insufficiency (non-azotemic cats with USG  $\geq 1.007$  but  $< 1.030$  and supportive history and diagnostics; 46), hyperthyroidism (46), neurological disease (25), respiratory disease (22), and non-gastrointestinal neoplasia (20).

The overall prevalence of positive cultures was 135/879 (15%). Cats with diabetes had positive cultures in 18/141 (13%), renal failure in 70/397 (18%), LUTD in 13/130 (10%), and miscellaneous disease in 34/211 (16%). The prevalence of positive urine cultures among the subdivisions of the miscellaneous group was as follows: GI, 4%; renal insufficiency, 20%; hyperthyroidism, 24%; neurologic disease, 24%; respiratory disease, 9%; and neoplasia, 20%. *Escherichia coli* was the most common urine culture isolate from diabetic and non-diabetic cats (61% and 51% of positive cultures, respectively.) The median USG in cats with positive cultures and diabetes was 1.034 (range, 1.012-1.057); renal failure, 1.011 (1.007-1.026); LUTD, 1.037 (1.014-1.060); and miscellaneous diseases, 1.024 (1.012-1.055). There was no significant difference ( $p > 0.2$ ) between USG and urine culture results among any cat group.

Concurrent disorders were identified in 48 diabetic cats and included renal failure ( $n=16$ ), acromegaly (9), neoplasia (7), hyperadrenocorticism (6), corticosteroid use (6), urinary tract calculi (4), hyperthyroidism (3), and positive FIV status (3). Urinary tract infections were found in 2 diabetics with concurrent renal failure and one diabetic each with hyperadrenocorticism, neoplasia, and hyperthyroidism. Of the 18 diabetic cats with positive urine cultures, 8 (44%) cats had lower urinary tract signs. The percentage of positive urine cultures in untreated and insulin-treated diabetics was 17% and 20%, respectively. Positive urine cultures were identified in 8 of 71 diabetic cats with poor control (11%), 8 of 55 diabetic cats with fair to good control (15%), and 2 of 15 diabetic cats overdosed with insulin (13%).

The overall prevalence of urinary tract infections in cultured diabetic cats is similar to that of other hospital patients. USG does not distinguish cats predisposed to infections. Level of diabetic control and signs of LUTD are insensitive predictors for urinary tract infections in diabetic cats.

#### **ABSTRACT #219**

**PANCREATIC HISTOPATHOLOGY OF DIABETIC BURMESE AND NON-BURMESE CATS.** R Lederer, JS Rand, IP Hughes, M Latter, O Wattle, School of Veterinary Science, The University of Queensland, Australia.

Burmese cats in Australia and New Zealand are at increased risk of developing diabetes. The purpose of this study was to describe pathological changes in the pancreas of Burmese and non-Burmese cats, and determine if there were changes that may be characteristic for Burmese cats.

Pancreas tissue from 98 cats was collected through pathology archives and private clinics. Nine cats were diabetic Burmese, 14 were non-diabetic Burmese cats, 21 were diabetic cats of other breeds (non-Burmese) and 54 were non-diabetic non-Burmese cats. All animals in the study were between 5 and 22 years old. The mean age at time of death was 13.3 years for diabetic Burmese and 12.2 years for cats of other breeds. Two thirds of diabetic cats in both groups were neutered females, one third were neutered males.

Serial sections were made from one randomly chosen area of each pancreas and stained with HE and special amyloid stains (Sirius Red and Congo Red). Sections were examined by a researcher blinded to the status of the cats. Islet amyloidosis was scored on a scale from 0-

3. Results were compared using Fishers exact test and binary logistic regression analysis.

Pancreatitis was significantly less common in diabetic Burmese cats (1 in 9 cats) than in diabetic cats of other breeds (10 in 21 cats;  $p=0.046$ ). The mean islet density per field in 10 randomly chosen high-power fields was significantly higher ( $p=0.03$ ) in diabetic non-Burmese cats (1.7/field) than in the non-diabetics of this group (1.4/field). This higher islet density in diabetics was also observed as a non-significant trend within the relatively small group of Burmese cats (1.7 versus 1.0/field;  $p=0.058$ ).

Diabetes was significantly linked to the occurrence of islet amyloidosis in both diabetic groups combined ( $p=0.004$ ). The severity of islet amyloidosis was also significantly higher ( $p=0.0002$ ) in diabetic non-Burmese cats than in the non-diabetics of this group. Increasing age was significantly linked to the occurrence of islet amyloidosis in non-diabetic cats ( $p=0.035$ ).

In conclusion, the only feature that was significantly different between diabetic Burmese and diabetic non-Burmese cats was a lower frequency of pancreatitis in diabetic Burmese cats. Diabetic cats in both groups tended to have a higher islet density and more severe islet amyloidosis than non-diabetic cats.

#### **ABSTRACT #220**

BASAL PLASMA INSULIN AND HOMEOSTASIS MODEL ASSESSMENT (HOMA) ARE SIMPLE INDICATORS OF INSULIN SENSITIVITY IN CATS. D.J. Appleton<sup>1</sup>, J.S. Rand<sup>1</sup>.  
<sup>1</sup>Centre for Companion Animal Health, University of Queensland, Brisbane, Australia.

Insulin sensitivity is reduced in diabetic cats, and underlying low insulin sensitivity increases the risk of impaired glucose tolerance developing with weight gain. Measurement of insulin sensitivity traditionally requires multiple blood samples and complex mathematical calculations, which are costly and time-consuming. The objective of this study was to identify a simple and reliable method for assessing insulin sensitivity in cats across differing body weights and glucose tolerance levels.

Thirty-two neutered cats (18 F, 14 M) with varying bodyweights (mean 5.4 kg, range 3.3-8.6kg), were used. This included a subset of seven overweight and obese cats with impaired glucose tolerance, but fasting euglycemia. Relationships were determined between Bergman's minimal-model derived insulin sensitivity index ( $S_I$ ), and various alternative measures of insulin sensitivity derived from the intravenous glucose tolerance test. Alternate measures included basal insulin concentration, mean of two basal insulin concentrations, the basal insulin to glucose concentration ratio, the Bennett Index, Homeostasis Model Assessment (HOMA), Quantitative Insulin Check Index (QUICKI), insulin concentrations 60- and 120-mins after a glucose injection, area under the insulin curve, and the ratio of area under the insulin curve to area under the glucose curve during an intravenous glucose tolerance test.

The most useful overall predictors of insulin sensitivity were basal insulin conc. ( $P < 0.001$ ,  $r = -0.59$ ) and HOMA ( $P < 0.007$ ,  $r = -0.47$ ), which is the product of basal insulin and glucose concentrations divided by 22.5. In the sub-group of seven overweight and obese, glucose intolerant cats, basal insulin conc. was also the most strongly correlated with  $S_I$  ( $P < 0.002$ ,  $r = -0.93$ ), and HOMA provided the next strongest relationship ( $P < 0.009$ ,  $r = -0.88$ ).

Increased plasma insulin concentration after withholding food gave an indication of reduced insulin sensitivity in individual cats across varying body weights and degrees of glucose tolerance, excluding fasting hyperglycemia. Because prolonged insulin resistance leads to pancreatic beta cell failure and decreased insulin secretion, estimating insulin sensitivity based on insulin concentrations alone may not give accurate data in all cats, especially those with hyperglycemia. A simple marker of insulin sensitivity that includes information on both

insulin and glucose concentrations such as HOMA provides additional information.

It is concluded that in cats fasted for 24 hrs, measurement of plasma insulin concentration in conjunction with HOMA could be used in clinical research projects, and by practicing veterinarians to screen for reduced insulin sensitivity. Such cats are likely at increased risk of developing diabetes mellitus, and would benefit from early detection, so preventative programs including weight loss, increased activity and dietary modifications can be instigated.

#### **ABSTRACT #221**

Abstract Withdrawn

### ABSTRACT #222

EDTA ANTICOAGULANT FALSELY INCREASES PLASMA CORTISOL CONCENTRATIONS. J.K. Klaassen, D.C. Lewis, A. Hamilton. Antech Diagnostics, Irvine, CA.

The purpose of this study was to investigate whether EDTA-anticoagulated plasma samples were suitable for measurement of cortisol concentrations.

Ten samples from dogs that had sufficient serum and EDTA-plasma submitted to Antech Diagnostics within the previous 12 hours were chosen sequentially. Cortisol was measured by radioimmunoassay (Coat-A-Count® Cortisol, DPC) immediately in each serum and EDTA-plasma sample. Aliquots of serum and EDTA-plasma samples from each dog were subsequently mixed, 1:2, with 4 cortisol standards ([0], [5], [20], & [50] ug/ml, in processed human serum) and the expected cortisol concentration for each mixture was calculated. The calculated cortisol concentration was compared to the measured cortisol concentration after sample storage for 24 hours at 5C.

Cortisol concentrations measured in serum and EDTA-plasma within 12 hours of sample submission were not statistically different (P=0.44).

Observations on serum vs EDTA cortisol concentrations	Serum	EDTA-plasma
# higher than expected value	21	34
# lower than expected value	19	6
Range of differences from expected value (%)	16% less to 28% greater	11% less to 36% greater
Range of differences from expected value (ug/dl)	1.7 ug/dl less to 1.4 ug/dl greater	0.7 ug/dl less to 8.8 ug/dl greater
Mean absolute difference (%)	7.1 %	17.7 %
Mean absolute difference (ug/dl)	0.6 ug/dl	2.4 ug/dl
% within 10% of expected value	70%	30%
% within 15% of expected value	90%	42%

Statistically, cortisol concentrations measured in EDTA-plasma were significantly greater than cortisol concentrations measured in serum (Wilcoxon signed rank test; P<0.001). Differences were most pronounced in samples with higher cortisol concentrations.

Storage of EDTA-plasma falsely increased cortisol concentrations by as much as 30% and may have lead to erroneous interpretation of cortisol results. Serum is the specimen of choice for measurement of cortisol concentrations.

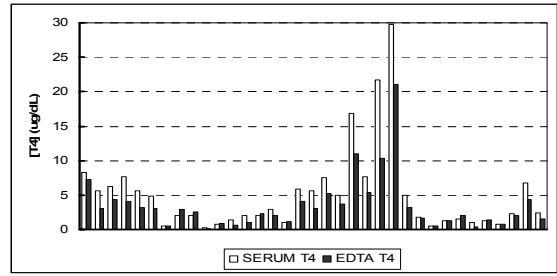
### ABSTRACT #223

EDTA ANTICOAGULANT FALSELY DECREASES PLASMA THYROXIN CONCENTRATIONS. J.K. Klaassen, D.C. Lewis, A. Hamilton, M Imran. Antech Diagnostics, Irvine, CA.

The purpose of this study was to investigate whether EDTA-anticoagulated plasma samples were suitable for measurement of total thyroxin (T4) concentrations.

Samples from dogs and cats that had sufficient serum and EDTA-plasma submitted to Antech Diagnostics within the previous 12 hours were chosen sequentially. Thyroxin was measured by a solid-phase I<sup>125</sup> radioimmunoassay (Coat-A-Count® Total T4, DPC) immediately in each serum and EDTA-plasma sample.

Sixteen canine and 20 feline samples were analyzed. One feline sample was excluded from analysis because of a large discrepancy in serum and EDTA T4 concentrations that was unable to be rechecked. T4 concentrations measured in EDTA-plasma were statistically significantly lower than T4 concentrations measured in serum for dogs (Wilcoxon signed rank test; P<0.015), cats (P<0.001), and for dogs and cats combined (P<0.001). The mean T4 difference was 0.9 ug/dL for dogs and 2.2 ug/dL for cats.



Thyroxin concentrations, when measured in EDTA-plasma by solid-phase I<sup>125</sup> radioimmunoassay, are falsely decreased. This may lead to over-diagnosis of hypothyroidism in dogs and under-diagnosis of hyperthyroidism in cats. Serum is the specimen of choice for T4 measurement.

### ABSTRACT #224

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS FUNCTION IN DOGS WITH NEOPLASIA. AL Boozer, EN Behrend, EM Whitley, AN Smith, KA Busch, RJ Kempainen. Auburn University College of Veterinary Medicine, Auburn, AL.

The hypothalamic-pituitary-adrenal axis (HPAA) may become dysregulated during illness. Diminished as well as exaggerated adrenal function has been reported in humans, cats and dogs with various illnesses or neoplastic conditions. Elevated or suppressed serum cortisol concentrations may have implications on tumor growth, host immune function, and the general health of the patient. The purpose of this study was to further examine and compare the HPAA in dogs with both lymphosarcoma (LSA) and non-hematological neoplasia (NHN).

Twenty dogs with LSA and 16 dogs with NHN were evaluated. Dogs that received exogenous glucocorticoids within 4 weeks prior to admission were excluded. Dogs underwent an ACTH stimulation test and measurement of plasma endogenous ACTH (eACTH) concentration. ACTH stimulation tests also were performed in 16 normal dogs. For the ACTH stimulation test, Cortrosyn was injected (5 mcg/kg IV) and samples taken before and 1 hr after injection. Traslolol was added to all blood samples used for measurement of eACTH, and samples were centrifuged within 15 minutes of collection and the plasma frozen at -20C until analysis. For measurement of cortisol, serum samples were used. Samples were centrifuged after clotting, and the serum separated and stored at -20C until analysis. For comparison of 2 and 3 groups, a Mann-Whitney and Kruskal-Wallis test was used, respectively. Significance was set at p≤0.05.

Of the 20 dogs with LSA, 3 (15%) had elevated basal serum cortisol concentration, 1 (5%) had an exaggerated response to ACTH, and 1 (5%) had an elevated eACTH concentration. Of the 16 dogs with NHN, 3 (19%) had elevated basal cortisol concentration, 2 (13%) had an exaggerated response to ACTH and no dogs had an elevated eACTH concentration. Decreased adrenal function was noted in a similar percentage. Of the dogs with LSA and NHN, 1 (5%) and 3 (19%), respectively, had decreased basal cortisol levels. In 1 dog, basal serum cortisol concentration was severely decreased (<1 nmol/L). Four dogs with LSA (20%) and 3 (19%) with NHN had a subnormal ACTH response. Endogenous ACTH levels were low in 2 dogs (10%) with LSA and 2 (13%) with NHN. Neither pre- nor post-ACTH serum cortisol concentrations were different between normal dogs and dogs with LSA or NHN. Basal plasma eACTH concentrations were not different between dogs with LSA and NHN. Serum basal cortisol concentration for neither group of tumor-bearing dogs correlated with plasma eACTH concentration.

Our results suggest that: 1. Secretion of cortisol is not related to plasma eACTH concentration in tumor-bearing dogs. 2. Standard measurements of HPAA function are abnormal in a subset of dogs

with neoplasia. 3. A subset of tumor-bearing dogs has decreased HPA function. Further study is needed to determine the clinical relevance of these findings.

#### ABSTRACT #225

MINMOD ANALYSIS OF INTRAVENOUS GLUCOSE TOLERANCE TESTS IN CATS: COMPARISON TO EUGLYCEMIC HYPERINSULINEMIC CLAMP. M. Hoenig<sup>a</sup>, K. Thomaseth<sup>b</sup>, J. Brandao<sup>a</sup>, D.C. Ferguson<sup>a</sup>, <sup>a</sup>University of Georgia College of Veterinary Medicine, Athens, GA and Authors and <sup>b</sup>Institute of Biomedical Engineering of the Italian National Research Council, Padova, Italy.

Although insulin sensitivity is best measured under steady-state conditions of a euglycemic hyperinsulinemic clamp (EHC), the practical aspects of the infusions and simultaneous glucose monitoring required by this test have led to the search for other tests such as the minimal model analysis (MINMOD). The purpose of this study was to perform MINMOD on well-defined lean and obese cats and to compare the practical aspects of its performance and analysis as well as the insulin sensitivity ( $S_I$ ) parameters derived to that of the EHC.

Both procedures were performed on 5 lean (3.8±0.5 kg body weight (BW) and 15.2 ± 3.3 % body fat (BF)) and 3 obese cats (6.6 ± 1.3 kg BW and 41.7 ± 4.4 % BF). The cats received an intravenous bolus of glucose (0.8 g/kg) at time 0 and of regular insulin (0.05 U/kg) at 20 minutes. Thirty-three blood samples were collected at -5,0,1,2,3,4,5,6,7,8,10,12,14,16,20,22,23,24,25, 28,30,40,50,60,70,80,90,100,110,120,160,180 and 240 minutes for the measurement of glucose and insulin. The MINMOD is a nonlinear 2-compartment model with 1 compartment models for glucose and insulin kinetics. The insulin compartment kinetics are described by its turnover rate constant  $P_2$ , which determines the lag associated with an insulin-induced increase in glucose disposal rate. Other parameters estimated were insulin sensitivity ( $S_I$ ,  $\text{min}^{-1}/(\text{pmol/L}) \times 10^4$ ) which quantifies the steady-state variation in glucose disappearance rate due to unitary increases in plasma insulin, and the glucose effectiveness ( $S_G$ ,  $\text{min}^{-1}$ ) which quantifies the fractional glucose clearance rate at basal insulin concentrations. From the zero-time intercept, it is possible to calculate an apparent initial glucose distribution volume ( $V_G$ , dl/kg). Logarithmic transformations were used for  $P_2$ ,  $S_I$  and  $S_G$ , and a nonlinear mixed-effects model was used to simultaneously analyze all data sets (178 observations over 8 animals) and account for random variability.

Confidence intervals for  $S_I$  determined by MINMOD did not overlap for all cats, and body weight was the only variable which influenced insulin sensitivity:  $S_I$  in obese cats averaged 0.126, 19% of that of lean cats at 0.654. The data was fit with  $P_2$  and  $S_G$  being identical for all cats, and  $V_G$  averaged 2.08 ± 0.36 dl/kg with no significant effect of BW. This value is similar to that observed in human patients (1.5-2 dl/kg). Despite the ability of MINMOD analysis to distinguish  $S_I$  between lean and obese cats, the correlation coefficient of the regression of  $S_I$  values determined by MINMOD to those determined by EHC was not significant ( $R=0.36$ ,  $p=0.32$ ).

In conclusion, it appears that MINMOD may be a feasible procedure for studying glucose kinetics and insulin action in cats. For reasons of maintaining catheter function and the lack of analytical value of such extensive sampling in the first 20 minutes, fewer samples are necessary during this period. With the MINMOD procedure, there is considerable intersubject variability which may lead to reduced ability to detect subtle differences in insulin sensitivity compared to the more complicated EHC procedure.

#### ABSTRACT #226

COMPARISON OF THE PHARMACODYNAMICS AND PHARMACOKINETICS OF SUBCUTANEOUS GLARGINE, PROTAMINE ZINC, AND LENTE INSULIN PREPARATIONS IN HEALTHY DOGS. VJ Stenner, LM Fleeman, and JS Rand. Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Brisbane, Australia.

The pharmacodynamic and pharmacokinetic effects of glargine, a recombinant human insulin analogue, protamine zinc beef-pork insulin (PZI), and a purified pork lente insulin preparation were evaluated in 9 healthy neutered dogs (4 male, 5 female). Serial serum glucose and insulin concentrations were determined over a 24-hour period following subcutaneous administration of 0.5 U/kg bodyweight of each of the insulin preparations. The three insulin treatments were administered 3 days apart in a sequence that was randomly allocated in a crossover rotation. All results were expressed as mean ± SEM and compared using ANOVA. Significance was set at  $P<0.05$ .

Serum glucose concentration was significantly decreased in all dogs following the administration of lente and PZI insulin, and in 7 of 9 dogs following glargine administration. Two dogs showed no significant change in serum glucose concentration after glargine administration and were excluded from the analysis of glargine pharmacodynamic data. Lente had a significantly earlier onset of action (0.6 ± 0.7hr) than PZI (3.1 ± 0.7hr) ( $P=0.003$ ). The onset of action for glargine (2.2 ± 0.9hr) was not significantly different from either lente or PZI. Lente had a significantly shorter time to glucose nadir concentration (2.4 ± 1.0hr) than either glargine (5.7 ± 1.2hr) ( $p=0.015$ ) or PZI (6.4 ± 1.0hr) ( $p=0.005$ ). PZI had the longest duration of action (19.0 ± 1.6hr) and was significantly longer than both lente (10.4 ± 1.5hr) ( $p=0.002$ ) and glargine (13.2 ± 1.9hr) ( $p=0.036$ ).

Lente produced a single insulin concentration peak at 1.2 ± 0.6hr in all 9 dogs. In the first 2hr following subcutaneous administration, lente resulted in significantly higher serum insulin concentrations than either PZI or glargine ( $p<0.0001$ ). Following PZI administration, serum insulin concentration had a consistent trend to peak at 0.5hr. Although serum insulin concentration exceeded the critical difference in only 2 of the 9 dogs following PZI, there was a significant glucose-lowering effect in all 9 dogs. Following glargine administration, there was a trend for peak insulin concentrations to occur between 0.5 and 6hr. The serum insulin concentrations did not exceed the critical difference in 3 dogs following glargine and in 2 of these dogs, there was no significant glucose-lowering effect.

We conclude that in healthy dogs: 1) Subcutaneous lente insulin produces a predictable insulin concentration peak and results in a shorter onset and duration of action than PZI. 2) PZI administration results in significant glucose-lowering effect and prolonged duration of action in all dogs. 3) Glargine administration results in an unpredictable serum insulin concentration response and, in some dogs, fails to produce a significant glucose-lowering effect.

#### ABSTRACT #227

PROTEIN C DEFICIENCY IN DOGS WITH LIVER DISEASE. O Toulza, SA Center, MB Brooks, K Warner, W Deal. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Protein C (PC), a critical anticoagulant protein, also is known to modulate inflammation and apoptosis. Synthesized in the liver with a short (< 10 hour) plasma half-life, PC deficiency develops in humans with portosystemic shunting and hepatic atrophy. We hypothesized that PC may serve as a clinical marker of liver disease in dogs and prospectively determined its utility compared to antithrombin III (AT), and serum bile acids (SBA).

Dogs suspected of having liver disease ( $n=97$ ) based on historical, physical, and clinicopathologic abnormalities and abdominal ultrasonography, had plasma collected for routine coagulation tests, AT, PC and SBA. Disease groups included: portosystemic vascular

anomaly (PSVA, n=32), microvascular dysplasia (MVD, n=10), chronic hepatitis (CH n=11), liver failure (LF, n=4), miscellaneous liver disorders (MLD, n=5), hepatic neoplasia (HN, n=4), and non-hepatic illnesses (n=31). Acquired liver disease was diagnosed by liver biopsy, PSVA by color flow ultrasonography, colorectal scintigraphy, and in some cases, radiographic portography. A reference range for PC activity (75-135%) was derived from assay of plasma from 37 healthy adult dog of a variety of pure breeds. Plasma PC activity was measured using a commercial chromogenic substrate kit. PC < 75%, AT < 70%, SBA > 25 µmol/L were considered abnormal.

Nonparametric methods were used to detect significant differences and associations using an  $\alpha$  of 0.05; median values and (range) are shown. Test diagnostic performance was expressed using specificity (SP), sensitivity (SS), positive and negative predictive values (+PV, -PV).

Percentage per group with low PC were: 100% LF, 94% PSVA, 30% MVD, 73% CH, 40% MLD, 25% HN, and 32% non-hepatic disorders. Percentage per group with low AT were: 100% LF, 28% PSVA, 0% MVD, 11% CH, 20% MLD, 50% HN, and 32% non-hepatic disorders. Significantly lower PC was found in dogs with LF [12(8-23)%] and PSVA[41(22-92) %] compared to other dogs with liver disease [73(8-288)%]. PC values normalized in 4 dogs with successful PSVA ligation. Only dogs with LF [35(16-51)%] had significantly reduced AT compared to other dogs with liver disease [81(23-108)%] and PSVA [76(42-102)%];  $p < 0.05$ . Significant positive correlations with PC were found for AT, fibrinogen, albumin, cholesterol, and glucose;  $p < 0.05$ . Significant negative correlations were found with SBA, total bilirubin, and AST;  $p < 0.05$ . Diagnostic accuracies for PC, AT and SBA for detection of liver disease were: SP: 68, 75, 64%; SS: 74,30,83%; PV+: 83,72, 91%; PV-: 55, 33, 47%, respectively.

We conclude that determination of plasma PC activity may assist in recognizing dogs with portosystemic shunting, hepatic failure, and other forms of liver disease. Results suggest that plasma PC activity may assist in differentiating PSVA from MVD and also may be useful for determining the success of PSVA ligation.

#### **ABSTRACT #228**

FASTING AND POSTPRANDIAL SERUM BILE ACIDS, & SINGLE AND SERIAL URINE BILE ACIDS IN DOGS WITH CONGENITAL PORTOSYSTEMIC VASCULAR ANOMALIES. SA Center, JF Randolph, KL Warner. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Urine bile acids (UBA) in randomly collected urine can screen for abnormally increased concentrations of serum bile acids (SBA). The UBA test shows promise as a convenient means of detecting acquired liver disease causing functional, cholestatic, or perfusion abnormalities. However, initial investigation recognized a reduced ability to detect liver dysfunction due solely to portosystemic shunting associated with congenital portosystemic vascular anomalies (PSVA). Reduced test efficacy in this disorder was hypothesized to reflect the dynamic and only transiently increased SBA associated with portosystemic shunting and their subsequent transient urinary elimination. We have more fully investigated the diagnostic utility of UBA in PSVA and their temporal association with fasting and postprandial intervals.

Randomly collected urine samples from dogs (n=50) were prospectively entered into this study after a diagnosis of PSVA was confirmed using Doppler assisted abdominal ultrasonography, colorectal scintigraphy, and, in some cases, radiographic portography; 47 of these dogs underwent liver biopsy. Serial UBA measurements (12-hr fasting and 1,2,3,4,6,8,10, and 12-hrs postprandially, voluntary micturition) were evaluated in seven dogs. SBA and UBA were measured using enzymatic linked reactions with colorimetric endpoints. Urine creatinine (UCr), measured using a

modification of the Jaffe reaction, was used to normalize UBA concentrations. Dogs with SBA  $\geq 25$  µmol/L or UBA/UCr [(µmol/mg) x 100]  $\geq 7.3$  were considered to have abnormal tests. The percentage of dogs with abnormal tests was calculated for fasting SBA (FSBA), postprandial SBA (PSBA), and UBA/UCr (including hourly intervals in dogs undergoing serial determinations). Nonparametric methods (Wilcoxon signed rank test) were used to determine significant differences between FSBA and PSBA concentrations and between the 12-hr fasting and hourly UBA/UCr values in dogs serially tested. An  $\alpha = 0.05$  and two tailed p values were applied, values represent median (range).

Of 50 dogs with PSVA, high FSBA developed in 84%, high PSBA in 98%, and high random UBA/UCr in 84%. Median FSBA [99 (0-444)] was significantly lower than PSBA [228 (23-605)];  $p < 0.0001$ . Median random UBA/UCr was 25.9 (0-286). Serially tested UBA/UCr significantly increased at the 2 to 4-hr postprandial intervals (46.9, 57.7, and 72.4) compared to the 12-hr fasting value (17.6); all  $p \leq 0.03$ . The percentage of high UBA/UCr values in serially tested dogs was 83-86% (0, 2, 3 hrs), 60% (1 hr), 100% (4, 6, and 8 hrs), 80% (10 hr), and 50% (12 hrs).

Results suggest that after morning urine elimination, urine collected 4 to 8 hours after feeding improves the diagnostic performance of UBA/UCr in dogs with PSVA.

#### **ABSTRACT #229**

INFLUENCE OF CHRONIC ORAL S-ADENOSYLMETHIONINE (SAME) ON BILIARY GLUTATHIONE AND BILE ACID CONCENTRATIONS IN HEALTHY CATS. SA Center, KL Warner, College of Veterinary Medicine, Cornell University, Ithaca, NY.

S-adenosylmethionine (SAME) is an important intermediary metabolite and glutathione (GSH) substrate donor. SAME therapy offers several potential benefits for cats with acquired liver disease considering that oral SAME in healthy cats significantly increases hepatic and erythrocyte GSH concentrations. Experimentally, SAME has been shown to impart a choleric response in various disease models, although mechanisms remain ill defined. Enhanced hepatobiliary exportation of GSH may be involved. Since acquired feline hepatobiliary disorders commonly manifest substantial cholestasis, a SAME induced choleresis could offer therapeutic advantage. However, the influence of SAME on feline biliary constituents has not previously been evaluated. We investigated whether chronic oral SAME administration in healthy cats influenced their biliary concentrations of bile acids and GSH as evidence of altered bile volume or flow.

Ten adult healthy intact female cats given 200 mg tablets of SAME (Denosyl-SD4<sup>®</sup>, Nutramax Laboratories) PO SID on an empty stomach for 118 days had bile collected on Days 0 and 118. Bile was collected after a 12-hour fast with cats were under general anesthesia at the time of other sampling procedures, using laparoscopically directed cholecystocentesis. Collected bile was snap frozen in liquid nitrogen and stored frozen at  $-80^{\circ}$  C until thawed for analyses. Biliary bile acids were measured using a validated enzymatic linked colorimetric reaction. Biliary total GSH was measured using a validated kinetic enzymatic method. Nonparametric methods were used for data analysis; the Wilcoxon signed rank test was used to investigate for significant differences between biliary bile acid and GSH concentrations between Day 0 and 118; an  $\alpha = 0.05$  and a two tailed pvalue were applied. Values are expressed as median and (range).

Median bile acid concentrations in bile on Day 0 were significantly higher than Day 118; Day 0: 138 (78-224) mm/L; Day 118: 120 (60-153) mm/L,  $p = 0.04$ . Biliary GSH concentrations were not significantly different between sampling intervals; Day 0: 8.5 (2.9-43.8) nm/ml, Day 118: 8.0 (2.2-16.5) nm/ml];  $p = 0.3$ .

Findings suggest that SAME administration increased bile flow without enhancing bile acid flux. Maintenance of biliary GSH concentrations in this circumstance is consistent with 1) the GSH substrate donor role of SAME, 2) increased hepatic GSH concentrations achieved with chronic SAME administration in cats, and 3) the role of hepatobiliary canalicular GSH exportation as a driving influence on bile flow. Results suggest that therapeutic doses of the stable SAME salt investigated may provoke a choleric response that could reduce bile lithogenicity and enhance biliary elimination of toxic and microbiologic agents that may complicate feline acquired liver disease.

#### **ABSTRACT #230**

**INFLUENCE OF ORAL URSODEOXYCHOLIC ACID ON SERUM AND URINE BILE ACID CONCENTRATIONS IN CLINICALLY NORMAL DOGS.** SA Center, JF Randolph, KL Warner. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Ursodeoxycholic acid (UDCA) has a molecular configuration detected by serum bile acid (SBA) and urine bile acid (UBA) assays. Therapeutic oral administration of UDCA at a dose of 15 mg/kg delivers approximately 38  $\mu\text{mol/kg}$  UDCA that enters the enterohepatic circulation and may spill into the systemic circulation. It remains unclarified whether therapeutic UDCA can significantly influence either SBA or UBA determinations. We investigated the influence of a therapeutic dose of UDCA on both SBA and UBA concentrations in clinically normal dogs.

Serial serum and urine bile acids were measured in 14 clinically normal dogs (wt: 8.5 - 37.8 kg; age: 2.5-11.0 years) with and without oral UDCA administration (15 mg/kg). A 4 day rest period was provided between serial samplings. Samples were collected at baseline after a 12-hr fast, and then postprandially at 1,2,3,4,6,8,10, and 12-hrs. Food was withheld after the morning feeding throughout the 12-hr sampling interval. The UDCA (in encapsulated form) was given at the time of feeding each dog its standard maintenance ration. SBA and UBA concentrations were measured using enzymatic linked reactions with colorimetric endpoints. Urine creatinine (UCr), measured using a modification of the Jaffe reaction, was used to normalize UBA concentrations.  $\text{SBA} \geq 25 \mu\text{mol/L}$  or  $\text{UBA/UCr} [(\mu\text{mol/mg}) \times 100] \geq 7.3$  were considered abnormal. Nonparametric methods were used to investigate for significant differences between hourly SBA and UBA/Cr values within (Wilcoxon signed rank test) and between (Wilcoxon rank sum test) serial samples, using an  $\alpha = 0.05$  and a two tailed p value.

Highest median SBA concentrations were achieved between 0-4 hrs without UDCA and between 2-4 hrs with UDCA administration. Without UDCA, all SBA values were within the normal reference range, except for a single dog at the 3-hr interval (27  $\mu\text{mol/L}$ ). However, following UDCA administration, six dogs developed SBA  $\geq 25 \mu\text{mol/L}$  (variably at the 1- through 6-hr intervals, range: 25 to 75  $\mu\text{mol/L}$ ). Although all median SBA concentrations remained within the reference range, higher values developed (on inspection) at all hr intervals with UDCA; significant increases in SBA concentrations with UDCA occurred at the 2, 3, and 4-hr sampling intervals ( $p=0.03$ , 0.003, and 0.003, respectively). All UBA/UCr values were  $< 5.5$  at hourly intervals without UDCA. Only one dog developed an abnormally increased UBA/UCr (14.8) with UDCA treatment (4-hr interval only). Although median UBA/UCr after UDCA remained within the reference range, they were significantly increased at the 6-hr interval compared to values with-out UDCA administration (1.64 vs 0.71),  $p = 0.02$ .

Findings in this study confirm that UDCA can significantly influence total SBA and UBA concentrations in clinically normal dogs. Similar but more pronounced effects would be expected in dogs with liver disease.

#### **ABSTRACT #231**

**INVESTIGATION INTO THE EXPRESSION OF THE FELINE HOMOLOG OF HER2 IN FELINE NEOPLASIAS.** Gisselman, K., Hayes, K., Mathes, L. Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio.

Feline mammary adenocarcinoma is the third leading cause of cancer in cats accounting for 17% of feline cancers. The mechanisms of transformation and tumor progression currently are unknown. However, early ovariectomy has been shown to be protective against the development of spontaneous mammary tumors. In humans, breast cancer is the number one cause of cancer in females. Over 25% of human mammary tumors over-express the type 1 receptor tyrosine kinase, human epidermal growth factor receptor 2 (HER2). In such tumors, over-expression of this receptor, which is activated ligand-independently, causes uncontrolled cell growth leading to tumor formation. Diagnosis of HER2-positive mammary cancer is associated with poor prognosis. Recently, a humanized monoclonal antibody to HER2 (Herceptin, Trastuzumab) has shown promise in clinical treatment of HER2-positive breast cancer in humans. The present study was designed to investigate the role of over-expression of the feline homolog of HER2 in the progression of feline neoplasias, including mammary tumors. For this purpose, the feline ER2 cDNA was cloned using a low stringency reverse transcription on consensus ER2 sequence information (Genbank) obtained from known human, rat, mouse, and canine sequences. The feline sequence obtained was compared to the known sequences of ER2 cDNA and found to have 90% sequence homology to the canine. A highly homologous region of the sequence, 190 base pairs long, was used to design primers for use in real time RT-PCR in order to begin screening a variety of feline tissues and tumors for levels of ER2 expression. The feline neoplasias initially evaluated included mammary adenocarcinomas, fibrosarcomas, and head and neck squamous cell carcinomas. Analysis of most neoplasias indicated some level of ER2 expression. Ongoing work includes quantifying ER2 mRNA expression levels in normal and neoplastic tissue.

#### **ABSTRACT #232**

**CLASSIFICATION OF CANINE LYMPHOMA USING THE REVISED WHO CLASSIFICATION SYSTEM.** WC Kisseberth<sup>1</sup>, VE Valli<sup>2</sup>, CE Kosarek<sup>1</sup>, and GJ Kociba<sup>1,3</sup>. <sup>1</sup>College of Veterinary Medicine, The Ohio State University, Columbus, OH, <sup>2</sup>College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, and <sup>3</sup>Veterinary Diagnostics Laboratory, Columbus, OH.

The World Health Organization (WHO) has adopted a new histological classification system for lymphoma in the dog. This classification system attempts to define distinct disease entities based upon morphology, immunophenotype, genetic and clinical features. In this study, we apply the new classification system to a large series of canine lymphoma biopsies. Pathology reports (1998-2002) from a commercial veterinary diagnostic laboratory were searched. 380 peripheral lymph node biopsies were identified from dogs that had a histological diagnosis of lymphoma, were retrievable, and contained sufficient tissue for tissue microarray (TMA) construction. TMAs were constructed from formalin-fixed, paraffin-embedded tissue using a manual arraying device (Beecher Instruments). Immunophenotyping was done using anti-CD3 (T-cell marker) and -CD79a (B-cell marker) antibodies. Histological classification using the revised (2003) WHO classification system was done by a single board-certified veterinary pathologist (VEV).

The median age of affected dogs was 8 years. 198 females, 154 males, and 28 dogs of unknown sex were affected. 59 breeds were represented, with the Boxer, Golden Retriever, and Labrador Retriever breeds being the most common. Based on anti-CD3 and -CD79a immunophenotyping, 97 (26%) were T-cell tumors, 254 (66%) were B-cell tumors, 4 (1%) were null tumors, and 25 (7%)

were undetermined. B-cell diagnoses were: diffuse large cell B-cell lymphoma 173 (68%), marginal zone lymphoma 46 (18%), marginal zone-like 13 (5%), and other 22 (9%). T-cell diagnoses were: diffuse large T-cell lymphoma 49 (52%), small lymphocytic T-cell lymphoma 15 (15%), T-zone lymphoma 12 (12%), lymphoblastic T-cell lymphoma convoluted 9 (9%), and other 12 (12%).

The revised WHO classification system was readily applied to this retrospective series of canine lymphoma biopsies. Construction of TMAs simplified immunophenotyping of this large case series. Future studies will need to address the prognostic significance of this classification system, particularly for marginal zone and marginal zone-like lymphomas, T-zone lymphoma, and small lymphocytic T-cell lymphoma, whose clinical behavior as unique diagnoses have yet to be determined.

#### **ABSTRACT #233**

VACCINATION WITH LIPOSOME-ENCAPSULATED MRNA PRIMES ANTI-TUMOR CYTOLYTIC T LYMPHOCYTE (CTL) RESPONSES AND IS ENHANCED BY CO-ADMINISTRATION OF GM-CSF MRNA. Paul R. Hess<sup>†\*</sup>, David Boczkowski\*, Smita K. Nair\*, David Snyder\*, and Eli Gilboa\*. <sup>†</sup>Dept. of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC. \*Dept. of Surgery, and Center for Genetic and Cellular Therapies, Duke University Medical Center, Durham, NC.

Nucleic acids have advantages over other antigen forms in anti-tumor vaccines, as they are easily purified and replicated, encode multiple MHC class I and class II epitopes unrestricted by haplotype, and can provide long-lasting antigen expression. Although plasmid DNA vaccines are well-characterized, few reports have examined immunization with mRNA, which may be a safer means of cancer vaccination. In this study we investigated the hypotheses that 1) injection of mRNA encoding a model tumor-associated antigen (TAA) could prime specific CTL responses capable of protecting against tumor growth, and 2) co-immunization with TAA and co-stimulatory or cytokine mRNAs could potentiate CTL activity.

C57BL/6 mice were injected intradermally (ID) or intravenously (IV) with cationic liposome-encapsulated, *in vitro* transcribed mRNA (0.3-4 µg) encoding chicken ovalbumin (OVA). Specific CTL activity in spleen and lymph nodes was measured by *in vitro* europium-release assay, and by *in vivo* cytotoxicity assay. The anti-tumor effects of mRNA immunization were assessed with OVA-expressing tumors in protection (EG.7) and therapeutic (F10.9-OVA) models. To evaluate the potential adjuvant effects of co-administration of mRNAs encoding immunologically-active molecules, B7.1, IL-2, or GM-CSF mRNAs (1µg) were mixed with OVA mRNA prior to vaccination. We found that ID injection of liposome-encapsulated OVA, but not control, mRNA into the pinnae readily elicited CTL activity detectable *in vitro* and *in vivo*, and protected mice from EG.7 challenge. Further, growth of the melanoma F10.9-OVA was delayed in mice vaccinated with OVA mRNA 3 and 10 days after tumor injection. With IV immunization, a single injection of as little as 1 µg of OVA mRNA primed CTL activity. Co-administration of OVA and GM-CSF mRNAs enhanced specific cytolytic responses 7 and 30 days after vaccination.

Injection of cationic liposome-encapsulated mRNA appeared to be a simple, safe, and effective means of eliciting anti-tumor CD8<sup>+</sup> T cell responses. This is the first study to show that vaccination with non-replicating mRNA could prime CTL to mediate tumor rejection, and that inclusion of GM-CSF mRNA has an adjuvant effect in such a vaccine.

#### **ABSTRACT #234**

COMPUTED TOMOGRAPHY OF THE NORMAL LUMBOSACRAL INTERVERTEBRAL DISC IN 22 DOGS.

T. Axlund, J. Hudson. Auburn University, Auburn, AL

The purpose of this study was to determine the degree of intervertebral disc (IVD) bulge in neurologically normal medium-sized dogs.

Computed tomography (CT) and magnetic resonance imaging are now the preferred modalities for imaging of disease of the cauda equina in both people and animals. Previously described CT abnormalities have included spondylosis deformans and osteophytosis as well as more subtle lesions such as bulging of the intervertebral disc. CT abnormalities, however, have been described in dogs without clinical evidence of neurologic disease. Similar observations made in our lab prompted us to examine recent data to determine the degree of IVD bulge in neurologically normal medium-sized dogs.

Twenty-two medium sized hound dogs between one and six years of age were included in the study. None of the dogs had any clinical evidence of lumbosacral (LS) disease and all were negative for inducible pain on palpation of the lumbosacral junction or dorsal elevation of the tail. Subsequently, the lumbosacral region of each dog was surgically explored as part of another study. In particular, surgeons noted any disc margin bulging or nerve root displacement. All procedures were approved by the Institutional Animal Care and Use Committee at Auburn University.

Ventrodorsal and lateral radiographs of the LS spine were made prior to computed tomography. For computed tomography (CT), the dogs were positioned in dorsal recumbency with the hindlimbs variably extended. CT was performed using a third generation CT scanner with technique settings of 120 kVp, 200 mA, 2.0 sec and small field-of-view. Contiguous transverse slices were obtained 1.5 mm apart with a tilt angle of 0 degrees.

Two sagittal reconstructions were made as close to the center of the canal as possible to allow measurement of the degree of bulging of the intervertebral disc. For each reconstruction, a line was traced from the caudodorsal aspect of the body of L7 to the craniodorsal aspect of S1. The bulge was measured from the line to its most dorsal point. The height of the vertebral canal (dorsoventral diameter) was measured from the midpoint of the line to the inner lamina of the roof of L7. The mean of the two sets of measurements were taken for each dog. The mean and standard deviation were then calculated for disc bulge, vertebral canal height, and % occupation (the percentage of the vertebral canal height occupied by the bulge of the intervertebral disc).

The width of the mid-portion of the L7-S1 intervertebral disc was  $0.5 \pm 0.07$  cm. All L7-S1 intervertebral discs were dorsally convex with the mean "bulge" measuring  $0.25 \pm 0.07$  cm. The height of the vertebral canal at L7-S1 was  $0.91 \pm 0.11$  cm. The mean percentage of the vertebral canal occupied by the bulge was  $26.89 \pm 5.05$  %.

Mild bulging of the L7-S1 IVD can be a normal finding in medium sized dogs. Therefore, one must not rely solely on the radiographic findings in making a diagnosis of LS stenosis.

#### **ABSTRACT #235**

RETROSPECTIVE STUDY OF CANINE INTRACRANIAL PRIMARY NEOPLASIA (171 CASES). Jessica Mikszewski, Christiane Massicotte, Charles Vite, Frances S. Shofer, and Thomas J. Van Winkle. Matthew J. Ryan Veterinary Hospital, University of PA, Philadelphia, PA.

The purpose of this study is to investigate the frequency, locations, and clinical findings associated with primary brain tumors in a large population of dogs that presented to University of Pennsylvania from 1986 to 2003.

Medical records of 170 dogs with 171 primary intracranial neoplasms confirmed on post-mortem examination were identified.



Parameters available were reviewed including signalment, clinical signs and duration, thoracic radiographs, cerebrospinal fluid analysis, and advanced imaging. Locations of primary brain tumors as well as other unrelated neoplasia identified on post-mortem examination were recorded. Metastatic neoplasia invading the brain, tumors of the skull (e.g. chondrosarcoma, multilobular tumor of bone), and other neoplasms affecting the brain by extension (e.g. nasal, pituitary, and cranial nerve tumors) were excluded from this report.

Of the 171 primary brain tumors, 79 (46%) were meningiomas, 30 (17%) were astrocytomas, 26 (15%) were oligodendrogliomas, 12 (7%) were choroid plexus tumors, and 7 (4%) were primary CNS lymphomas. Smaller numbers of glioblastomas (n=5), primitive neuroectodermal tumors (5), vascular hamartomas (4), poorly differentiated gliomas (2), and one ependymoma were identified. One dog had a meningioma and an astrocytoma. The average age at diagnosis was  $9.47 \pm 3.37$  years. Mixed breed were most often represented (33%), followed by Golden retrievers (13%) and Boxers (10%).

Seizures were the most common clinical presenting complaint for meningiomas (57%), glioblastomas (60%), oligodendrogliomas (69%), and vascular hamartomas (50%). Mentation changes were common clinical signs in astrocytoma (48%) and choroids plexus tumors (75%). Primitive neuroectodermal tumors were most often associated with vestibular system dysfunction (80%). Most tumors were located in the telencephalon (62% of meningiomas, 55% of astrocytomas, 64% of choroid plexus tumors, 81% of oligodendrogliomas, and 100% of vascular hamartomas). Meningiomas and oligodendrogliomas were located in the olfactory area in 19% and 23% of cases, respectively. Of 166 tumors for which a brain location was recorded, 79 were found in more than one brain division. Other neoplasms unrelated to the primary brain tumor were identified on post-mortem examination in 40 dogs (24%). Intra-thoracic and intra-abdominal neoplasms were present at necropsy in 13 and 22 cases, respectively. Abnormalities were present in 17 of 95 dogs (18%) that had thoracic radiographs performed.

In conclusion, most dogs in this study had tumors of the telencephalon and seizures as their primary presenting complaint. This is an area that is often accessible for biopsy and surgical treatment of the tumor. Based on the results of this study, thoracic radiographs and abdominal ultrasonography are indicated prior to advanced imaging of the brain or intracranial surgery to look for extracranial neoplasia.

#### ABSTRACT #236

**BILATERAL CAVERNOUS SINUS SYNDROME (CSS) IN 6 DOGS.** Rossmeis JH, Higgins M, Inzana KD, Herring IP, Grant DC. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

CSS is a rare syndrome characterized by dysfunction of cranial nerves (CN) III, IV, VI, and the ophthalmic and maxillary branches of CN V as they course together through the basilar cavernous venous sinus (CS). Previous canine reports of CSS have primarily identified cases with unilateral disease. The purpose of this retrospective study is to describe the clinical, diagnostic imaging, and pathologic features of bilateral CSS in 6 dogs.

Patient data is summarized in the table. The mean duration of signs prior to diagnosis was  $31 \pm 5$  days, and 5/6 patients initially presented with owner complaints referable to both eyes. No dog had clinical evidence of concurrent dysfunction of both the ophthalmic and maxillary branches of CN V. All dogs (3/6) with metastatic head and neck neoplasms had clinical evidence of multiple cranial neuropathies in addition to CSS. The median survival time for the 4/6 dogs that were not euthanized at diagnosis was 178 days (range, 16-392 days).

Dog	Neuro-ophthalmic Signs	Other Neuro Signs	Diagnosis
Boxer, M, 10 years	OD- External/Internal ophthalmoplegia OS- External plegia/Internal paresis	Temporalis/Masseter atrophy, dysphagia	Suspected chemodectoma
Mixed breed, MN, 2 years	OD- External/Internal ophthalmoparesis OS- External/Internal ophthalmoplegia OU- Reduced corneal sensation	Temporalis/Masseter atrophy, dysphagia	Chemodectoma
Mixed breed, FS, 7 years	OD- External plegia/Internal paresis OS- External paresis/Horner's	None	Undifferentiated sarcoma
Mixed breed, MN, 1 year	OU- External/Internal ophthalmoplegia	Nasofacial analgesia	Lymphoma
Mixed breed, FS, 11 years	OU- External/Internal ophthalmoplegia, Reduced corneal sensation	Temporalis/Masseter atrophy, dysphagia	Thyroid carcinoma
German Shepherd dog, FS, 10 months	OU- External/Internal ophthalmoplegia	Partial seizures, Nasofacial hypalgesia	Primitive neuroectodermal tumor

Diagnostic imaging (4/6 dogs) or necropsy examination (1/6 dogs) revealed bilateral mass lesions confined to the CS in 2/6 dogs, and bilateral, contiguous mass lesions within and outside of the CS in 3/6 dogs. Of the 3 dogs with extrasinusoidal lesions, the mass invaded the overlying forebrain in one dog, and demonstrated significant intracranial as well as extracranial extension in the other 2 dogs. Besides neuroanatomic location, the only other consistent neuroimaging feature identified was variably intense, heterogenous enhancement of the CS mass lesions. A definitive diagnosis of neoplasia was made by biopsy of extracranial portions of the CS mass in 2 dogs, biopsy of remote but related lesions in 2 dogs, and necropsy in one dog.

In conclusion, malignant neoplasia should be suspected in any dog with bilateral CSS. Dogs with CSS and additional cranial nerve deficits not localizable to the CS should be systematically evaluated for a distant primary tumor.

#### ABSTRACT #237

**NEURONAL CEROID-LIPOFUSCINOSIS IN A BORDER COLLIE.** Hiroshi Koie, Nihon University, Fujisawa City, Kanagawa, Japan.

Neuronal ceroid-lipofuscinosis (NCL), the accumulation of lipofuscin granules in cells such as nerve cells and fibroblasts is an extremely rare enzyme defect gene disease. Generally, neurological symptoms such as character changes, visual disturbance and epilepsy appear. NCL is a fatal disease in which most of all dogs die within three years old. We encountered the first NCL case which was proven by pathologic examination in Japan. The dog was examined by image diagnoses including MRI which revealed an intravital clinical symptom of NCL.

**Material and Method:** The case was one year and 11 months of age, a castrated male, blue color border collie. He was referred to the Nihon University Animal Medical Center by the behavioral abnormality and the visual disturbance.

**Medical examination:** There was no abnormality in blood and neurological examinations. He had the 1,280 times distemper IP antibody and the 550 times neutralizing antibody of the serum, and less than 10 times IP antibody and less than 3 times neutralizing antibodies of the cerebral spinal fluid (CSF). The MRI examination revealed the slight dilated sulcus of brain and the left ventricular enlargement. Abnormality was not especially identified in examinations of the optical fundus and electroretinography (ERG). In addition, there was no abnormality of CSF examination. This case deteriorated gradually, following the neurological symptoms of gait and feeding difficulties, and then three months later died following the hyperthermia.

**Pathology:** The accumulation of lipofuscin granules was confirmed by the transmission electron microscope examination in the nerve cells. This case was diagnosed from these findings as NCL.

**Conclusion:** The NCL was very rare in dogs. This case became the first report on border collie in Japan, and also our investigation by MRI was the first report in dogs. As new NCL canine cases have been identified in Japan after our case, the MRI examination for NCL cases would be possible to establish as an optimal diagnosis and a differential diagnosis.

#### ABSTRACT #238

THE ANTICOAGULANT EFFECTS OF WARFARIN IN HEALTHYCATS. Masami Uechi, Yayoi Osaki and Yumi Ishikawa. Veterinary Teaching Hospital, Kitasato University, Towada, Japan.

Warfarin was administered to cats to investigate its anticoagulant effect by measuring prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fib), bleeding time (BT) and activated clotting time (ACT) over several days. Using eight healthy cats, warfarin was orally administered at 0.05, 0.1 or 0.2 mg/kg for 10 days. The blood was sampled from the jugular vein under isoflurane anesthesia before and 1, 3, 5, 7 and 10 days after the start of administration to determine PT, APTT, Fib, BT, ACT and CBC. The blood test was continued after discontinuation of warfarin until the result returned to the normal range. With the 0.05 mg/kg dosage, no change was observed in coagulation status or clinical signs. With the 0.1 mg/kg dosage, PT was significantly prolonged by 1.5 to 2-fold at 5 days post-administration. BT did not show a significant difference, while ACT was significantly prolonged. 0.2 mg/kg warfarin increased PT starting the next day, and was discontinued in 2 cats as PT reached 75 sec 5 days post-administration. Thus, we found that in cats 0.05 mg/kg of warfarin was not sufficient, but 7-day administration of 0.1 mg/kg had a favorable anticoagulant effect. At 0.2 mg/kg, PT exceeded the detection limit in 2 cases, suggesting that we should be aware of possible adverse effects. This indicated that a PT measurement is recommended at 7 to 10 days of 0.05 - 0.1 mg/kg warfarin to review the dosage.

#### ABSTRACT #239

THR  $\beta$ -BLOCKING EFFECT OF CARVEDILOL IN CATS. Masami Uechi, Takashi Kodama, Takeshi Toyofuku and Yumi Ishikawa. Veterinary Teaching Hospital, Kitasato University, Towada, Japan.

There is no report in cats on the  $\beta$ -blocking effect of Carvedilol (CAR). In this study, we report the  $\beta$ -blocking effect of CAR in cats using seven clinically healthy adult cats (5 females and 2 males, 2.5-4.0 kg). After administration of placebo, 0.1, 0.2 or 0.4 mg/kg of CAR, an isoproterenol infusion (0.04, 0.08 and 0.16  $\mu$ g/kg/min) was given at 3, 6, 12 and 24 hr to determine the  $\beta$ -blocking effect. At the time of isoproterenol infusions, the mean arterial pressure (MAP) and the heart rate (HR) were measured via a catheter inserted to the femoral artery. In addition, at 0.08  $\mu$ g/kg/min of isoproterenol infusion at 3 hr after the administration of 0.1 or 0.2 mg/kg of CAR, MAP and the left ventricular (LV) pressure were measured to calculate LV dP/dt. Significant differences ( $p < 0.05$ ) in the blocking effect of CAR against  $\beta$ -adrenergic stimuli by isoproterenol were observed until 12 hr after the administration of 0.1 mg/kg CAR and 24 hr after the 0.2 and 0.4 mg/kg CAR. At 3 hr after the administration of 0.2 mg/kg CAR, elevation of HR and LV dP/dt in response to the  $\beta$ -adrenergic stimuli of isoproterenol was suppressed, although there was no significant difference in MAP. Since the oral dose of 0.2 mg/kg/day CAR successfully blocked  $\beta$ -adrenergic stimuli for 24 hr, 0.2 mg/kg/day may be clinically sufficient to maintain the  $\beta$ -blockage. Because 0.2 mg/kg of CAR also suppressed the positive chronotropic and inotropic actions of isoproterenol, we suggest increasing the dosage gradually when administering to veterinary patients with cardiac failure.

#### ABSTRACT #240

CANINE HEART VALVE INTERSTITIAL CELLS: PHENOTYPIC AND FUNCTIONAL PROPERTIES OF NORMAL AND MYXOMATOUS VALVES. Zimmerman SA, Orton EC. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Myxomatous mitral valve disease (MVD) is a leading cause of heart disease in aging dogs. Therapy is directed at management of secondary congestive heart failure, not underlying mechanisms of valvular degeneration. Little is known about the cellular events that trigger and mediate MVD. Thus, we determined the cytoskeletal immunophenotype and proteolytic activity of heart valve interstitial cells (VIC) of mitral valves from apparently normal dogs ( $n = 4$ ) and dogs with advanced MVD ( $n = 5$ ). Mitral valves were collected post-mortem, fixed in formalin, and embedded in paraffin for standard H & E histology and immunohistochemistry. A panel of monoclonal antibodies against cytoskeletal elements ( $\alpha$ -actin, vimentin, desmin, smooth muscle myosin and non-muscle myosin) were used to immunophenotype the VIC. Matrix metalloproteinase - 1 (MMP-1) and - 13 (MMP-13) were evaluated by immunohistochemistry to determine functional changes in collagenase activity in VIC. Epitope retrieval methods included heat with or without incubation with a proteinase solution. VIC from normal mitral valves strongly expressed non-muscle myosin compared to VIC from myxomatous valves. VIC from myxomatous mitral valves strongly expressed  $\alpha$ -actin, desmin, smooth muscle myosin and MMP-1. VIC from normal and myxomatous valves stain equally for vimentin and MMP-13. In conclusion, MVD in dogs is associated with a shift in the cytoskeletal phenotype of VIC and an increase in cellular collagenase expression as evaluated by immunohistochemistry. These and other cellular changes may signal the presence of a myxomatous cellular phenotype and may be used in the future to investigate triggering mechanisms of MVD.

#### ABSTRACT #241

NEW LEAD SYSTEM FOR THE ELECTROCARDIOGRAPHY ON THE BASIS OF THE LONG AXIS OF THE HEART IN DOGS. Masami Uechi, Masahiro Takeuchi and Yasutomo Hori. Veterinary Teaching Hospital, Kitasato University, Towada, Japan.

New lead system (NLS) that places the ECG electrodes on the surface of the dog's chest in accordance with the long axis of the heart was devised and studied comparatively using dogs with cardiovascular disorders and normal mixed-breed dogs as well as pug dogs. 25 clinically healthy mixed-breed dogs and 4 pug dogs were placed in right lateral recumbency and the ECG of standard limb leads system (SLLS) and NLS (RA: right medial scapular caudal cardiac base, LL: left medial scapular caudal cardiac base, LL: sternal apex) were recorded. QRS in lead II in normal mixed-breed dogs was divided into qR, QR, qRS, and QRs in SLLS, and QR was predominant. On the other hand, it was divided in qR, R, RS and qRS in NLS wherein RS was predominant. In SLLS of pug dogs, it was classified into QR, qRs, and QRs, whereas all the 4 dogs were RS in NLS. SLLS and NLS of the ECG were recorded with 12 dogs which were diagnosed as heart worm disease, 10 dogs diagnosed as mitral regurgitation, and 2 dogs diagnosed as dilated cardiomyopathy. QRS was divided in qR and QR in SLLS of MR, wherein NLS, it was classified broadly in R and Rsr'. In SLLS of heart worm disease, 5 dogs were RS, 2 dogs were Rsr', and the others showed various patterns. On the other hand, 11 dogs were RS and 1 dog was Rsr' in NLS, and appearance ratio of RS was significantly high ( $p < 0.05$ ). It was demonstrated that NLS may reflect the conformational change of the heart more straight compared to the SLLS.

## ABSTRACT #242

SPIRONOLACTONE AS A DIURETIC AGENT IN THE DOG: IS THE WATER BECOMING MUDDY? Jeunesse E<sup>#</sup>, Woehrlé F<sup>#</sup>, Schneider M<sup>#</sup>, Thoulon F<sup>#</sup>, Lefebvre HP<sup>\*</sup>, \*UMR 181 Physiopathologie et Toxicologie Expérimentales INRA-ENVT, National Veterinary School, Toulouse; <sup>#</sup>Vétoquinol, Lure, France.

Spirolactone is used as a diuretics in canine medicine, especially for the treatment of pulmonary edema associated with congestive heart failure, ascites secondary to cardiac or hepatic failure, and nephrotic syndrome. The dose level recommended for such indications are 2-4 mg/kg/day (sid or bid). However, no evidence has been published in the veterinary literature about the diuretic efficacy of such a dosage regimen. The aim of this study was to document the efficacy of spironolactone as a diuretic agent in the dog.

Eight adult healthy Beagle dogs weighing 13 to 16 kg were used for 2 separate 2\*2 cross-over designs. In the 1st cross-over, 4 dogs received orally spironolactone at 1 and 2 mg/kg for 8 days. The other 4 dogs were given similarly spironolactone at 4 and 8 mg/kg. The 24-h urine was collected for 3 days before dosing and during all the treatment period. Blood was sampled everyday for HPLC assay of plasma spironolactone and canrenone (the main active metabolite). Daily urine weight (UW), urine specific gravity (USG), daily water consumption (UWC), daily urinary excretion of sodium (UNa) and potassium (UK) were assessed. Results are expressed as mean +/- SD.

Spirolactone was absorbed and transformed into canrenone (plasma concentration: 92 +/- 51.4 ng/mL 2 h after the 8<sup>th</sup> administration at 8 mg/kg). No effect of spironolactone on the tested variables was detected whatever the dose level. For example, the mean +/- SD values observed in the control and treatment period at the dose level of 2 mg/kg were 253 +/- 90 and 218 +/- 85 g for UW, 1.041 +/- 0.011 and 1.044 +/- 0.011 for USG, 505 +/- 164 and 486 +/- 128 g for UWC, 9.2 +/- 4.9 and 7.3 +/- 3.7 mmol for UNa, and 12.8 +/- 6.4 and 10.0 +/- 5.4 mmol for UK, respectively.

In conclusion, spironolactone has no detectable diuretic effect in the healthy adult dog. Further studies however are required to assess potential effects in diseased dogs with hyperaldosteronism.

## ABSTRACT #243

NONINVASIVE ULTRASOUND COLOR M-MODE ESTIMATION OF PULMONARY VASCULAR RESISTANCE IN DOGS. Hsiu-Mei Yao<sup>1</sup>, Janice M. Bright<sup>1</sup>, June A. Boon<sup>1</sup>, and Robin Shandas<sup>2</sup>, <sup>1</sup>Department of Clinical Sciences, Colorado State University, Fort Collins, CO, and <sup>2</sup>The Children's Hospital, University of Colorado Health Sciences Center, Denver, CO.

Pulmonary hypertension (PHT) has been noted as a primary disorder and in association with many cardiopulmonary diseases in dogs. Determination of pulmonary vascular resistance (PVR) is necessary for evaluation of treatment and assessment of prognosis in the patients with PHT. Recently, a novel noninvasive technique of estimating PVR by using color M-mode, based on the concept of velocity propagation ( $Vel_{prop}$ ), has been validated in an *in vitro* mathematical model and in *in vivo* studies in children. Here we tested the hypothesis that the newly developed method can be used in dogs to obtain reproducible measurements of  $Vel_{prop}$  of pulmonary artery (PA) flow and to verify that  $Vel_{prop}$  correlates to PVR in this species.

The study was performed in 6 anaesthetized normal research dogs at baseline and after infusion of 3 different doses of a thromboxane A<sub>2</sub> mimetic vasoconstrictor (U46619). At the same time the hemodynamic data were being obtained, color M-mode images of PA flow were recorded for off-line analysis of  $Vel_{prop}$  under each condition.  $Vel_{prop}$  was calculated from the isovelocity slope [ $Vel_{prop} = (d_2 - d_1) / (t_2 - t_1)$ ] by tracing at the abrupt color change caused by the velocity exceeding the Nyquist limit.

Better correlation was found between  $Vel_{prop}$  and PVR [ $Vel_{prop} = 10.29 - 0.42PVR$ ;  $R^2 = 76.2\%$ ] than between  $Vel_{prop}$  and

pulmonary artery pressure (PAP) [ $Vel_{prop} = 15.42 - 0.35PAP_S$ ;  $R^2 = 51.9\%$ ;  $s = \text{systolic}$ ]; [ $Vel_{prop} = 16.66 - 0.56PAP_D$ ;  $R^2 = 63.0\%$ ,  $D = \text{diastolic}$ ]; [ $Vel_{prop} = 16.95 - 0.48PAP_M$ ;  $R^2 = 64.0\%$ ,  $M = \text{mean}$ ] ( $N = 25$ ). Single beat variability and beat-to-beat variability were 5.07 and 11.02, respectively.  $Vel_{prop}$  showed the ability to distinguish between high resistance and high pressure and promised to be a useful noninvasive method to evaluate PVR in dogs.

## ABSTRACT #244

ELETROCARDIOGRAPHIC EVALUATION OF HEALTHY DOGS UNDERGOING DOBUTAMINE STRESS TEST. Marlos Gonçalves Sousa<sup>1</sup>, Roberta Carareto<sup>1</sup>, Gláucia Bueno Pereira Neto<sup>1</sup>, Daniel Guimarães Gerardi<sup>1</sup>, Aparecido Antonio Camacho<sup>1</sup>. <sup>1</sup> Faculty of Agronomical and Veterinary Sciences – São Paulo State University – Campus of Jaboticabal – São Paulo – Brazil.

Pharmacologic stress tests are commonly used in human medicine aiming to precipitate hemodynamic alterations that could help diagnosing cardiovascular diseases. Such tests are especially useful in elderly or traumatized patients, as well as those with neurologic deficits. Many drugs have been used in stress testing, but dobutamine is still one of best choices due to its inotropic properties with little chronotropic action.

As there is not much available about pharmacologic stress tests in veterinary medicine, this work was conceived to evaluate electrocardiographic features of dogs undergoing dobutamine stress test. The EKG evaluation included heart rate (HR), duration and height of P wave (Pms and PmV), duration of QRS complex (QRSms), height of R wave (RmV), PR interval (PRms), QT interval (QTms) and ST segment (STms).

For such, five adult mongrel females dogs, with mean weight of 19 Kg, were evaluated before (1) and during the infusion of dobutamine in the following rates (in  $\mu\text{g}/\text{kg}/\text{min}$ ): (2) 10; (3) 20; (4) 30; and (5) 40. Statistical analysis of the data by ANOVA showed a significant variation only for HR ( $P < 0,0001$ ), PRms ( $P = 0,0006$ ), QTms ( $P = 0,0044$ ) and STms ( $P = 0,0040$ ). After applying Tukey's test to these parameters, it was also possible to verify that the variation was significant only when the infusion of dobutamine reached the rate of at least 30  $\mu\text{g}/\text{kg}/\text{min}$  ( $P < 0,01$  for HR and  $P < 0,05$  for PRms, QTms and STms).

Results allowed to conclude that when the rate of infusion of dobutamine is much superior to that used clinically, it can exert a positive chronotropic effect besides its positive inotropic effect. This is proportionally greater accordingly to the increase in infusion rate. It was also observed the absence of malignant arrhythmias or abnormal deflections in EKG even when dobutamine rate reached the highest rates. So, it was also concluded as a safe and feasible test to be performed in dogs.

## ABSTRACT #245

EVALUATION OF ARTERIAL BLOOD PRESSURE, CARDIAC OUTPUT, SHORTENING AND EJECTION FRACTIONS IN HEALTHY DOGS SUBMITTED TO DOBUTAMINE STRESS TEST. Marlos Gonçalves Sousa<sup>1</sup>, Gláucia Bueno Pereira Neto<sup>1</sup>, Daniel Guimarães Gerardi<sup>1</sup>, Roberta Carareto<sup>1</sup>, Aparecido Antonio Camacho<sup>1</sup>. <sup>1</sup> Faculty of Agronomical and Veterinary Sciences – São Paulo State University – Campus of Jaboticabal – São Paulo – Brazil.

Exercise stress tests have been routinely used in human being to make the heart overwork. Thus, it is possible to non-invasively detect and functionally assess ischemic diseases that were imperceptible during rest. However, some patients are unable to perform adequate exercise due to debility or neurologic deficits, among others. Such patients can undergo stress testing by the use of drugs that cause an overwork to the heart. Amongst several available drugs, dobutamine has emerged as a good choice for stress tests due to the predominant

augmentation of myocardial contractility with minimal effects on systemic pressure.

Due to the lack of data about dobutamine stress test in dogs, this work was conceived in order to provide some data about the use of this diagnostic tool, which can also be useful in veterinary medicine.

For such, 5 adult mongrel female dogs, with mean weight of 19 Kg, undergone echocardiographic evaluation, as well as pressure assessment before (1) and during the infusion of dobutamine in the following rates (in  $\mu\text{g}/\text{kg}/\text{min}$ ): (2) 10; (3) 22; (4) 32; and (5) 42.

Data were submitted to statistical analysis by ANOVA method and revealed a very significant increase in fractional shortening ( $P=0,0001$ ), which was also seen for ejection fraction ( $P=0,0006$ ). A second analysis by Tukey's test showed that this variation was significant for both parameters when the infusion rate reached at least  $20 \mu\text{g}/\text{kg}/\text{min}$  ( $P<0,01$ ). By the other hand, cardiac output obtained by Doppler of aortic flow did not show a significant variation along the increasingly rates of dobutamine infusion ( $P=0,5143$ ). Systolic arterial pressure variation was also not significant ( $P=0,2024$ ), but Mean and Diastolic arterial pressure had a significant decrease accordingly to the rate of infusion ( $P=0,0306$  and  $P=0,0315$ , respectively). Again, after applying Tukey's test to these pressure parameters, it was seen that the variation was significant when the infusion reached  $40 \mu\text{g}/\text{kg}/\text{min}$  ( $P<0,05$ ).

Results allowed concluding that dobutamine can exert effects on systemic vasculature when it is infused at much superior rates comparing to its clinical use. This may be probable due to a misbalance in its beta-2 and alpha-1 agonist effects, which might be dangerous in some patients that can not face a drop in arterial pressure. Concerning the echocardiographic parameters, the augmentation of myocardial contractility resulted in good increase of ejection fraction. However, it was not enough to increase cardiac output, which remained stable along the infusion of dobutamine. Thus, dobutamine stress test seem to be a feasible and safe test to be performed in dogs and could help in diagnosing cardiac changes when the disease it is not completely settled yet.

#### **ABSTRACT #246**

DECREASED PLATELET FUNCTION IN DOGS WITH SUBAORTIC STENOSIS. L Tarnow, T Falk, AT Kristensen, LH Olsen, L Haubro, HD Pedersen. Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Platelets are thought to be involved in the pathophysiology and progression of heart disease in dog and man. Activation of platelets can predispose to thromboembolic events and release proinflammatory and vasoactive substances. Shear stress has, on the other hand, been shown to deactivate and exhaust platelets and the high shear hemodynamics of aortic valve stenosis has been shown in humans to cause a decrease in platelet function and subsequent bleeding tendencies. Decreased platelet function has previously been reported in dogs with asymptomatic and symptomatic mitral regurgitation. The aim of the study was to evaluate platelet function and aggregation response in dogs with subaortic stenosis (SAS).

Ten dogs with clinically inapparent SAS (mean age $\pm$ SD:  $3.1\pm 1.9$  yrs) and 15 control dogs ( $5.2\pm 3.2$  yrs) were included. The dogs with SAS all had aortic flow velocities  $\geq 2.5$  m/s measured by subcostal echocardiography. Control dogs were enrolled based on a normal physical examination, CBC, biochemistry profile and echocardiography. All dogs were evaluated by cardiac auscultation, echocardiography, CBC, biochemistry profile, manual platelet counts, whole blood platelet aggregometry and platelet function. Platelet aggregation was assessed in citrated anti-coagulated whole blood using an impedance aggregometer (Chrono-Log, model 500VS) with adenosine-diphosphate (ADP) (final concentration  $20 \mu\text{M}$ ), collagen ( $0.1 \text{ mg}/\text{ml}$ ) and arachidonic acid (AA) ( $0.5 \text{ mM}$ ) as agonists. The maximal aggregation amplitude in Ohms was recorded. Whole blood platelet function was measured by the PFA100 closure

time (CT), i.e. the time it takes before a platelet plug closes an aperture in a membrane coated with ADP and collagen as agonists.

No difference was found in manual platelet counts, hematocrit or WBC between dogs with SAS and control dogs. Closure times were significantly longer in dogs with SAS (median and 25-75 percentile: 107 s; 81-165 s) compared to control dogs (62 s; 58-68 s) ( $p<0.0001$ ). Maximal aggregation response was significantly lower in dogs with SAS when collagen (mean $\pm$ SD:  $6.5\pm 2.5\Omega$ ) and AA ( $4.6\pm 3.2\Omega$ ) was used as agonists compared to control dogs ( $11.3\pm 4.1\Omega$  and  $8.3\pm 4.3\Omega$ ) ( $p=0.003$  and  $p=0.03$ ). There was no difference in aggregation response between the 2 groups when ADP was used as agonist. Three control dogs and 3 dogs with SAS had no or very little ( $<1 \Omega$ ) aggregation response to ADP.

In conclusion, dogs with SAS had longer closure times and lower aggregation response to 2 out of 3 tested agonists, suggesting that dogs with SAS have decreased platelet function. The mechanism behind this is speculated to be high shear damage to the platelets caused by turbulent blood flow through a stenotic aorta. The clinical relevance and risk of bleeding in dogs with SAS warrant further studies.

#### **ABSTRACT #247**

COMPARISON OF QUANTITATIVE METHODS FOR ESTIMATING LEFT VENTRICULAR MASS, VOLUME, AND WALL THICKNESS IN FELINES: ANATOMIC VALIDATION FROM TWO DIMENSIONAL AND M-MODE ECHOCARDIOGRAPHY. Gidlewski JM, and Fox PR. Animal Medical Center, New York, NY.

Background: Left ventricular mass (LVM) and volume (LVV) are altered in a variety of acquired and congenital heart diseases. While noninvasive measurement of these parameters provides valuable diagnostic and prognostic information in people, necropsy comparison studies testing these methods have not been reported in cats. In view of this, we set out to compare several quantitative methods to estimate LVM, LVV, and LV wall thickness determined by echocardiography, with respective post mortem measurements.

Methods: To test the ability of 2-D and M-mode echo to estimate LVM, LVV, and LV thickness compared with respective post mortem measurements, we prospectively studied 14 cats (12 died or were euthanized for myocardial disease, and died 2 for noncardiac conditions). Each had echocardiographic examination  $< 14$  days before death. LVM was estimated by 3 geometric models: 1) truncated ellipsoid (T-E), 2) area-length (A-L), and 3) M-mode cubed; 12 additional T-E and A-L methods were assessed by modifying short axis to include/exclude LV papillary muscles, and/or epicardial tracing to include/exclude pectinate muscles on the RV IVS border. The LVV was estimated by bi-plane method of discs and by single plane A-L techniques. At postmortem: LVV was measured, LVM was determined by dissecting and weighing LV; and LV wall thickness was measured at a plane judged to be similar for echo measurements. Statistical analyses included paired t-test, Pearson product moment correlation, and one way ANOVA.

Results: Optimal estimation of LVM was obtained using the Area-Length model modified in short axis to include LV papillary muscles and epicardial tracing incorporating RV pectinate muscles along the IVS border. Postmortem LVM ( $11.1\pm 3\text{g}$ ) was not different ( $P<0.0001$ ) from these estimations obtained by A-L in diastole (mean $\pm$ SD; 95%CI:  $11\pm 3.2\text{g}$ ; 9.9 to  $11.8\text{g}$ ) or by A-L in systole ( $11\pm 3.5$ ; 9.8 to  $11.9\text{g}$ ). Correlation coefficients were each 0.93 ( $P<0.0001$ ). All other 2-D methods significantly underestimated postmortem LVM, while the M-mode cubed method significantly overestimated LVM. Measurement of LVV at post mortem was not different from LVV estimated by method of discs in systole (mean $\pm$ SD; 95%CI:  $1.2\pm 0.75\text{ml}$ ;  $0.53$ - $1.74$ ;  $P=0.83$ ) and single plane A-L in systole ( $1.18\pm 0.9\text{ml}$ ;  $0.59$ - $1.78$ ;  $P=0.69$ ). Correlation coefficient for the method of disc estimate was 0.61 ( $P=0.028$ ) and

for the single plane A-L measurement, 0.72 ( $P=0.003$ ). M-mode end-systolic measurements of LV wall ( $7.3\pm 1.6$ ) most closely approximated postmortem LV wall thickness ( $7.4\pm 1\text{mm}$ ) ( $P=0.8$ ). M-mode end-systolic measurement of IVS ( $6.6\pm 2.1$ ) most closely approximated postmortem IVS thickness ( $7.4\pm 2.1\text{mm}$ ) ( $P=0.16$ ).

Conclusions: Anatomically accurate LVM estimation was best with a modified 2-D A-L method. Post-mortem ventricular volume and thickness is influenced to a great degree by rigor mortis.

#### **ABSTRACT #248**

INFLUENCE OF GENDER AND SIZE OF DOGS IN THE SIX-MINUTE WALK TEST. F. Campagner, P.H. Tanno, P.P.V.P. Diniz, A.S. Carvalho Filho, A.J. Crocci, D.S. Schwartz. Department of Veterinary Clinics. FMVZ-UNESP-Botucatu (School of Veterinary Medicine, São Paulo State University), Botucatu, SP, Brazil.

It is known that exercise intolerance is one of the most frequent sign observed by the owner of dogs with cardiac, pulmonary disease and obesity, but it is difficult to quantify. The six-minute walk test has been used in humans to assess exercise capacity of the patient. It is a submaximal capacity test, easy to perform and inexpensive, based on the distance walked in six minutes. It has been applied to dogs, but it is not known how size and gender affect the test.

The purpose of this study was to standardize the test in healthy dogs of different sizes and gender, to be applied in later studies of exercise intolerance and to evaluate the variation of heart rate (HR) and respiratory rate (RR), before and after the 6MWT. Sixty adult dogs (males and females) were divided into three groups depending on body weight: GI = less than 10 kg; GII = 10 to 24 kg; GIII = 25 kg or more. The dogs were conducted on a leash by one of the authors or by the owner, walking for 6 minutes in a 50 meter hallway, marked every 2 meters. The dogs walked on their own pace, only guided by the conductor. The test was repeated on different days and the average distance was used for analysis. Morphometric variables were measured [as nose-to-tail length (L), height (H), thorax (TD) and abdomen (AD) diameters, rotula-to-heel distance (RH) etc.]. HR and RR were measured before the test, right after and 5 minutes later. To evaluate the effect of gender and size on the walked distance, two-way ANOVA was applied. Forward Stepwise Regression Analysis was used to evaluate the morphometric variables influencing the distance walked. There was a strong and significant correlation ( $p < 0.05$ ) between body size and the distance walked in six minutes. A linear regression equation was obtained ( $\text{Distance} = 55,3 + 8,3 \text{ TD} + 0,9 \text{ L} + 2,1 \text{ RH}$ ;  $r^2=0.73$ ;  $r=0.85$ ) to predict the distance walked in relation to morphometric variables. No effect of gender was observed on the distance walked. There was no variation in the distance walked in different days associated to groups. The HR and RR were higher after the walk test and decreased after five minutes of rest, as expected. In conclusion, the distance walked in 6 minutes was related to body size and 73% of variability in the distance walked was explained by thorax diameter, body length and rotula-to-heel distance. The test was repeatable and should be useful in estimating exercise intolerance in dogs.

#### **ABSTRACT #249**

RR-QT INTERVAL RELATIONSHIP OBTAINED BY AUTONOMIC VARIATION IN CONSCIOUS DOGS. P.H. Tanno, F. Campagner, A.S. Carvalho Filho, P.P.V.P. Diniz, D.S. Schwartz. Department of Veterinary Clinics. FMVZ-UNESP-Botucatu (School of Veterinary Medicine, São Paulo State University), Botucatu, SP, Brazil.

The QT interval, which represents ventricular depolarization and repolarization period, has clinical and toxicological importance, since QT prolongation not related to heart rate (HR) predisposes to arrhythmias due to electrical instability, and may lead to sudden death. There is controversy regarding QT correction in humans, even

considering the great number of studies in this subject, although most studies use Bazett's or Fridericia's formulas. Despite some studies on the relation between HR and QT interval in dogs, the different approaches lead to variable results, what increases the need for more research to determine the best way to calculate QTc in this specie. Crescent doses of atropine ( $6\mu\text{g}/\text{kg}$ ,  $12\mu\text{g}/\text{kg}$ ,  $24\mu\text{g}/\text{kg}$  and  $40\mu\text{g}/\text{kg}$  IV) and propranolol ( $20\mu\text{g}/\text{kg}$  to  $120\mu\text{g}/\text{kg}$  IV) were used in order to study the relation between QT and RR interval. Sixteen mature mongrel dogs (8M, 8F, 5-12Kg) were used. Dogs were kept in right lateral recumbency and computerized ECGs were recorded at baseline, during atropine and propranolol infusions, and after atropine associated with propranolol. For different heart rates obtained with the protocol, QT intervals were measured on screen, considering the end of the T wave where it crossed baseline. The average of three consecutive QT intervals was used for the mean RR interval of the period (mean HR) to avoid errors due to sinus arrhythmia. Linear, logarithmic, polynomial, power and exponential equations were obtained from the relation between RR and QT intervals. The curvilinear equations showed stronger and significant correlation ( $P < 0,0001$ ). Linear regression also showed good correlations, probably because the curve has a significant linear portion. The results suggest that the relationship between RR and QT intervals after pharmacological autonomic tone variation follows a curve more than a line, indicating that linear formulas are not the most indicated for QT normalization in dogs.

#### **ABSTRACT #250**

UTILITY OF NASAL CYTOLOGIC BRUSH AND BIOPSY SAMPLES FOR MOLECULAR STUDIES. Rebecca C. Windsor, Lynelle R. Johnson, Jane E. Sykes, Christian M. Leutenegger. University of California, Davis, CA.

Biopsy is the gold standard for characterization of pathological lesions within the nasal cavity. Although biopsy provides important diagnostic information, it requires general anesthesia and causes trauma to the nasal mucosa and turbinates. Brush cytology has demonstrated variable diagnostic value in identifying disease processes within the nasal cavity, however to the authors' knowledge, the utility of brush cytology in molecular studies has not been assessed. We hypothesized that analysis of gene transcription could be adequately performed using cytology brush samples in place of tissue biopsies.

Bilateral nasal brush (Cytosoft™) and biopsy samples (Karl Storz 2mm cup forceps) were obtained from 5 healthy dogs. Samples were stored in 1.5 mL RNase-free Eppendorf tubes for DNA/RNA extraction using a 6700 automated nucleic acid extraction workstation. Taqman PCR was utilized to compare the level of transcription of universal bacteria, universal fungi, and various cytokines (IL-1 $\beta$ , IL-6, IL-10, IL-12p40, IFN- $\gamma$ , and TNF- $\alpha$ ) in brush and biopsy samples. Statistical analysis was performed using the Mann-Whitney U test for non-parametric data. Significance was set at  $P < 0.05$ .

Gene transcription of microbes and cytokines did not differ significantly between the left and right nasal cavity for either brush cytology or nasal biopsy samples. Detection of fungal and bacterial gene transcription and IL-1 transcription was significantly greater in superficial cytology brush samples than in tissue biopsy samples ( $P < 0.05$ ). A significant difference in expression of other cytokines was not detected between the two sample collection methods. Overall, gene transcription in cytology brush samples displayed larger ranges of values than did biopsy samples.

Based on results in samples examined here, we conclude that cytology brush specimens contain significantly higher levels of contaminating or infectious micro-organisms in comparison to biopsy specimens. Increased transcription of IL-1 in these samples may be a result of immune activation of surface cells by topical antigens or

microbes. Finally, the methodology used for sampling the nasal cavity impacts the results obtained using molecular techniques.

**ABSTRACT #251**

UTILITY OF ECHOCARDIOGRAPHIC INDICES OF DIASTOLIC FUNCTION AND SELECT PLASMA NEUROHORMONES IN PREDICTING PROGNOSIS IN DOBERMAN PINSCHERS WITH DILATED CARDIOMYOPATHY. ML O'Sullivan, MR O'Grady, SL Minors, R Horne. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Idiopathic DCM is a common myocardial disease in Doberman pinschers. Echocardiographic indices of diastolic function and circulating neurohormones have great utility in predicting prognosis and assessing therapeutic response in human DCM patients. The objective of this study was to determine the utility of diastolic function indices and select plasma neurohormones in predicting 1) time to onset of CHF or sudden death (SD) in Dobermans with occult DCM (free of clinical signs), and 2) survival times in Dobermans with overt DCM (DCM and CHF).

Dobermans were determined to have occult or overt DCM on the basis of history, clinical signs, echocardiographic assessment of LV size and systolic function, and the presence of pulmonary edema on thoracic radiographs in the case of the overt group. Ten dogs in each group were enrolled for study. Each dog underwent an echocardiogram for assessment of diastolic function, incorporating transmitral flow (TMF), isovolumic relaxation time, pulmonary venous flow, LV flow propagation velocity by color M-mode, and mitral annular motion by tissue Doppler imaging, and venous blood sampling for measurement of plasma norepinephrine (NE), epinephrine, aldosterone, atrial natriuretic peptide, and big endothelin-1 (ET-1). Dogs in the overt DCM group underwent a repeat examination incorporating all of the above at one month. Outcome data were collected on all dogs. Statistical analysis was limited to univariate Cox proportional hazards analysis with censoring. Level of significance was set at  $p \leq 0.05$ .

In the occult group, 7/10 dogs reached CHF or SD. The mean and median times to this combined endpoint were 177 days and 143 days, respectively (range 47 to 357 days). Predictors of time to CHF or SD included deceleration time of the TMF E wave (RR 0.83,  $p = 0.03$ ) and plasma aldosterone (RR 1.07,  $p = 0.03$ ). In the overt group, 9/10 dogs died due to CHF or SD. The mean and median times to cardiac death were 77 days and 62 days, respectively (range 13 to 214 days). Age (RR 0.55,  $p = 0.03$ ), LV internal dimension in diastole (RR 1.15,  $p = 0.02$ ), and LV internal dimension in systole (RR 1.24,  $p = 0.005$ ) at enrollment were predictive of survival time. None of the diastolic indices or neurohormones at enrollment were significant univariate predictors of survival. However, absolute and % change in NE (RR 2.0,  $p = 0.03$  and RR 3.9,  $p = 0.03$ , respectively), and absolute and % change in big ET-1 (RR 1.17,  $p = 0.007$  and RR 2.8,  $p = 0.01$ , respectively) from enrollment to the one-month recheck were significant predictors of survival.

While the small sample size certainly limits the scope of this work, some diastolic function indices and plasma neurohormones may have prognostic utility. Continued enrolment of many more dogs is necessary to allow multivariate analysis and thus more thorough investigation of the value of these indices.

**ABSTRACT #252**

THORACIC RADIOGRAPHIC FINDINGS IN DOGS WITH CARDIAC TAMPONADE. E Côté<sup>1,2</sup>, LA Schwarz<sup>1,3</sup>, F Sithole<sup>2</sup>, RL Malakoff<sup>1</sup>, NJ Laste<sup>1</sup>, NK Harpster<sup>1</sup>, NK Fenollosa<sup>1</sup>. <sup>1</sup>Angell Memorial Animal Hospital, Boston, MA <sup>2</sup>Atlantic Veterinary College, Charlottetown, PEI, Canada <sup>3</sup>Veterinary Emergency and Specialty Center of New England, Waltham, MA.

An enlarged, globoid cardiac silhouette is the classic radiographic abnormality of pericardial effusion (PE) in dogs, but the sensitivity and specificity of this and other parameters in cases of hemodynamically important PE (cardiac tamponade, CT) in dogs has not been established. We evaluated thoracic radiographs of adult dogs with confirmed CT (chief complaint consistent with CT; PE associated with atrial collapse on echocardiography; and any 2 of the following physical findings: tachycardia, ascites, weak pulse, muffled heart sounds) in a retrospective, randomized, blinded, controlled fashion.

Forty-six cases and 23 controls were included, with controls selected from 2 pools: normal thoracic radiographs (n=10) and thoracic radiographs of patients with heart disease other than CT (n=13). Vertebral heart score (VHS) was measured routinely, and the upper limit of normal was set at 10.7. The cardiac silhouette was assessed subjectively for the presence or absence of a globoid appearance. The presence or absence of an exceptionally straight caudal cardiac border on the lateral projection (Straight caud.) also was assessed; anecdotally, this finding has been associated with the presence of pericardial effusion in dogs. There was a conflicting appearance of the cardiac silhouette between lateral and DV (or VD) views in 4 cases, which were then excluded from analysis for globoid appearance. The resultant data are presented below (LL= lower limit of 95% confidence interval [CI]; UL= upper limit of 95% CI).

	VHS > 10.7			Globoid			Straight caud.		
	%	LL	UL	%	LL	UL	%	LL	UL
<i>Sensitivity</i>	80	68.3	91.7	59.5	43.6	75	76.2	63.3	89.1
<i>Specificity</i>	48	27.4	68.2	77.8	58.6	97	79.2	62.9	95.4
<i>True prevalence</i>	66	54.9	77.4	67.3	54.9	80	63.6	52	75.2
<i>Apparent prevalence</i>	71	59.8	81.4	47.3	34.1	61	56.1	44.1	68
<i>Predictive value +</i>	75	62.8	87.3	84.6	70.7	99	86.5	75.5	97.5
<i>Predictive value -</i>	55	33.2	76.8	48.3	30.1	67	65.5	48.2	82.8

We conclude that no single radiographic parameter is highly (>90%) sensitive or specific for detection of PE causing CT in dogs. The lack of an enlarged cardiac silhouette is expected in 20% of cases, despite overt manifestations of CT.

**ABSTRACT #253**

INDEX OF MYOCARDIAL PERFORMANCE AND SYSTOLIC TIME INTERVALS AS ESTIMATES OF GLOBAL RIGHT VENTRICULAR FUNCTION IN NORMAL DOGS. Baumwart RD, Meurs KM, Bonagura JD. The Ohio State University, Columbus, Ohio.

Right ventricular dysfunction (RVD) causes exercise intolerance, hypotension, syncope, and heart failure in dogs with cardiac and respiratory disorders. While RVD often is suspected, identification is difficult with current non-invasive studies. Doppler-echocardiographic methods might identify and quantify RVD; however, established methods and reference values are needed before these become clinically useful for recognizing RVD or assessing treatments designed to improve it. This study determined Doppler-derived reference values that reflect global right ventricular function in healthy dogs. We measured systolic time intervals and a right ventricular Index of Myocardial Performance (IMP) defined previously for the left ventricle by Tei.

Clinically healthy dogs (n=27) between 8 months and 8 years of age were studied. Based on body size, three groups of 9 dogs each were identified: 3-15 kg (mean=7); 16-30 kg (mean=22); and 31-55 kg (mean=40). Mean ages (in years) for the three groups were 3, 4, and 3 years, respectively. Echocardiograms were obtained in left lateral recumbency without sedation. Pulsed wave Doppler recordings of mitral and tricuspid inflow and of aortic and pulmonary ejection were acquired with an ECG at 200 mm/s sweep speed. Prejection period (PEP), ejection time (ET), PEP/ET, and IMP for both the right ventricle (RV) and the left ventricle (LV) were determined from 5 consecutive beats. IMP was calculated as: {(isovolumetric contraction + isovolumetric relaxation) / ET}.

Differences in mean RV and LV variables for all 27 dogs were identified by paired t-test and differences across the three arbitrary weight groups were identified by one-way ANOVA. The relationship between RV IMP and body weight, age, heart rate, PEP, ET and PEP/ET was explored by linear regression. Significance was set at  $\alpha=0.05$ .

Compared to the LV, the mean right ventricular IMP was significantly smaller (RV:  $0.23 \pm 0.02$ , 95% CI 0.18 to 0.25; LV:  $0.38 \pm 0.02$ , 95% CI 0.33 to 0.41), and was always less than the corresponding LV value in each dog. Mean ET (msec) for the RV was significantly longer than for the LV (RV: 186,  $\pm 3$ , 95% CI 180 to 192; LV: 173,  $\pm 3$ , 95% CI 167 to 178); however, PEP or PEP/ET were not different between ventricles. Mean ( $\pm$ SD) of heart rate during measurements of IMP and systolic time intervals were 98 ( $\pm 17$ )/min for the RV and 96 ( $\pm 21$ )/min for the LV. Linear regression did not identify a clinically relevant correlation between right ventricular IMP and body weight, heart rate, or between RV or LV ET, PEP or PEP/ET. A significant difference was observed between the three arbitrary weight groups for body weight, PEP (LV and RV) and PEP/ET (LV and RV), but not for age, heart rate, ET (RV or LV) or IMP (RV or LV). We conclude that the IMP for the RV is relatively independent of body weight and heart rate within the ranges studied, and is consistently lower than values derived from the LV in healthy dogs. These data provide initial reference values for the canine RV and emphasize the need for using specific right ventricular values for identification of RVD in dogs.

#### ABSTRACT #254

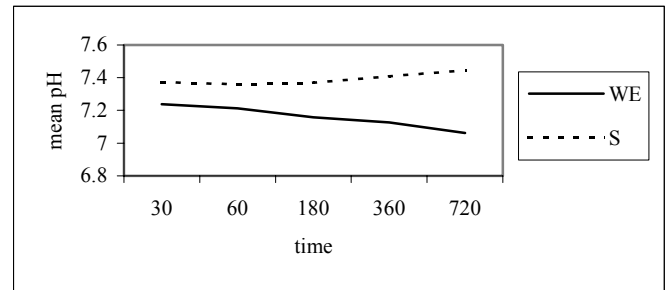
EFFECT OF CENTRIFUGATION AND OF STORAGE AT ROOM TEMPERATURE ON pH, PO<sub>2</sub>, PCO<sub>2</sub>, AND CONCENTRATIONS OF LACTATE AND BICARBONATE IN PATHOLOGIC PERICARDIAL EFFUSIONS OF DOGS. SJ Miller<sup>1</sup>, E Côté<sup>1</sup>, PJ Ewing<sup>1</sup>, M Letsoalo<sup>2</sup> <sup>1</sup>Angell Memorial Animal Hospital, Boston, MA <sup>2</sup>Medical Research Council, Pretoria, South Africa.

Biochemical analysis of naturally-occurring pericardial effusion (PE) and its supernatant (S) has not been undertaken uniformly in various clinical studies of dogs with PE. We evaluated the effects of centrifugation and of time at room temperature on the pH, PO<sub>2</sub>, PCO<sub>2</sub>, and lactate and HCO<sub>3</sub> concentrations of PE.

Eleven dogs underwent pericardiocentesis for treatment of PE. For each dog, PE was divided into 2 aliquots. The first aliquot was whole effusion (WE). It was immediately fractionated into 6 samples that were analyzed 15, 30, 60, 180, 360, and 720 minutes after collection while the second aliquot was centrifuged (10 minutes) and the S fractionated into 5 samples that were analyzed at 30, 60, 180, 360, and 720 minutes after collection. All samples were contained in heparinized, capped, sterile, plastic syringes at room temperature, and all analyses were performed on a portable blood gas and lactate analysis instrument (I-Stat [Heska Corp]). ANOVA was used to compare samples between groups and over time. Results were considered significant if  $p < 0.05$ .

All PEs were grossly hemorrhagic. Comparing groups, mean values for pH (figure), PO<sub>2</sub>, and PCO<sub>2</sub> differed significantly between the WE and S groups at all time points measured (PO<sub>2</sub>: S group higher; PCO<sub>2</sub>: WE group higher). Mean lactate concentration in the WE and S groups was similar initially (30, 60 min) but differed thereafter (significantly higher in the WE group). Mean HCO<sub>3</sub> concentration was not significantly different between groups at any time. Over time, mean pH (figure), PO<sub>2</sub>, and PCO<sub>2</sub> measurements were not significantly different at 60 min compared to 30 min, but measurements at 180, 360, and 720 min were significantly different from measurements at 30 min for all 3 analysis categories in both the WE and S groups. There was no significant difference in HCO<sub>3</sub> concentration over time in either group.

These results mirror those obtained from previous studies using whole blood and represent additional variables to be addressed in studies that evaluate these biochemical parameters in dogs with PE.



#### ABSTRACT #255

CONOTRUNCAL DEFECTS IN THE BORDER TERRIER. KN Boddy, P Werner, P Henthorn, MM Sleeper. The Matthew J Ryan Veterinary Hospital at The University of Pennsylvania, Philadelphia, PA.

Through epidemiologic, pathologic and genetic research with the Keeshond it has been shown that, at least in this particular breed, there are many grades of pathology found ranging from subclinical abnormalities that have to be diagnosed on pathology to advanced tetralogy of Fallot and that the defect is in a single autosomal locus. Based on this research, if other breeds were to act similarly, it can be seen that there are likely many carriers of pathologic yet quiescent disease that would make the disease easy to perpetuate.

Recently there have been an increased number of Border Terrier puppies being assessed for murmurs and subsequently found to be affected with conotruncal defects (CTD). Over the past few years there have been 8 puppies diagnosed, echocardiographically, with the following CHD lesions: 1 small ventricular septal defect (VSD) and mitral valve dysplasia (MVD), 2 mild-moderate subclinical pulmonic stenosis (PS), 1 severe clinical PS, 1 moderate bi-directional tetralogy of Fallot (ToF), 2 severe cyanotic ToF and 1 severe large VSD with aortic stenosis (valvular) and mild MVD. Seven out of 8 (87.5%) of these pups have lineage that traces back to a common ancestor known to have had ToF. One dog, the MVD with a small VSD, is not related to the known ToF dog and has since had spontaneous closure of the VSD and, in fact, may not be a true CTD puppy. Interestingly, out of the 7 related, there are 2 groups of three siblings each known to be affected. Additionally, these two groups of siblings have a high kinship coefficient indicating that the level of inbreeding in this group is high.

Based on the current pedigree analysis alone we are still unable to say the mode of heritability present in the Border Terrier but there is a suggestion for a genetic predisposition in this breed. With so many siblings affected there would be a high suspicion for a dominant mode of inheritance but with the level of inbreeding present one cannot fully rule out a recessive mode. Further genetic and pathologic studies need to be done to completely understand the CTD in the Border Terrier breed.

#### ABSTRACT #256

PHYSIOLOGIC VALVE REGURGITATION IN NORMAL CATS. Adin, DB, McCloy, K. University of Florida, College of Veterinary Medicine, Gainesville, Florida.

Physiologic valve regurgitation (PVR) occurs commonly in normal dogs, however the percentage of normal cats with PVR has not been previously reported. The purpose of this study was to retrospectively and prospectively evaluate echocardiograms from normal cats for the presence of PVR.

Echocardiograms were retrospectively evaluated from normal purebred cats in screening clinics over a 1 year period and

prospectively evaluated from normal student- and staff-owned cats at the University of Florida. Signalment and echocardiographic findings were recorded for each cat. PVR was diagnosed if the valve was structurally normal and regurgitant color flow was subjectively trivial. The color jet area and chamber area were traced and expressed as a percentage for each view. Results are expressed as mean  $\pm$  SD.

Echocardiograms were retrospectively evaluated from 46 clinically normal purebred cats (25 F, 21 M) (38 Maine coons, 3 Turkish angoras, 2 Scottish folds, 2 Ragdolls, 1 Devon rex). Cats were  $2.1 \pm 1.5$  yrs (range 0.3-7 yrs). All echocardiograms were normal. Physiologic mitral regurgitation (PMR) was detected in 4 cats (9%; 4 Maine coons) from the right parasternal long axis view (RPLAV) (in 1 cat PMR was also seen from the left apical view). PMR area from the RPLAV was  $4 \pm 1\%$ . Physiologic pulmonary regurgitation (PPR) was detected in 1 cat (2%) in the right parasternal short axis view (RPSAV). Physiologic tricuspid regurgitation (PTR) was detected in 27 cats (57%; 23 Maine coons, 3 Turkish angoras, 1 Ragdoll). PTR area was  $6 \pm 3\%$  from the RPLAV,  $11 \pm 8\%$  from the RPSAV and  $7 \pm 5\%$  from the left cranial view (LCV). PTR was detected in 3 views in 15%, in 2 views in 34% and in 1 view in 52% of cats.

Echocardiograms were prospectively evaluated from 58 clinically normal student- and staff-owned normal cats (22 Fs, 36 Mc) (54 mixed breed, 2 American shorthair, 1 Maine coon, 1 Devon rex). Cats were  $4.9 \pm 2.8$  yrs (range 1-12 yrs, normal distribution) and weighed  $5.2 \pm 1.2$  kg. All echocardiograms were normal. PMR was detected in 3 cats (5%; 2 mixed breed, 1 Devon rex) from the RPLAV and the area was  $10 \pm 2\%$ . PPR was detected in 1 cat (2%; mixed breed) in the RPLAV. PTR was detected in 41 cats (71%; 38 mixed breed, 2 American shorthair, 1 Maine coon). PTR area was  $9 \pm 5\%$  from the RPLAV,  $11 \pm 6\%$  from the RPLAV and  $9 \pm 4\%$  from the LCV. PTR was detected in 3 views in 44%, in 2 views in 34% and in 1 view in 22% of cats.

Retrospective and prospective data were pooled (104 cats) and 7 cats (7%) had PMR, 2 cats (2%) had PPR and 68 cats (65%) had PTR. Aortic regurgitation was not detected in any cat.

PTR is common in normal cats. PTR was not detected in all views in every cat and the average regurgitant jet area was small compared to the chamber area.

#### ABSTRACT #257

CARDIAC ENZYME CONCENTRATIONS IN NORMAL DOGS AND CATS USING A BEDSIDE ANALYZER. Adin DB, Berger KD, Engel C, Salute M, Milner RJ. University of Florida, College of Veterinary Medicine, Gainesville, Florida.

Circulating cardiac enzymes are non invasive markers of cardiomyocyte injury. Cardiac troponin I (cTnI) is the most sensitive and specific marker, however in human medicine cTnI is often evaluated with other markers such as myoglobin (MG) and CK-MB. Several studies have reported reference ranges for cardiac troponin I in normal dogs and cats, however results are specific to each analyzer and analyzers are expensive. The objective of this study was to develop reference ranges for cardiac enzymes (cTnI, MG and CK-MB) in normal dogs and cats using an inexpensive bedside analyzer (Triage Meter® ; Biosite Inc, San Diego, CA).

Clinically normal dogs and cats owned by students and staff at the University of Florida were evaluated by physical examination, ECG and echocardiography. EDTA plasma was obtained for analysis of cTnI, MG and CK-MB using the Triage Meter®. Purified canine cTnI (Advanced ImmunoChemical, Inc, Long Beach, CA) was diluted with canine plasma ( $25 - 0.04$  ng/ml) to confirm detection of canine cTnI and to assess assay linearity. Each dilution was run on 3 test kits to assess test variability.

The 55 dogs were  $4.8 \pm 3.1$  yrs and  $24.4 \pm 11.2$  kg (27 Mc, 19 Fs, 5 M, 4 F). The 58 cats were  $4.9 \pm 2.8$  yrs and  $5.1 \pm 1.1$  kg (36 Mc, 22 Fs). Age was normally distributed for both populations. All animals

were normal by physical examination, ECG and echocardiography. Measured values of purified canine cTnI closely matched the calculated concentration of cTnI across the range of dilutions used. The coefficient of variance was 0.43 to 9.12% with higher variability at the lower concentrations. Table 1 shows the median and range for each cardiac enzyme for dogs and cats. The lower limits of detection for the assays are 0.05 ng/ml for cTnI, 10 ng/ml for MG and 1.0 ng/ml for CK-MB.

Table 1	Median cTnI (ng/ml)	Range cTnI (ng/ml)	Median MG (ng/ml)	Range MG (ng/ml)	Median CK-MB (ng/ml)	Range CK-MB (ng/ml)
Dogs	<0.05	<0.05 - 0.21	<10.0	<10.0 - 136.0	<1.0	<1.0 - 1.9
Cats	<0.05	<0.05 - 0.22	<10.0	<10.0 - 13.8	<1.0	<1.0 - 4.1

This study provides reference ranges for cTnI, MG and CK-MB in dogs and cats using the Triage Meter®. Additionally, detection of canine cTnI by this analyzer was confirmed using commercially available purified canine cTnI. The Triage Meter® is an affordable bedside analyzer and therefore the availability of reference ranges for this machine may increase clinical use and research of these markers in veterinary medicine.

#### ABSTRACT #258

ASSESSMENT OF THE ABILITY OF PIMOBENDAN TO INCREASE THE FREQUENCY OF VENTRICULAR ECTOPY IN DOGS WITH CHF DUE TO DCM AND CHRONIC MITRAL VALVE INSUFFICIENCY. MR O'Grady, SL Minors, ML O'Sullivan, R Horne. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Congestive heart failure (CHF) is commonly associated with the development of sudden death. We presume that most cases of sudden death result from the development of ventricular tachyarrhythmias. Furthermore, the presence and frequency of ventricular premature beats (VPCs) may predict individuals at risk for the development of these tachyarrhythmias and sudden death. Positive inotropes have been implicated in the development of ventricular tachyarrhythmias and sudden death in people with CHF. Pimobendan has recently been advocated in the management of CHF due to DCM and chronic mitral valve insufficiency (CMVI) in the dog. The objective of this study was to determine whether pimobendan, a novel positive inotrope, increases the frequency of ventricular premature beats.

The study population consists of Doberman Pinschers with CHF due to DCM (5 dogs), and small breed dogs with CHF due to CMVI (9 dogs). These cases were selected from ongoing studies into the use of pimobendan to treat CHF in dogs. The Dobermans were randomly assigned to receive pimobendan (0.25 mg/kg BID PO) or placebo (0.25 mg/kg BID PO) in addition to furosemide and ACE inhibition. The CMVI dogs were randomly assigned to receive pimobendan (0.25 mg/kg BID PO) or benazepril (0.5 mg/kg BID PO) in addition to furosemide. CHF and the etiology for CHF were determined on the basis of history, clinical signs, echocardiography and the presence of pulmonary edema on thoracic radiographs. Ambulatory 24-hour (Holter) recordings were obtained from all dogs on the day of enrolment (all dogs were naïve to pimobendan at enrolment) and at 1 month, for the DCM dogs, or 4 months, for the CMVI dogs. All Holter recordings underwent manual editing. The percent change in the frequency of VPCs from the first exam to the second exam was determined. For the DCM dogs, no comparison with the placebo group was possible as only one placebo dog was available at 1 month. For the CMVI dogs, a t test was used to compare the percent change in the frequency of VPCs between the first and second exam between the pimobendan and benazepril dogs.

In the DCM group the mean change in the percent of VPCs/hr between the two examinations was 214% (range = -94% to 1041%). In the CMVI group, there was no difference in the percent change in number of VPCs/hr (mean for pimobendan group [6 dogs] = 654%; mean for the benazepril group [3 dogs] = 647%).



Although the sample sizes are quite small, there appears to be no significant increase in the frequency of VPCs induced by pimobendan when compared to other therapies. More dogs should be studied to extend these findings.

#### **ABSTRACT #259**

CAN ECHOCARDIOGRAPHY PREDICT SURVIVAL IN BOXERS WITH VENTRICULAR ECTOPY? Dewey H. Carpenter and Anthony H. Tobias. University of Minnesota, St Paul, MN.

Left bundle branch block (LBBB) morphology ventricular premature complexes (VPC's) and fatty infiltration of the right ventricular (RV) free wall is associated with sudden death in Boxers. This combination of disorders closely resembles arrhythmogenic right ventricular cardiomyopathy (ARVC) of humans, a disease that is associated with RV structural and functional abnormalities. However, in humans, LBBB VPC's also result from idiopathic right ventricular tachycardia (IRVT). IRVT is purely a conduction disorder that leads to palpitations, occasionally syncope, and rarely sudden death. Differentiating between ARVC and IRVT is based primarily on subjective assessment of the RV free wall by echocardiography; structural and functional abnormalities are present with AVRC but not IRVT. Further, humans with ARVC have abnormalities of both tricuspid valve (TV) inflow velocities and tissue Doppler-derived (TD) indices of RV systolic and diastolic function. These abnormalities are not present with IRVT.

It is not known whether LBBB VPC's in Boxers result from one or more diseases. However, diffuse fatty infiltration of the RV free wall may lead to structural and functional abnormalities that can be identified by echocardiography. Consequently, we hypothesized that echocardiography would allow separation of Boxers with LBBB VPC's into two groups: those with RV structural and functional abnormalities (analogous to ARVC) and those without (analogous to IRVT). We further hypothesized that the group with RV abnormalities would have shorter survival when compared to the group in which no RV abnormalities were detected.

Sixteen Boxers with LBBB VPC's evaluated at the University of Minnesota Veterinary Medical Center from 2000 to 2003 had echocardiograms that were suitable for subjective assessment of RV structure and function. TV inflow velocities and TD indices of right ventricular systolic and diastolic function were measured in 8 of these dogs. Mean age for all 16 Boxers was  $6.9 \pm 2.9$  yrs, and mean weight was  $30.1 \pm 5.9$  kg. RV structural and functional abnormalities were identified in half ( $n = 8$ ) of the dogs. Median survival time (MST) for the 16 Boxers was 628 days. MST for Boxers with RV structural and functional abnormalities was 589 days, versus 628 days for Boxers in which RV echocardiography was unremarkable. A log-rank test disclosed no difference in survival between the 2 groups ( $P = 0.8$ ).

Our results indicate that echocardiographic assessment of RV structure and function does not provide important prognostic information in Boxers with LBBB VPC's. These data also suggest that, in contrast to humans, LBBB VPC's in Boxers result from a single disease entity.

#### **ABSTRACT #260**

ASSESSMENT OF THE CHRONIC HEMODYNAMIC RESPONSE TO PIMOBENDAN IN DOBERMAN PINSCHERS WITH CHF DUE TO DILATED CARDIOMYOPATHY. MR O'Grady, SL Minors, ML O'Sullivan, R Horne. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Dilated cardiomyopathy (DCM) is a common myocardial disorder in the dog, particularly in large breed dogs and within these dogs, particularly the Doberman Pinscher breed. Pimobendan has recently been advocated in the management of CHF due to DCM. The objective of this study was to evaluate the chronic hemodynamic

effects of pimobendan treatment in Dobermans with CHF due to DCM.

Doberman Pinschers with CHF due to DCM were randomly assigned to receive pimobendan (0.25 mg/kg BID PO) or placebo (0.25 mg/kg BID PO) in addition to furosemide and ACE inhibition. CHF due to DCM was determined on the basis of history, clinical signs, echocardiography and the presence of pulmonary edema on thoracic radiographs. Dogs were examined on the day of enrolment (naïve to pimobendan but usually not to ACE inhibitors) and at 1 month, providing the dogs were still alive and treatment failure had not occurred by this time. Treatment failure was defined as failure of 5 mg/kg TID PO furosemide to control abnormal respiratory signs. Examinations consisted of an echocardiographic examination that included the measurement of: left ventricular (LV) internal dimension at end diastole (LVID-D) and end systole (LVID-S) by M-mode; and LV end diastolic and end systolic volume by Simpson's rule. From the above data, fractional shortening and ejection fraction were derived. Sixteen dogs were enrolled in this treatment trial. Eight dogs were enrolled into the pimobendan arm. Of these, 5 dogs had examinations performed on both day 0 and at 1 month as defined. The mean and range of responses are described. No comparison with the placebo group was possible as only one placebo dog was available at 1 month.

There was an increase in fractional shortening and ejection fraction at 1 month compared with enrolment (48% [-9% to 138%]; and 9% [-3% to 19%] respectively). There was a reduction in LVID-D and LVID-S at 1 month compared with enrolment (2% [-6.2% to 8.5%] and 11% [1% to 17%] respectively).

The small sample size certainly limits the scope of this work. Our goal was to determine if the pimobendan group would demonstrate improved contractility as compared with the placebo group. Such a comparison was not possible as the placebo arm demonstrated a high mortality and treatment failure rate compared with the pimobendan arm restricting analysis. A modest improvement in systolic function occurred with pimobendan. More dogs should be studied to strengthen these findings.

#### **ABSTRACT #261**

COMPARISON OF THE CHRONIC HEMODYNAMIC RESPONSE OF PIMOBENDAN VERSUS BENAZEPRIL IN DOGS WITH CHF DUE TO CHRONIC MITRAL VALVE INSUFFICIENCY. MR O'Grady, SL Minors, ML O'Sullivan, R Williams, R Horne. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Chronic mitral valve insufficiency (CMVI) is the most common myocardial disorder in the dog. Pimobendan has recently been advocated in the management of CHF due to CMVI. The objective of this study was to compare the chronic hemodynamic effects of pimobendan and benazepril treatment in dogs with CHF due to CMVI.

Small breed dogs with CHF due to CMVI were randomly assigned to receive pimobendan (0.25 mg/kg BID PO) or benazepril (0.5 mg/kg BID PO) in addition to furosemide. CHF due to CMVI was determined on the basis of history, clinical signs, echocardiography and the presence of pulmonary edema on thoracic radiographs. Dogs were examined on the day of enrolment (naïve to pimobendan but usually not to ACE inhibitors) and at 4 months, providing the dogs were still alive and treatment failure had not occurred by this time. Treatment failure was defined as failure of 5 mg/kg TID PO furosemide to control abnormal respiratory signs. Examinations consisted of an echocardiographic examination that included the measurement of: left ventricular (LV) internal dimension at end diastole and end systole by M-mode; forward flow across the mitral and aortic valves using the area and velocity time integral of flow at the annulus; mitral regurgitant volume by the flow convergence method; LV end diastolic and end systolic volume by Simpson's rule;

and the velocity of regurgitant flow across the tricuspid valve. From the above data, fractional shortening, forward stroke volume, mitral regurgitant volume, mitral regurgitant fraction, ejection fraction, and pulmonary artery systolic pressure were derived. Twenty-five dogs have been enrolled to day in this treatment trial. Ten dogs, 6 in the pimobendan arm and 4 dogs in the benazepril arm, had examinations performed on both day 0 and at 4 months as defined. Statistical analysis was limited to a t test comparing the percent change in parameters at 4 months versus baseline between both treatment arms. Level of significance was set at  $p \leq 0.05$ .

There was no significant difference in the response of the pimobendan group compared with the benazepril group at 4 months with respect to any of the measured or calculated parameters described.

The small sample size certainly limits the scope of this work. Our goal was to determine if an interim analysis would yield a more favorable hemodynamic response in the pimobendan group. As this is an ongoing study we will endeavour to enrol more dogs to determine whether a stronger hemodynamic effect is observed with pimobendan as compared with benazepril to account for the improved benefit reported with pimobendan.

#### **ABSTRACT #262**

THE EFFECTS OF THE CALCIUM-CHANNEL BLOCKER DILTIAZEM ON CARDIOVASCULAR FUNCTION IN HORSES: A DOSE-FINDING STUDY. C.C. Schwarzwald, J.D. Bonagura. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Atrial fibrillation (AF) is the most common cardiac arrhythmia impacting performance in horses. Quinidine is an effective treatment for AF, but accelerates ventricular response rate prior to conversion. Diltiazem controls heart rate response to AF in other species, but has not been studied in horses. This investigation examined the effects of diltiazem on heart rate (HR) and rhythm, atrioventricular conduction, and mean arterial blood pressure (ABP) with the intent of identifying clinically effective doses in normal, standing, non-sedated horses.

Eight healthy horses were treated intravenously every 15 minutes with diltiazem to achieve cumulative doses of 0.00 (baseline), 0.05, 0.175, 0.425, 0.925, 1.425, and 1.925 mg/kg. The HR and cardiac rhythm, PR interval, and ABP were recorded continuously throughout injection and measured at the midpoint of each treatment period. Diltiazem plasma concentrations were determined at each sampling period. The horses were monitored for clinical adverse effects during and for the duration of 8 hours after the experiment. Statistical analysis of measured variables was performed using repeated measures ANOVA with Tukey's or Dunn's post-test. The level of significance was  $p < 0.05$ .

Diltiazem caused dose-dependent increases in HR, from 33/minute at baseline to 40/minute at a dose of 0.925 mg/kg ( $p=0.008$ ). Mean ABP decreased by 25 mm Hg at the highest dose ( $p<0.001$ ). The PR interval increased minimally ( $p=0.036$ ) and inconsistently. However, atrioventricular or sinoatrial blocks were observed consistently at the two highest doses. At these doses, diltiazem plasma concentrations averaged  $720 \pm 43$  ng/ml and  $874 \pm 113$  ng/ml, respectively. Diltiazem plasma concentrations at each treatment period varied considerably among horses. One horse developed high-degree sinus arrest with signs of hypotension. No other adverse effects were noted in any of the horses.

These data indicate that diltiazem, dosed at 1 to 2 mg/kg intravenously, depresses sinus and AV nodal function in healthy horses at plasma concentrations between 700 and 1000 ng/ml. Effective doses may vary among horses, and may be limited by hypotension caused by peripheral vasodilation or bouts of bradycardia. Presumably the slight increase in mean HR observed at the higher doses was caused by activation of the baroreceptor reflex, which mitigated some of the depressant effects of diltiazem on nodal

tissues, but did not prevent periods of sinus arrest or atrioventricular block. The ultimate cardiovascular effects of diltiazem are likely mediated by competing actions: direct drug depression on cardiac and vascular calcium entry countered by reflex activation of the sympathetic nervous system in response to falling ABP. Further studies are needed to investigate diltiazem pharmacokinetics and drug effects on ventricular function, central hemodynamics, peripheral vascular resistance, and peripheral blood flow.

#### **ABSTRACT #263**

PERFORMANCE EVALUATION OF AN IMPEDANCE-BASED POINT-OF-CARE HAEMATOLOGY ANALYSER USING FLOATING DISCRIMINATORS IN THE ANALYSIS OF EQUINE BLOOD SAMPLES. Gittan Gröndahl<sup>1</sup>, Bram Bottema<sup>2</sup>, Inger Lilliehöök<sup>3</sup>, <sup>1</sup>Dept of Large Animal Clinical Sciences, <sup>3</sup>Dept of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, <sup>2</sup>Boule Medical AB, Stockholm, Sweden.

In critical care, reliable and fast patient-side analysis of erythrocytes, leukocytes, and platelets is essential for appropriate diagnosis and clinical management. Because of the great physical individual variations in blood cells, an impedance-based haematology analysing system using floating discriminators for the differentiation of blood cell populations designed specifically for veterinary applications has been developed, Medonic™ CA620-VET / HESKA® CBC-Diff Hematology System (Boule Medical, Stockholm, Sweden). In human haematology, this approach has proven to be more accurate than the use of fixed discriminators, especially for pathological samples. In veterinary haematology with its large range of species, breeds and types, floating discriminators and specifically modified veterinary reagents are necessities for high accuracy.

The purpose of this study is to compare results from this veterinary haematology analyser to results from the Cell-Dyn® 3500 (Abbott Laboratories, North Chicago, IL, US), haematology instrumentation used in many veterinary reference laboratories, and to manual microscopic differential counts of white blood cells. Blood was collected from healthy and sick horses to assure a wide range of values within the studied parameters. These included red blood cell count (RBC), mean cell volume (MCV), haemoglobin concentration (HGB), haematocrit (HCT), white blood cell count (WBC), lymphocyte count (LYM), granulocyte count (GRAN) and platelet count (PLT). The number of equine blood samples was 30 in the correlation between the instruments, and 46 in the comparison to microscopic counts.

The correlation between the two different instruments was excellent for the red and white blood cell parameters. R-squared values for RBC, MCV, HGB, and HCT in horse blood samples were 0.98, 0.96, 0.97, and 0.93, respectively. Total counts of WBC, LYM, and GRAN had R-squared values of 0.98, 0.97, and 0.98, respectively. For PLT, it was 0.58. Also in comparison to the manual method, the correlation was good. The percentages of LYM and GRAN showed  $R^2$  of 0.79 and 0.74, respectively.

Platelet analysis is more difficult in horses on all instrument systems including the Cell-Dyn® analyser because of an inherent tendency for aggregation in the equine thrombocyte. Therefore, the platelet concentration correlation observed for horses was satisfactory. Both methods are regarded as reliable for detecting clinically important thrombocytopenia.

In conclusion, performance of the impedance-based haematology analysing system using floating discriminators and reagents designed for veterinary application was interpreted as very good. With its high accuracy, it proved to be a useful and reliable tool for the veterinary practice dealing with haematology in horses.

## ABSTRACT #264

IS THE IMMUNE SYSTEM OF THE EQUINE NEONATE GROWN UP? M. Julia B.F. Flaminio, Rebecca L. Tallmadge, Bettina R. Wagner, Mary B. Matychak, Julie M. Hillegas, and Diana Ruano, from the Department of Clinical Sciences, and the Baker Institute for Animal Health, Cornell University, Ithaca, NY.

Previous immunological studies demonstrated that the equine fetus is able to generate an antigen specific immune response, and considered the foal immunocompetent at birth. However, the susceptibility to certain organisms in early life suggests limitations in the immune system of the equine neonate. This study describes additional information regarding the activation and maturation status of the immune system of the foal at birth.

Peripheral blood samples from 11 normal foals were collected within 1 hour of birth and before the ingestion of colostrum for serum immunoglobulin (Ig) isotype analysis. Peripheral blood mononuclear cells and lymphoid tissues of 6 normal foals of less than 2 hours of age were tested for the expression of cell markers and cytokines. Samples from 6 normal adult horses were tested in parallel for comparison. Serum Ig isotype levels were measured using ELISA. The expression of cell-surface Ig isotypes and clusters of differentiation in mesenteric lymph node, spleen and thymus was tested using immunohistochemistry. The mRNA expression of major histocompatibility complex class I and II (MHC I and MHC II) and beta-2 microglobulin (b2M) molecules in spleen, lymph node and thymus was measured using polymerase chain reaction (PCR). The mRNA expression of cytokines in isolated peripheral blood mononuclear cells, spleen and lymph nodes was tested using quantitative real-time PCR.

Serum IgM, IgG<sub>A</sub> and IgG<sub>B</sub> were detected in the pre-suckle foals, and levels were inferior than the adult horses ( $p < 0.05$ ); serum IgG<sub>C</sub> and IgG(T) levels were essentially undetectable. In the newborn foals, the expression of the cell surface Ig in secondary lymphoid tissues was positive for IgM in the areas concomitantly positive for the B cell marker. No other Ig isotype expression was detected on the cell surface. The spleen of foals revealed organized B cell and T (CD4<sup>+</sup> and CD8<sup>+</sup>) cell areas, and the lymph nodes demonstrated a paucity of CD4<sup>+</sup> T cells. The expression of MHC class II molecule at the protein and molecular levels was more abundant in the spleen and lymph node in comparison to MHC class I. The expression of b2M molecule was similar to that of MHC class I. The cytokine IL-2, IL-4, IL-10, IFN-gamma, and IL-12p35 mRNA expressions in the spleen and lymph node of the newborn foals were compared to the adult horses and revealed dissimilar distribution.

These results indicated that, under physiological conditions and with negligible antigenic stimulation, the spleen of the equine fetus becomes organized and immunologically active throughout gestation, with the expression of MHC class II molecules, B cell germinal centers and T cell zones, primary IgM humoral response, B cell isotype switch for the production of IgG<sub>A</sub> and IgG<sub>B</sub>, and cytokine production. The interaction between the innate and adaptive immune systems is under investigation.

## ABSTRACT #265

HUMORAL IMMUNITY IS NOT CRITICAL TO PROTECTION AGAINST EXPERIMENTAL INFECTION WITH *SARCOCYSTIS NEURONA* IN B-CELL DEFICIENT MICE. Sharon G. Witonsky<sup>1</sup>, Robert M. Gogal, Jr.<sup>2,3</sup> Robert B. Duncan<sup>2</sup>, and David S. Lindsay<sup>2</sup>. <sup>1</sup>Department of Large Animal Clinical Sciences, <sup>2</sup>Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, <sup>3</sup>Via College of Osteopathic Medicine, Virginia Polytechnic Institute and State University (Virginia Tech) Blacksburg, VA.

Equine protozoal myeloencephalitis, due to *Sarcocystis neurona* infection, is one of the most common causes of neurologic disease in the United States. Even though national seroprevalence is greater

than 50%, less than 1% of all horses develop clinical disease. The protective immune response to infection has not been well defined. Specifically, the role of the humoral response has not been elucidated until now.

In this study, B-cell deficient (uMT) mice were infected with *Sarcocystis neurona* merozoites in order to assess the role of the humoral response to active infection. Interferon-gamma (IFN-gamma) knockout (GKO) and immunocompetent C57BL/6 mice were infected simultaneously with the same inoculum as controls. Using a direct agglutination test, all C57BL/6 and GKO knockout infected mice seroconverted to *S. neurona* merozoite antigens by day 14 post-infection (PI). However, B-cell deficient (uMT mice) did not seroconvert. GKO infected mice, which were euthanized by day 28 post-infection (PI), developed encephalitis, confirming activity of the *S. neurona* merozoites. B-cell deficient (uMT) mice developed mild splenomegaly and bilateral symmetrical lymphadenopathy by day 14 PI, which completely resolved by day 60 PI. All uMT infected mice survived until day 60 PI, at which time they were euthanized. Histopathologic changes at day 14 PI revealed mild to moderate multifocal cellular infiltrates in the liver, and mild increase in follicles without germinal center development in the spleens. Changes had resolved by day 60 PI. Flow cytometry was used to correlate gross and histopathological changes with changes in immune cell subsets. B-cell staining confirmed the lack of B-cells in peripheral blood, spleen and lymph nodes. At day 14 in the blood, there was a mild increase in the percentage of CD4 cells present, and by day 60 there was an increased percentage of CD4, CD8 and memory CD44/CD4 and CD44/CD8 cells present. There was an increased percentage of CD4 cells and CD44/CD8 memory cells present in the spleen at day 60 PI. In summary, we propose that in this mouse model, humoral immunity is not critical to protection against *S. neurona* infection.

## ABSTRACT #266

EVALUATION OF THE EFFICACY OF DISINFECTANT FOOTBATHS. Morley PS, Morris N, Hyatt DR. College of Veterinary Medicine and Biomedical Sciences, Colorado State University: Fort Collins, Colorado.

Objective: To evaluate the efficacy of two disinfectants when used in footbaths for reducing bacterial contamination of footwear. Materials and Methods: New rubber boots were used for the study after they were cleaned and disinfected with 70% ethanol. Boots were contaminated using a standardized process; an investigator wearing rubber boots walked in a serpentine pattern for 2 minutes through straw bedding in a stall contaminated through routine stabling of an adult cow. Footbaths were filled with the same volume of freshly diluted disinfectant solution such that there was approx. 4 in of disinfectant standing in the tubs. Disinfectant solutions were prepared using the manufacturer's instructions. The following disinfectants were used: quaternary ammonium (A464N® [mixture of methyl ammonium compounds], Airkem Professional Products) and a peroxygen compound (Virkon-S® [potassium peroxymonosulfate], Antec International). Tap water was used as a control treatment for comparison. Samples were collected for analysis using sterile swabs pre-moistened with broth containing neutralizers for common disinfectants and swabbing 4 marked areas (20 cm × 1 cm) on each boot. Pre-disinfection bacterial counts were estimated by sampling sites on one boot immediately after contamination. The other boot was placed in the footbath for approximately 3 sec. Post-disinfection sampling was performed on the second boot 7 min after stepping out of the footbath. Ten pairs of boots were sampled for each type of solution tested. Swabs were placed in 10 ml of the sterile broth containing disinfectant neutralizers and held at room temperature until processed. Surface bacterial load was quantified by making serial dilutions and plating to blood agar and MacConkey's agar. Plates were incubated for 24 hr at

37°C, colonies were counted, and results were expressed as colony forming units (CFU) per cm<sup>2</sup>. Differences between treatments were analyzed using regression analysis. Statistical analyses used GEE to control for the hierarchical and repeated nature of the data. Least square means and variance estimates for log<sub>10</sub> bacterial counts were determined from these models and used to compare differences associated with the experimental treatments. Results: There was no statistically detectable reduction in bacterial counts from boots after A464N treatment, while more than 70% reduction in bacterial counts was observed after treatment with Virkon-S. There was a detectable increase in bacterial counts on control boots treated with water compared to pre-disinfections counts on untreated boots. Conclusion: Results of this study suggest that Virkon-S is superior to A464N when used in footbaths for rapid disinfection of footwear in a veterinary hospital. This assumes that large amounts of gross fecal contamination typically will not be found on the surface of footwear in veterinary hospital environments.

**ABSTRACT #267**

SURVEILLANCE FOR *SALMONELLA* SHEDDING IN LARGE ANIMAL PATIENTS. Morley PS, and Dunowska M, College of Veterinary Medicine and Biomedical Sciences, Colorado State University: Fort Collins, Colorado.

Objective: To characterize the risk of shedding *Salmonella enterica* among large animal patients admitted to the James L. Voss Veterinary Teaching Hospital (JLV-VTH) at Colorado State University. Design: Prospective longitudinal study. Procedures: Fecal samples were obtained from all large animal patients admitted to the JLV-VTH for ≥1 day between July 1, 2002, and July 21, 2003, and cultured to identify *Salmonella enterica*. Samples were collected at the time of admission, and every Mon, Wed, and Fri throughout hospitalization. Antimicrobial susceptibility and serogroup and serotype of *Salmonella* isolates were evaluated. Patient signalment and hospitalization information was collected from medical records. Information was also collected regarding each patients' health and management during the 48 hrs preceding each sample collection, including categorical assessment of the patients' overall systemic illness status, soft fecal consistency or diarrhea, fever, leukopenia, anesthesia or surgery, antimicrobial therapy, significant reduction in dietary intake, body systems affected, stabling location when sampled, and attending hospital service. Factors potentially associated with *Salmonella* shedding were analyzed using logistic regression, using generalized estimating equations to control for repeated sampling of horses. Factors associated with the likelihood of *Salmonella* recovery in univariable models ( $P<0.20$ ) were included in multivariable modeling, using backward stepping to determine which variables were retained in the final model ( $P<0.10$ ). Results: A total of 3,504 samples were submitted from 1,417 patients (1,508 separate admissions) as a part of the surveillance program (average = 2.5 samples per animal, median=2, range 1-73). *Salmonella* was recovered from 3.5% of samples (124/3504), and 5.5% of patients (77/1396). The patient characteristic most strongly associated with increased rates of *Salmonella* recovery was the subjective characterization of severity of patients' systemic illness, regardless of which body systems were affected. Diarrhea occurring within 48 hrs of sampling was also associated with an increased rate of *Salmonella* recovery, but having other GI disease was not. After controlling for these clinical factors, bovine patients, older patients, and patients housed in isolation were more likely to shed *Salmonella*, which is consistent with prior conceptions regarding shedding risk at the JLV-VTH as indicated by our existing empirical *Salmonella* control procedures. It is also interesting to note variables that were not retained in the final model, such as fever, leukopenia, history of anesthesia or surgery, reduction in feed intake, body system affected, and gender. Conclusions: *Salmonella* infections represent a major risk to the JLV-VTH. In general, food animal and equine patients are

segregated, as are patients with gastrointestinal disease. Additional biosecurity precautions should be used with patients having moderate or major systemic illness or diarrhea. Reliance upon these factors alone did not fully predict *Salmonella* shedding in this population.

**ABSTRACT #268**

SEROLOGIC DIAGNOSIS OF EQUINE BORRELIOSIS: EVALUATION OF AN IN-CLINIC ELISA (SNAP<sup>®</sup>3Dx<sup>®</sup>). R Chandrashekar, D. Daniluk. IDEXX Laboratories, Westbrook, Maine.

*Borrelia burgdorferi*, the causative agent for Lyme disease, infects a wide range of mammalian hosts. Serological studies in horses indicate that incidence of Equine *Borrelia* infection to be 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.

SNAP<sup>®</sup>3Dx<sup>®</sup> (IDEXX Laboratories, Westbrook, ME) is a commercially available in-office test kit for the simultaneous detection of antibodies to *B. burgdorferi* and *Ehrlichia canis* and *Dirofilaria immitis* antigen in dogs. The test kit is an ELISA that uses a synthetic peptide (C<sub>6</sub>) derived from the IR<sub>6</sub> region within the *Borrelia* membrane protein VlsE. Studies with canine samples suggests that SNAP<sup>®</sup>3Dx<sup>®</sup> is particularly useful in Lyme-endemic areas because it can be conveniently and reliably used in the clinic to determine the infection status of a dog irrespective of its vaccination history.

We evaluated the performance of SNAP<sup>®</sup>3Dx<sup>®</sup> for the detection of antibodies to *B. burgdorferi* in equine serum samples from northeastern United States. A total of 164 samples from horses were tested both by SNAP<sup>®</sup>3Dx<sup>®</sup> and QualiCode<sup>™</sup> *B. burgdorferi* IgG/IgM Western Blot Kits (Immunitics, Cambridge, MA). Of the serum samples tested, 109 were positive for Lyme by SNAP<sup>®</sup>3Dx<sup>®</sup>. However, only 106 of 164 samples were positive by Lyme Western Blot Assay. The three discordant samples were positive by IFA with a low titer of 1:64. Thus, relative to Western Blot Assay, SNAP<sup>®</sup>3Dx<sup>®</sup> had a sensitivity of 100% and specificity of 95%.

Performance of SNAP <sup>®</sup> 3Dx <sup>®</sup>			
		QualiCode <sup>™</sup> Western Blot	
		+	-
SNAP <sup>®</sup> 3Dx <sup>®</sup>	+	106	3*
	-	0	55
Relative to Western Blot			
% Sensitivity		100	
% Specificity		95	
*Positive by IFA			

These results indicate that SNAP<sup>®</sup> 3Dx<sup>®</sup> can be successfully used to detect antibodies to *B. burgdorferi* in infected horses.

**ABSTRACT #269**

LIPOPOLYSACCHARIDE RECEPTOR-LIGAND INTERACTIONS IN EQUINE MONOCYTES. KL Lohmann<sup>1</sup>, MH Barton<sup>1</sup>, TF Murray<sup>2</sup> and JN Moore<sup>1</sup>. Dept Large Animal Medicine<sup>1</sup> and Physiology and Pharmacology<sup>2</sup>, College of Veterinary Medicine, University of Georgia, Athens, Georgia.

Understanding of the cellular response to lipopolysaccharide (LPS) is critical to the development of new treatments for endotoxemia. Structurally variant LPS may be useful for investigating interactions between ligand and receptor and may act as antagonists to enteric LPS. Rhizobiaceae are nitrogen-fixing plant bacteria that produce LPS of unique structure, which differs greatly from LPS of enteric bacteria such as *Escherichia coli* (*E. coli*). Here, we compare the response of equine monocytes to LPS from *E. coli* and 2 rhizobial

bacteria (*Rhizobium galegae* and *Rhizobium sin-1*) and characterize receptor-ligand interactions by means of radioligand binding studies and selective receptor protein transfection in a reporter cell system.

Equine monocytes isolated by gradient density centrifugation were stimulated with LPS and production of tumor necrosis factor (TNF) was measured by bioassay. Maximum TNF production and EC<sub>50</sub>, i.e. the LPS concentration yielding half-maximal response, were compared between ligands. Cells were co-incubated with *E. coli* LPS and rhizobial LPS to evaluate the antagonistic potential of rhizobial LPS. Binding affinities of *E. coli* LPS and rhizobial LPS were estimated by determining values of IC<sub>50</sub> (the concentration of unlabeled competitor that inhibits 50% of the binding of a radioligand) in equilibrium competition experiments using radiolabeled *E. coli* K12 LPS. Equine CD14, TLR4 and MD-2 were transiently expressed in human embryonic kidney (HEK) cells. HEK cells were also transfected with luciferase reporters, and cellular activation by LPS was measured using a double luciferase assay.

Average maximal TNF production and EC<sub>50</sub>, respectively, were 1005.6 units/ml and 0.057 ng/ml for *E. coli* LPS, 611.9 units/ml and 333.3 ng/ml for *R. galegae* LPS, and 938.3 units/ml and 236.1 ng/ml for *R. sin-1* LPS. LPS from *E. coli* was significantly more potent than rhizobial LPS. In 3 of 7 experiments, LPS from *R. galegae* inhibited cellular response to *E. coli* LPS by 51 to 65% with an IC<sub>50</sub> of 5.6 to 37.9 ng/ml, while no inhibition was observed in the remaining experiments. LPS from *E. coli*, *R. galegae* and *R. sin-1* competed for binding of radioligand LPS with an average IC<sub>50</sub> of 0.5 µg/ml, 11.6 µg/ml and 7.8 µg/ml, respectively. IC<sub>50</sub> of LPS from *R. galegae* and *R. sin-1* were not significantly different from each other, but were significantly higher than IC<sub>50</sub> of *E. coli* LPS. Transfection of HEK cells showed that the presence of all 3 receptor proteins was required to render cells responsive to LPS, and that fetal bovine serum, presumably through soluble CD14, could substitute for expression of membrane CD14.

We conclude that LPS from *R. galegae* and *R. sin-1* are low-potency, low-affinity agonists in equine monocytes, while *R. galegae* LPS may be classified as a partial agonist in some individuals. The cellular response of equine cells to these LPS is dependent on ligand interaction with the currently recognized LPS receptor proteins.

#### ABSTRACT #270

EFFECTS OF FATTY ACID CHAIN-LENGTH ON GASTRIC EMPTYING OF A GLUCOSE SOLUTION IN HORSES. V. Walsh\*, R. Geor†, J. Cant\*, S. Pratt\*, J. McCutcheon‡. University of Guelph, Depts of Biomedical Sciences†, Pathobiology‡ and Animal & Poultry Science\*, Guelph, ON Canada.

In monogastric species the systemic availability of ingested nutrients is dependent upon the rate of gastric emptying (GE). In human subjects, GE is faster after ingestion of a solution containing glucose and a medium chain fatty acid (MCFA) when compared to glucose alone. Conversely, long chain fatty acids slow GE of a glucose solution. Currently, there is little information regarding the effects of dietary fat on the rate of GE in horses. The objectives of the present study were to: 1) determine the effects of fatty-acid chain length (short, C3; medium, C8; long, C16-18) on the rate of GE after administration of a glucose solution to horses; and 2) evaluate the effects of these fatty acid treatments on glycemic response, an indicator of systemic glucose availability.

Seven mature Standardbred horses participated in each of 5 trials in which the following treatments were administered by gastric gavage after an overnight (12 h) fast: 1) GLUC – 1 g glucose per kg bwt. as a 20% (w/v) solution; 2) SCFA (short-chain fatty acids) – the addition of triacetin to the glucose solution; 3) MCFA (medium-chain fatty acids) – octanoic acid and glucose solution; 4) LCFA (long-chain fatty acids) – corn oil and glucose solution; and 5) CON – a negative control treatment of H<sub>2</sub>O (equivalent volume). The dose of all fatty acid treatments was 0.25 g/kg bwt. In each treatment, acetaminophen

(20 mg/kg bwt) was added as a marker of liquid phase gastric emptying. Blood samples for measurement of plasma acetaminophen, glucose, and immunoreactive insulin were obtained at -15 and -5 min before and at frequent intervals for 7 hours after treatment. Peak acetaminophen concentration (P<sub>ac</sub>) and time to P<sub>ac</sub> (T<sub>peak</sub>), and incremental areas under the plasma glucose (AUC<sub>G</sub>) and insulin (AUC<sub>I</sub>) vs. time curves were calculated as indices of GE rate and glycemic response, respectively. Data were analyzed by repeated measures analysis of variance ( $\alpha=0.05$ ).

There was a significant (P<0.001) effect of treatment on GE. The P<sub>ac</sub> was significantly (P<0.01) lower in GLUC (mean ± SD; 16.0 ± 0.7 µg/dl), SCFA (14.6 ± 0.9), MCFA (8.1 ± 0.9) and LCFA (13.5 ± 1.1) than in CON (30.6 ± 1.2). The P<sub>ac</sub> was lower (P<0.05) in MCFA than in all other treatments. Similarly, T<sub>peak</sub> was significantly (P<0.001) greater in MCFA when compared to CON, GLUC, SCFA and LCFA. This apparent delay in GE in the MCFA treatment was reflected in alterations in the glycemic response. AUC<sub>G</sub> was smaller (P<0.05) in MCFA (2624 ± 105 mM) when compared to the other treatments (GLUC 3771 ± 212; LCFA 3915 ± 172; SCFA 3842 ± 277). AUC<sub>I</sub> was also lower (P<0.01) in MCFA than in the other treatments. However, glycemic responses in SCFA and LCFA were not different from GLUC. It is concluded that: 1) when compared to water, a 20% glucose solution delays liquid-phase gastric emptying in horses; 2) MCFA, but not SCFA and LCFA, slow the rate of GE of a 20% glucose solution; and 3) a slowing in GE results in a blunted glycemic response.

#### ABSTRACT #271

PANCREATIC β-CELL RESPONSE TO GRADED HYPERGLYCEMIA IN HORSES. R. Geor\*, S. Pratt†, J. McCutcheon‡, L. Read‡. University of Guelph, Depts. of Biomedical Sciences\*, Animal & Poultry Science† and Pathobiology‡, Guelph, Ontario, Canada.

Glucose tolerance is a function of pancreatic β-cell secretion and insulin action in target tissues. In horses, oral or intravenous glucose loading is most commonly used for study of glucose tolerance. However, interpretation of β-cell responses is made difficult by the non-steady-state nature of these methods. In the present descriptive study, a three-step hyperglycemic clamp protocol was used for evaluation of the dose-response relationship between blood glucose and pancreatic β-cell secretion, as reflected by plasma concentrations of insulin and C-peptide.

Eight Standardbred horses (7 mares, 1 gelding; 3-8 years of age) maintained on a forage diet undertook a three-step (each of 75 min duration) hyperglycemic clamp (8, 16 and 24 mM). At each step, a priming injection of glucose (50% dextrose) was given by syringe pump to achieve the target blood glucose concentration. Blood glucose concentrations were measured at 5 min intervals and the rate of glucose infusion (GIR) adjusted to maintain the target concentration. Blood samples for measurement of immunoreactive insulin and C-peptide (by RIA) were obtained at 5 min intervals between 0-15 min of each step, and every 15 min thereafter. Hormone concentrations at 75 min of each step were plotted as a function of blood glucose concentration. The data were analyzed by one-way repeated measures analysis of variance to compare the different phases of the clamp ( $\alpha=0.05$ ). Data are presented as means ± SD.

Mean values for blood glucose, immunoreactive insulin and C-peptide, averaged over the last 30 min of each step, are shown in Table 1. At each step, a steady-state in blood glucose concentration was achieved after ~25 min. The GIR (mg/kg/min) required to maintain blood glucose at 8, 16 and 24 mM were, respectively, 2.86 ± 0.42, 6.49 ± 0.72 and 9.87 ± 0.88. There were progressive increases in insulin and C-peptide; values averaged over the last 30 min of each step were significantly higher compared to the previous step. Linear relationships were found between blood glucose and insulin

( $r^2=0.885$ ,  $P=0.05$ ) and C-peptide ( $r^2=0.919$ ,  $P=0.04$ ). The C-peptide-to-insulin molar ratio was lower ( $P<0.05$ ) at 24 mM when compared to the other steps, possibly a reflection of decreased hepatic clearance of insulin at higher insulin concentrations.

Table 1: Dose-response relationship for glucose-stimulated insulin and C-peptide responses

Variable	Basal	Target Glucose Concentration (mmol/l)		
		8	16	24
Glucose (mmol/l)	3.7 ± 0.50	8.1 ± 0.39	16.6 ± 0.98	24.5 ± 0.74
Insulin (pmol/l)	71.8 ± 19.6	280.3 ± 64.8	371.2 ± 71.3	436.6 ± 68.4
C-peptide (nmol/l)	0.42 ± 0.13	1.12 ± 0.22	1.48 ± 0.30	1.92 ± 0.39

Pancreatic  $\beta$ -cell secretion in horses, as reflected by plasma concentrations of immunoreactive insulin and C-peptide, is linear over a range of glucose concentrations between ~4 and ~24 mM.

### ABSTRACT #272

EFFECT OF DIETS DIFFERING IN STARCH AND FAT CONTENT ON INSULIN SENSITIVITY DURING A EUGLYCEMIC-HYPERINSULINEMIC CLAMP IN HORSES. S. Pratt<sup>†</sup>, R. Geor<sup>‡</sup>, J. McCutcheon\*. Departments of Animal and Poultry Science<sup>†</sup>, Biomedical Sciences<sup>‡</sup> and Pathobiology\*, University of Guelph, Guelph ON, Canada.

The ability of insulin to promote glucose disposal is affected by dietary macronutrient composition. In humans, a diet high in hydrolysable carbohydrate (hCHO) improves insulin sensitivity (Si), whereas high fat diets can result in decreased Si. Conversely, in a recent study horses had lower Si when fed a supplement high in hCHO when compared to a fat and fiber supplement (Hoffman et al. J Anim Sci 2003; 81:2333). To extend these observations, the present study was undertaken to assess the effects of diets differing in hCHO and fat content on Si in horses, measured by use of the euglycemic-hyperinsulinemic clamp (EHC) method

Fourteen unconditioned Standardbred horses (8 mares, 6 geldings, 2-5 years of age) were studied. During a 4-wk pre-test period, horses were adapted to a diet of forage cubes (fed at approximately 2% BW), and provided with free-choice water and salt. Following this baseline period, insulin sensitivity was assessed via an EHC in which insulin was infused at a rate of 3.0  $\mu$ U/kg/min for 180 min with a variable rate of glucose infusion (50% dextrose) designed to maintain whole blood glucose concentrations at approximately 5 mmol/L. The mean glucose infusion rate (GIR) during the final 90-min period of the EHC was calculated and used as an index of Si. Horses were then randomly assigned to one of two groups ( $n=7$ ; 4 mares, 3 geldings per group) based on the hCHO and fat content of the concentrate added to the diet: a high hCHO concentrate (CHO: 12.5% CP, 2.7% fat, 45% starch) or a low hCHO concentrate (FAT: 12% CP, 13% fat, 7.5% starch). Concentrates were fed isocalorically (~1% BW). After a 7-week adaptation to the diet, the EHC procedure was repeated. Data were analyzed by repeated measures analysis of variance ( $\alpha=0.05$ ) and are presented as mean  $\pm$  SD.

There was a significant increase in mean bodyweight within the 7-week dietary treatment period (417  $\pm$  34 kg to 434  $\pm$  31 kg, and 427  $\pm$  27 kg to 445  $\pm$  27 kg for the CHO and FAT diets, respectively). Similarly, mean body condition score was increased ( $P<0.05$ ) after 7 weeks on the CHO (4.8  $\pm$  0.2 to 5.3  $\pm$  0.7) and FAT (4.8  $\pm$  0.2 to 5.2  $\pm$  0.6) diets. There were no differences between CHO and FAT for resting concentrations of plasma glucose or immunoreactive insulin, or the insulin-to-glucose ratio in samples obtained before the two EHC procedures. Mean GIR (mg glucose/kg/min) at baseline (forage diet) did not differ between diet treatments (CHO: 6.45  $\pm$  1.58, FAT: 6.17  $\pm$  1.60). Similarly, after the diet phase mean GIR did not differ between the CHO (6.05  $\pm$  1.52 mg/kg/min) and FAT (7.03  $\pm$  2.26 mg/kg/min) treatments. In both groups, mean GIR was also unchanged when comparing the baseline and dietary phases. It is concluded that insulin sensitivity in horses, as assessed by the EHC method, is unaffected by the short-term (7 weeks) feeding of

concentrates differing in hydrolysable carbohydrate (starch) and fat content.

### ABSTRACT #273

GENDER BUT NOT AEROBIC CAPACITY AFFECTS INSULIN SENSITIVITY IN UNTRAINED HORSES. S. E. Pratt<sup>†</sup>, R.J. Geor<sup>‡</sup> and L. J. McCutcheon\*. Departments of Animal and Poultry Science<sup>†</sup>, Biomedical Sciences<sup>‡</sup> and Pathobiology\*, University of Guelph, Guelph ON, Canada.

Insulin sensitivity, defined as the capacity of insulin to promote glucose disposal, is affected by several factors. Age, gender, diet, obesity and physical fitness (aerobic capacity) are all factors that influence insulin sensitivity in human subjects. Consistent with data from other species, obesity in horses is associated with insulin resistance. However, there are minimal data on other determinants of insulin sensitivity in this species. Therefore, the objective of this study was to examine the relationship of insulin sensitivity to gender and aerobic capacity in untrained horses.

Fourteen unconditioned Standardbred horses (8 mares, 6 geldings, 2-5 years of age) were studied. For a 4-week period prior to testing, horses were adapted to a diet consisting of forage cubes (fed at approximately 2% of bodyweight); water and salt were available free choice. After the adaptation period, in vivo insulin sensitivity was assessed by measurement of glucose disposal during a euglycemic-hyperinsulinemic clamp (EHC). For the EHC, insulin was infused at a rate of 3.0  $\mu$ U/kg/min for 180 min with a variable rate of glucose infusion (50% dextrose) to maintain whole blood glucose concentrations at approximately 5 mmol/L. Glucose uptake rate in each horse was quantitated as the mean glucose infusion rate (GIR) over the final 90-min period of the EHC. After the EHC, horses underwent 2-3 days acclimation to running on a high-speed treadmill. Thereafter, horses completed an incremental treadmill (3° incline) exercise test for determination of the peak rate of oxygen consumption ( $VO_{2peak}$ ). Oxygen consumption ( $VO_2$ ) was measured throughout exercise by use of an open-circuit indirect calorimeter, and the highest value achieved was taken as the  $VO_{2peak}$ . Unpaired Student's t-test was used to compare mean GIR and  $VO_{2peak}$  between mares (M) and geldings (G) ( $\alpha=0.05$ ). Pearson's product-moment correlation coefficient was used to examine the relationship between GIR and  $VO_{2peak}$ . Data are presented as mean  $\pm$  SD.

Mean bodyweight (M: 421.4  $\pm$  27.9 kg; G: 420.3  $\pm$  35.1 kg) and condition score (M: 5.5  $\pm$  0.7; G: 5.0  $\pm$  0.2) did not differ between the genders. Mean GIR (mg glucose/kg/min) during the final 90 min of the EHC was 60% higher ( $P<0.01$ ) in G (8.01  $\pm$  1.29) than in M (4.97  $\pm$  0.93). Neither aerobic capacity ( $VO_{2peak}$ , liters  $O_2$  per min; M: 49.1  $\pm$  4.2; G: 47.7  $\pm$  3.2) nor running speed at  $VO_{2peak}$  (m/s; M: 9.9  $\pm$  1.1; G: 10.4  $\pm$  1.0) differed between gender groups. There was no significant correlation between  $VO_{2peak}$  and mean GIR during the EHC in either gender group (M:  $r^2=0.039$ ,  $P=0.748$ ; G:  $r^2=0.282$ ;  $P=0.278$ ). In this group of untrained Standardbred horses, insulin sensitivity assessed by the euglycemic-hyperinsulinemic clamp method was substantially higher in geldings when compared to mares of similar age, bodyweight and body condition. However, insulin sensitivity was unrelated to aerobic capacity in either gender. Further studies are required to elucidate the mechanisms of this apparent gender difference in insulin sensitivity.

### ABSTRACT #274

THE USE OF ELECTRONEUTRALITY EQUATION APPLIED TO PLASMA/SERUM BIOCHEMICAL PROFILE REPORTS AS AN ADDITIONAL QUALITY CONTROL SYSTEM OF MEASURED STRONG ELECTROLYTES, WEAK ACIDS AND TOTAL  $CO_2$ . HR. Stämpfli<sup>1</sup>, A.J. Stevenson<sup>2</sup>, L. Brooks<sup>2</sup>, M.P. Weber<sup>3</sup>. <sup>1</sup>Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, <sup>2</sup>Canadian Pari-Mutuel Agency, Nepean, Canadian Pari-Mutuel Agency, Jerseyville, Ontario.

The quantitative approach to acid-base regulation emphasises that the concentrations of  $[H^+]$  and  $[HCO_3^-]$  is determined by three independent variables. 1) carbon dioxide tension  $[pCO_2]$ ; 2) strong ion difference  $[SID] = [strong\ cations] - [strong\ anions]$ , and 3) the total weak acid concentration  $[A_{tot}]$  such as proteins and inorganic phosphates (P<sub>i</sub>). The law of electroneutrality requires that the sum of the cations has to balance the sum of the anions at all times  $\{[Na^+ + K^+] - [Cl^- + Lac^- + HCO_3^- + A^-] = 0\}$  where A<sup>-</sup> is the net anionic charge of plasma protein (J Appl Physiol. 2003; 95(2):620-630). The electrical neutrality equation may be applied, as an added quality control, to reports of automated systems such as blood gas machines, serum/plasma biochemical machines reporting electrolytes, pCO<sub>2</sub>, total protein and tCO<sub>2</sub>.

50 plasma samples were collected from 50 STB race horses 20 minutes prior to racing during random tCO<sub>2</sub> testing on Ontario racetracks in 2002. One aliquot was analyzed in duplicates on a multi-analyzer system for concentrations of: HCO<sub>3</sub><sup>-</sup>, (enzymatic assay), Na, K, Cl, (ion specific electrodes ISE) Lactate (enzymatic assay), and total protein (colorimetric assay). A second aliquot was analysed in duplicates on a blood gas analyser measuring the following parameters: pCO<sub>2</sub>, pH, Na, K, Cl and lactate (all measured with ISE), calculated HCO<sub>3</sub><sup>-</sup>. The SID = (Na<sup>+</sup> + K<sup>+</sup>) - (Cl<sup>-</sup> + Lactate<sup>-</sup>) was calculated for both machines and the electroneutrality equation was tested and statistically compared between both methods of measurements.

The electroneutrality (E) made much more physiologic sense in the blood gas machine analysis, was significantly and on average 6 mEq/L different to the multi-analyzer. ( $E_{blood-gas\ machine} = -1.7 \pm 2.32$ ;  $E_{multi-analyser} = -7.5 \pm 1.19$  mEq/L). Closer scrutiny of the data revealed that the SID, specifically the Na read significantly lower on the multi analyzer, and was not reading normally on the ISE. ( $SID_{multi-analyser} = 36.11 \pm 1.90$ ;  $SID_{blood-gas\ machine} = 41.95 \pm 2.55$  n=50; p<0.05;  $Na_{multi-analyser} = 135.24 \pm 1.71$ ;  $Na_{blood-gas\ machine} = 141.21 \pm 1.97$ ; n=50; p<0.05).

Checking indices for acid-base balance (SID, A<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>) may be additionally used as a quality control to detect measuring errors in analyzer systems. All values of plasma samples measured with both analyzer methods were well within the normal reference ranges of calibration accuracy of both machines. In future electroneutrality and SID might be added to reports of serum biochemistry patient data as an additional quality control.

#### ABSTRACT #275

EVALUATION OF TRADITIONAL VERSUS A SELF-LEARNING COMPUTER MODULE IN TEACHING HOW TO PASS A NASOGASTRIC TUBE IN THE HORSE. Sameeh M, Abutarbush<sup>1</sup>, Gale Parchoma<sup>2</sup>, Lyall Petrie<sup>1</sup>, and Jonathan Naylor<sup>1</sup> <sup>1</sup>Department of Large Animal Clinical Sciences, WCVM. <sup>2</sup>Extension Division. University of Saskatchewan

Computer assisted learning (CAL) is a relatively new approach to teaching that is being increasingly used in veterinary medicine. It has potential advantages to teaching techniques including reducing the number of animals used for demonstration, improving animal welfare, the ability to present complex audio and visual materials, self directed learning and unobstructed material presentation. Potential problems include a lack of reality and immediacy. Traditional teaching has potential advantages of reality, immediacy, bonding with a live instructor, and the opportunity to ask a wide range of questions. However, a student's view may be obstructed in large groups and the instructor determines the rate and direction of learning. With a computer assisted module a more time consuming effort is made initially but future preparation time can be reduced. A computer assisted nasogastric intubation learning module was developed that included sections on indications, equipment, anatomy, performing the procedure, common errors and complications. Each

section consisted of a text supplemented with video clips of internal and external views, audio, still photographs, or illustrations.

To evaluate its effectiveness, a double blinded, monocentric study was performed. Forty eight third year students were randomly assigned to one of two groups. The traditional group (TM) was instructed how to pass a NG tube using traditional methods, with an instructor explaining the concept and performing a live demonstration. The students in the self learning computer module (SLCM), were given a CD rom, which they studied for the same amount of time as the traditional group. The students were then united in one session to practice passing the NG tube. Every student was expected to perform the procedure. The students were given a knowledge quiz and a questionnaire about their level of comfort and confidence with the teaching method. The data were analyzed by non parametric tests. The students in the SLCM group performed better on the test of knowledge (P<0.001), median scores out of 10, were 9.67 and 8.1, for SCLM and TM respectively. In the questionnaire, which was graded on a likert scale (0-5), they felt that they learnt more with the SCLM (P<0.001), median scores were 4.0 and 3.0, for SCLM and TM, respectively. There was no significant difference between the two groups in their feeling of being prepared to perform the procedure, or their feeling of understanding of the procedure. Computer assisted learning in passing the NG tube seems to be an acceptable and effective method to train students with potential welfare and knowledge advantages.

#### ABSTRACT #276

ASSOCIATION OF A MUTATION IN THE RYANODINE RECEPTOR 1 GENE WITH EQUINE MALIGNANT HYPERTHERMIA. Aleman, M., Riehl, J., Aldridge B.M., LeCouteur R.A., Stott, J.L. and Pessah, I.N. Veterinary Medical Teaching Hospital, University of California, Davis.

Malignant hyperthermia (MH) is a potentially fatal pharmacogenetic disorder of skeletal muscle elicited by exposure to volatile anesthetics, depolarizing muscle relaxants and stress. Most cases of human and all cases of porcine and canine MH are associated with mutations in the skeletal muscle isoform of the ryanodine receptor (RyR1). Since the introduction of inhalation anesthesia in the horse in the 1970's, several horses with suspected MH episodes have been reported but never genetically confirmed. The purpose of our study is to determine if mutations in the candidate gene RyR1 are associated with MH in the horse. Two Quarter Horses that underwent halothane anesthesia with no pre-medication for other research purposes, exhibited a fatal MH episode clinically comparable to those observed in other species. Both cases developed hyperthermia, hypercapnia, lactic acidosis, and muscle rigidity. Multiple muscle biopsies were collected for histochemistry, genetic, functional and expression analysis. Mild non specific myopathic changes were observed on histochemistry. DNA sequencing revealed a common polymorphism that resulted in an amino acid change in these two horses (<sub>MH</sub>RyR1); the polymorphism was not observed in 80 breed-matched controls (<sub>WT</sub>RyR1). Screening tests were developed for detection of heterozygous and homozygous individuals. There were no differences between <sub>MH</sub>RyR1 and <sub>WT</sub>RyR1 in activation by caffeine and calcium, and inhibition by calcium and magnesium under physiological buffer conditions. However, the affinity to [<sup>3</sup>H]-ryanodine of <sub>MH</sub>RyR1 was significantly higher than <sub>WT</sub>RyR1. Western blot analysis showed no difference in expression levels of <sub>MH</sub>RyR1 and <sub>WT</sub>RyR1. This is the first report of equine MH associated with a mutation in the RyR1 gene.

### ABSTRACT #277

THE PHYSICO-CHEMICAL APPROACH TO ACID-BASE INTERPRETATION: DEVELOPMENT OF SOFTWARE FOR A HAND-HELD COMPUTER. HR Stämpfli<sup>1</sup>, PV Jaspers-Fayer<sup>2</sup>. <sup>1</sup>Clinical Studies, <sup>2</sup>Information Technology Services, Ontario Veterinary College, University of Guelph, Guelph, Ontario Canada.

Traditional acid-base interpretation has relied heavily on the Henderson Hasselbalch equation and has largely ignored the importance of relative electrolyte concentration and protein concentration in the determination of acid-base balance and anion gap assessment. The quantitative approach to acid-base regulation (Stewart, 1983) states that  $[H^+]$  and  $[HCO_3^-]$  of aqueous biological solutions are determined by three independent variables:  $pCO_2$ , strong ion difference [SID], and the total weak acid concentration  $[A_{TOT}]$ , mainly proteins and phosphates. Serum profile reports contain SID and total protein concentrations as well as  $tCO_2$ . Once this information is fed to a programmed hand-held computer the dependent variables pH and  $HCO_3^-$ , anion and strong ion gap may easily be calculated and acid-base interpretation becomes available. This paper demonstrates and validates one such software program developed for a Palm™ type device.

The complex non-linear polynomial equation system from Stewart was used, and a previously developed excel spreadsheet (Equine Vet J Suppl. 1999, 30:438-42) math program was adapted for a hand-held computer device of the Palm® series. Two program types were developed; one for the practitioner with  $tCO_2$  input and one for academic and teaching purposes with  $pCO_2$  input. The input variables for the program include total  $CO_2$  ( $tCO_2$ ) or  $pCO_2$ , Na, K, Cl, and total protein (or total solids from a refractometer reading). All of these variables may be derived from a serum biochemical profile and/or automated blood gas machine. The output variables include calculated values for pH, SID, anion gap, strong ion gap, net negative charge on proteins (A),  $HCO_3^-$ , visual plot of a Gamble gram, and calculated osmolarity. A data set of blood gas values (n=40) of exercising horses previously published (Equine Vet J Suppl. 18, 261-265, 1995) was used to statistically compare calculated pH,  $HCO_3^-$  with measured pH,  $HCO_3^-$  on stat profile blood gas analyser.

The palm program yielded identical calculated values as the previously developed excel program. There was excellent correlation of calculated compared to measured pH, and calculated  $HCO_3^-$ . The Palm program may be an excellent tool for anaesthesiologists and clinicians to demonstrate quantitative impacts of changes in independent variables SID (electrolytes),  $pCO_2$  and total proteins. Species-specific programs are currently available for dog, cat, cow, horse, and human profile and blood gases. This program assists students and teachers to understand pathophysiology of acid base changes in patients quantitatively by integrating electrolytes and proteins and other weak acids into a acid base equation system. Program tour and access is available at

<http://www.ovcnet.uoguelph.ca/ClinStudies/faculty/HenryStaempfli.shtm>

### ABSTRACT #278

PHARMACOKINETICS OF ONCE-DAILY AMIKACIN IN CLINICALLY NORMAL AND HOSPITALIZED EQUINE NEONATES. Erica Paige Bucki and Steeve Giguère. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL.

The objectives of this study were to investigate the pharmacokinetics of once-daily amikacin in clinically healthy and hospitalized equine neonates, and to determine the minimum inhibitory concentrations (MIC) of amikacin against Gram-negative isolates obtained from blood cultures collected from equine neonates. Mean half-life, clearance, and volume of distribution (Vd) after administration of a single intravenous bolus of amikacin (25 mg/kg) to 5 healthy 2- to 3-day-old foals were 5.08 h, 1.76 ml/min/kg, and

0.773 L/kg, respectively. There was a statistically significant decrease in area under the curve, mean residence time, and trough plasma amikacin concentrations between 2- to 3-day-old foals and the same foals at 10- to 11 days of age. Forty-nine hospitalized foals were studied. Eight hospitalized foals were premature, 10 were hypoxicemic, 28 were septic, and 19 did not fall within any of these categories. Amikacin concentrations were measured 0.5 h (peak) and 24 h (trough) post-administration. The median age at time of measurement of amikacin peak and trough concentrations was 3 days (range 1-15 days). The median duration of amikacin therapy was 6 days (range 3-23 days). The median dose of amikacin administered was 23.5 mg/kg (range 16.7-28.0 mg/kg). There was a positive correlation between amikacin dose and peak amikacin concentration as well as between amikacin dose and Vd. There was no correlation between other pharmacokinetic variables and age, dose of amikacin, creatinine concentration, BUN, sepsis score, or dose number. Hospitalized foals had significantly lower peak plasma amikacin concentrations and lower volume of distribution than healthy foals. Sepsis, prematurity, and hypoxemia did not alter the disposition of once-daily amikacin. MIC at which 90% of all Gram-negative isolates from equine neonatal blood cultures were inhibited by amikacin was 4 µg/ml, suggesting that peak amikacin concentrations of 40 µg/ml should be targeted to achieve recommended peak/MIC ratio of 10:1. The proportion of foals with peak serum concentration  $\geq 40$  µg/ml was significantly higher in foals receiving a dose  $> 23.5$  mg/kg (median dose: 25 mg/kg; 22/24 or 92%) than in foals receiving a dose  $< 23.5$  mg/kg (median dose 21 mg/kg; 9/25 or 36%) while there was no difference in the proportion of foals with trough concentrations  $\geq 3$  µg/ml between the 2 groups. Based on the results of this study, an initial dose of 25 mg/kg would be recommended for once-daily amikacin in sick equine neonates. Therapeutic drug monitoring should be performed to allow individual adjustment of dose and dosing interval.

### ABSTRACT #279

THE PHARMACOKINETICS OF ITRACONAZOLE IN THE HORSE. Jennifer L. Davis, Brian C. Gilger, Mark G. Papich. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Itraconazole is an oral triazole antifungal drug used for the treatment of infections caused by *Aspergillus* sp., *Histoplasma* sp., and *Blastomyces* sp. in humans and animals. Itraconazole is extremely lipophilic and has a very high affinity for tissues, including lung, kidney, brain, skin and esophageal tissue, with concentrations often higher in the tissue than in plasma. Fungal infections can be difficult to treat and often have devastating consequences. Very few systemic antifungal drugs have been studied in the horse, so treatment options are limited. Itraconazole at a dose of 3 mg/kg orally, twice a day has been recommended for the treatment of fungal diseases in horses, but no pharmacokinetic data is available. The purpose of this study is to test the hypothesis that itraconazole will be absorbed after oral administration in horses and exhibit a long half-life to allow for intermittent dosing to produce therapeutic concentrations for the treatment of fungal diseases.

Horses were given itraconazole capsules (Sporanox, Janssen Pharmaceuticals, Inc.) by mouth at 3 mg/kg twice a day for 5 days, or 5 mg/kg once. Plasma samples were collected at 0, 30, and 60 minutes and 2, 4, 8, 12, and 24 hours following the last dose administered. Concentrations of itraconazole and its active metabolite, hydroxy-itraconazole, in the plasma were determined by reverse-phase high performance liquid chromatography (HPLC) with solid-phase extraction using an assay developed in our laboratory. The assay was linear for itraconazole and hydroxy-itraconazole at concentrations between 0.02 and 10 µg/mL in plasma. Plasma protein binding of itraconazole and hydroxy-itraconazole was determined using an *in vitro* microcentrifugation technique



(Centrifree™ Micropartition system, Amicon). Pharmacokinetic parameters were determined using a one-compartment model (WinNonlin, Version 4.0, Pharsight).

Following multiple oral doses of 3 mg/kg, itraconazole exhibited a slow absorption phase ( $T_{max} = 2.6$  hr), a maximum plasma concentration of 0.16  $\mu\text{g/mL}$ , and a long elimination half-life ( $T_{1/2} = 32$  hr). Following a single oral dose of 5 mg/kg, itraconazole showed similar pharmacokinetic properties. The metabolite, hydroxy-itraconazole, was not detected in any plasma samples. Plasma protein binding of itraconazole and hydroxy-itraconazole were >99% and >93%, respectively, which may have implications regarding clearance and tissue distribution. No adverse effects were noted following administration of the drug.

The results of this study indicate that itraconazole is absorbed following oral administration in the horse. Furthermore, its pharmacokinetic properties, such as a long half-life may make it possible to use intermittent pulse dosing, which will make treatment convenient and affordable. This drug is safe for use in horses at a dose of 5 mg/kg orally, once daily, with no detectable side effects.

#### ABSTRACT #280

POPULATION PHARMACOKINETICS OF MARBOFLOXACIN IN HORSES. M. Peyrou\*, M.Y. Doucet\*, A.Vrins\*, M. Schneider<sup>+</sup>, D.Concordet<sup>§</sup>, A.Bousquet-Melou<sup>§</sup>. \*: Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada. +: Vétouquinol, Lure, France. §: Ecole Nationale Vétérinaire de Toulouse, Toulouse, France.

Population pharmacokinetics of marbofloxacin were investigated on 21 healthy and 16 diseased horses to assess interindividual variability of drug exposure. Demographic, physiologic and disease covariables were tested using mixed effects models. As a preliminary analysis, this study has demonstrated that none of the tested covariables were significant in regression models of compartmental volumes or distribution clearance, but the clinical status of the horse (healthy/diseased) was a significant covariable ( $p < 0.01$ ) for systemic clearance. Clearance had a lower mean and a higher variance for diseased horses than healthy horses, with respectively a mean of 0.209 and 0.284 L/h/kg and a coefficient of variation of 52% and 15%. Consequently, variability of AUC was greater in diseased horses. Considering an AUC/MIC ratio below 60h as a prediction of poor efficacy, a dosage regimen of 2 mg/kg intravenous was deemed to be inadequate for 19% of diseased horses if the MIC of the bacteria was 0.1  $\mu\text{g/mL}$  (sensitivity breakpoint). However 93% of diseased horses could achieve a ratio above 125h, predicting a very good efficacy, for the  $MIC_{90}$  of Enterobacteriaceae (0.027  $\mu\text{g/mL}$ ). These data, which should be validated with a larger group of horses, suggest that the use of marbofloxacin is reliable for treatment of Enterobacteriaceae-related diseases in horses with a 2mg/kg dosage regimen.

#### ABSTRACT #281

IN VITRO DISTRIBUTION AND ELIMINATION OF 20% PHENYLBUTAZONE SOLUTION ADMINISTERED ORALLY TO FASTED HORSES. Watson DM, Walesby HA, Barker SA, Short CR. Louisiana State University, LA.

The pharmacokinetics of phenylbutazone (20%) solution administered orally have not been described. Oral use of PBZ solution allows for exact dosage of horses weighing less than 150 kg. Increased accuracy decreases risk for over-dosage and toxicosis that exists with common oral formulations such as paste, powder, gel, or tablet. We hypothesized that oral administration of the same PBZ dose to fasted horses would yield statistically equivalent  $C_{max}$ ,  $T_{max}$ ,  $T_{1/2}$ , and AUC values for both formulations. Horses ( $n=6$ ) were weighed, fasted, and orally dosed with solution or paste (control) at 2.2 mg/kg BWT. Blood was collected at time 0, 0.5, 2, 4,

8, 12, and 24 hours post-administration (HPA). After a 14 day washout period, horses were fasted, dosed with the opposite formulation, and sampled as described. Serum samples underwent solid-phase acid extraction and quantification by high performance liquid chromatography.  $C_{max}$  values for paste (7.35  $\mu\text{g/mL}$ ) differed significantly ( $P < 0.05$ ) from injectable (5.65  $\mu\text{g/mL}$ ).  $T_{max}$  values for paste (4.97 HPA) and injectable (3.03 HPA) did not differ significantly. AUC for paste (101.75) was 30% greater than AUC for injectable (67.09).  $T_{1/2}$  for both formulations occurred at 12 HPA. The results indicate that oral absorption of both PBZ formulations is highly variable when compared to the literature. Oral administration of PBZ (20%) solution is an accurate route of administration for horses weighing less than 150 kg; more work needs to be done to determine the dose that will yield  $C_{max}$  and AUC values equal to PBZ paste.

#### ABSTRACT #282

PRE-EXERCISE INDUCTION OF METABOLIC ALKALOSIS IN THOROUGHBRED HORSES DOES NOT AFFECT EXERCISE-INDUCED ARTERIAL HYPOXEMIA BUT DECREASES DESATURATION OF ARTERIAL HEMOGLOBIN. T. E. Goetz, M. Manohar, and A. S. Hassan. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Prior work reported that  $\text{NaHCO}_3$  administration exaggerates exercise-induced arterial hypoxemia during submaximal and maximal exertion of racehorses (*Equine Veterinary Journal* 25:125-129, 1993). However, these studies did not quantify the effect of alkalosis on arterial hemoglobin- $\text{O}_2$  saturation. These observations are in sharp contrast with findings in human subjects exercising after administration of  $\text{NaHCO}_3$ , wherein exercise-induced arterial hypoxemia is largely unaffected, but the associated alkalosis helps limit the desaturation of arterial hemoglobin. An exaggeration of exercise-induced arterial hypoxemia following  $\text{NaHCO}_3$  administration to horses may adversely affect  $\text{O}_2$  supply, and in turn, athletic performance.

Thus, the present study reexamined the effects of pre-exercise induction of metabolic alkalosis with  $\text{NaHCO}_3$  on arterial oxygenation in racehorses performing short-term high-intensity exercise. Two sets of experiments, namely placebo (intravenous [IV] physiological saline) and IV  $\text{NaHCO}_3$  (250 mg/kg), were carried out on 13 healthy, sound Thoroughbred horses in random order, 7 days apart. Blood-gas/acid-base variables were examined at rest and during incremental exercise leading to 120 s of galloping at 14 m/s on a 3.5% uphill grade, which elicited maximal heart rate and induced pulmonary hemorrhage in all horses in both treatments.

$\text{NaHCO}_3$  administration caused significant alkalosis and hemodilution in standing horses, but arterial  $\text{O}_2$  tension and hemoglobin- $\text{O}_2$  saturation were unaffected. Thus,  $\text{NaHCO}_3$  administration caused a significant reduction in arterial  $\text{O}_2$  content at rest, although the arterial to mixed-venous blood  $\text{O}_2$  content gradient was unaffected. During maximal exercise in both treatments, significant arterial hypoxemia, desaturation of hemoglobin, hypercapnia, acidosis, hyperthermia and hemoconcentration developed. Although the extent of exercise-induced arterial hypoxemia was similar, there was a significant attenuation of the desaturation of arterial hemoglobin in the  $\text{NaHCO}_3$  treated horses, which had significantly higher arterial pH. Despite these observations, the arterial  $\text{O}_2$  content of exercising horses was significantly less in the  $\text{NaHCO}_3$  experiments because of the hemodilution, and a significant attenuation of the exercise-induced expansion of the arterial to mixed-venous blood  $\text{O}_2$  content gradient was observed.

It was concluded that pre-exercise  $\text{NaHCO}_3$  administration does not affect the development and/or severity of arterial hypoxemia in Thoroughbreds performing short-term high-intensity exercise.

### ABSTRACT #283

THE USE OF NEUTROPHIL-CHEMOATTRACTANT ACTIVITY IN BRONCHOALVEOLAR LAVAGE FLUID (BALF) AS A QUANTITATIVE PARAMETER TO DISTINGUISH RECURRENT AIRWAY OBSTRUCTION (RAO) HORSES FROM NORMAL HORSES. Dana Hoyt, Clarissa Zuver, Jean A. Hall, John W. Schlipf, Jr. Oregon State University, College of Veterinary Medicine, Corvallis, Oregon.

RAO, also known as heaves is one of the most common conditions affecting the equine lung and ultimately performance. Differentiating RAO, inflammatory airway disease (IAD), and normal horses can be challenging based on clinical signs and routine laboratory data. Percent neutrophils (PMNs) in BALF have been the gold standard used to differentiate (RAO) horses, normal horses and horses with (IAD). Some horses do not fit clearly into a category based strictly on percent PMNs and clinical signs. Additional objective laboratory data is needed to improve diagnostic accuracy and help assess treatment response.

Twenty adult horses (4 to >20 years) were enrolled in this study based on owner history and physical examination findings. Each horse was sedated with xylazine and a bronchoalveolar lavage tube was passed until it gently lodged in a small airway. Warm physiologic saline (100 ml) was infused and immediately aspirated; this was repeated a total of three times. An aliquot of the BALF was used for differential cell count. The remaining BALF was centrifuged and aliquots of the supernatant frozen for later analysis.

Chemoattractant activity was determined using a modified Boyden chamber assay. A 96 well plate was overlaid with a polycarbonate filter (5µm pore size). BALF with suspected chemoattractant activity was placed in the bottom wells, and equine neutrophils were placed on top of the filter. Cells that migrated through the filter into the bottom wells were pelleted by centrifugation and quantified by measurement of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenol tetrazolium bromide (MTT). The number of migrating cells corresponded to the amount of MTT reduced, which was measured with an ELISA plate reader.

Based on history, physical examination, and BALF cytology normal horses were categorized as having no clinical signs or history consistent with RAO and <10% PMNs in BALF. RAO horses had clinical signs and a history consistent with airway disease and >20% PMNs in BALF. Horses with IAD had either no clinical signs, but BALF with 10-20% PMNs, or clinical signs consistent with airway disease and <20% PMNs in BALF. The chemoattractant activity of BALF differed between RAO vs. normal (P=0.0003) and normal vs. IAD (P=0.08) horses.

Three populations of horses (normal, RAO, and IAD) were distinguished based on clinical signs and percent of neutrophils in BALF. BALF chemoattractant activity was greatest in horses with RAO and IAD.

BALF chemoattractant activity may be beneficial in differentiating RAO and IAD horses with minimal to no clinical signs and equivocal BALF cytological changes from normal horses.

### ABSTRACT #284

EFFECT OF A LUNG-DIRECTED TYPE V PHOSPHODIESTERASE INHIBITOR AND NITRIC OXIDE ON EXERCISE-INDUCED PULMONARY HEMORRHAGE IN THOROUGHBRED RACEHORSES. MM Durando, BJ Perry, B Murray, C Miller, and EK Birks. University of Pennsylvania, Kennett Square, PA.

This clinical trial examined the effects of a lung-directed treatment regime on exercise-induced pulmonary hemorrhage (EIPH) in Thoroughbred racehorses. EIPH is observed in the majority of racehorses, and is thought to contribute to decreased athletic performance. Studies have shown that one of the major factors in the development of EIPH is very high pulmonary vascular pressures that

occur during intense exercise. We have shown that this treatment combination (type V phosphodiesterase inhibitor, E4021, followed by inhaled nitric oxide) reduces both pulmonary artery pressures and EIPH in Thoroughbred horses during high-speed treadmill exercise. The purpose of this study was to examine the effect that this treatment may have on EIPH in horses in their normal training environment.

Sixteen horses actively racing at the Macau Jockey Club were enrolled, with 13 horses completing this study. These horses received treatment or placebo, in 2 separate trials, 2 weeks apart, in a randomized, crossover, double-blind design. They were administered either E4021 (6 mg) or vehicle IV, followed in 30 minutes by inhaled nitric oxide (NO) (100 breaths of 5000 ppm NO during inhalation only, giving 80 ppm peak NO) or room air (100 breaths). Horses then performed a near maximum intensity workout 30-60 minutes following treatment. Endoscopic examination for visual scoring of blood and tracheal washes for quantification of erythrocytes was conducted 45-60 minutes after exercise. Video recording of the exam to the level of the carina was performed, and 3 veterinary clinicians blinded to treatments subjectively evaluated the videotapes for the amount of blood observed in the airways on a scale from 0-4 as previously described. Erythrocytes were enumerated using standard hemocytometric techniques. Results were analyzed by ANOVA with significance  $p < 0.05$ . Values given are means  $\pm$  SEM.

Although there was no significant difference between combined endoscopic scores from all horses during the first or second trial (1.77 $\pm$ 0.35 vs 1.88 $\pm$ 0.28, respectively), treatment (E4021+NO) significantly decreased visual bleeding score vs placebo (1.04 $\pm$ 0.23 vs 2.62 $\pm$ 0.22). E4021+NO treatment also significantly decreased the number of erythrocytes recovered in tracheal wash fluid vs placebo (900 $\pm$ 654/ $\mu$ l vs 8154 $\pm$ 1753/ $\mu$ l, respectively). As with the visual scores, there was no significant difference in combined erythrocyte recoveries between the first and second trials (3887 $\pm$ 1806/ $\mu$ l vs 5223 $\pm$ 2042/ $\mu$ l, respectively), suggesting that the elapsed time between trials had no influence on the amount of hemorrhage. These data suggest that the lung-directed treatment, E4021+NO, decreases the severity of EIPH in horses exercising maximally at the racetrack.

### ABSTRACT #285

THE IN VITRO CONTRACTILE RESPONSE OF NON-GRAVID EQUINE CIRCULAR AND LONGITUDINAL MYOMETRIAL SMOOTH MUSCLE FROM THE UTERINE HORN TO ENDOTHELIN-1. Walesby HA, Venugopal CS, Hosgood G, Eades SC, Moore RM. Louisiana State University, SVM, Baton Rouge, LA.

This study was performed to characterize the in vitro response of circular (CH) and longitudinal (LH) myometrial layers of the equine uterine horn to endothelin-1 (ET-1) and compare the effectiveness of ET<sub>A</sub> (BQ-123) and ET<sub>B</sub> (IRL-1038) receptor antagonists to inhibit ET-1 induced myometrial response. Muscle strips from CH and LH of ten non-gravid female horses were suspended in tissue baths and connected to force-displacement transducers interfaced with a physiograph. The strips were incubated for 45-minutes with no antagonist (control), BQ-123, IRL-1038, or BQ-123 + IRL-1038 at 3 different concentrations (10<sup>-9</sup>, 10<sup>-7</sup>, and 10<sup>-5</sup>M) before concentration-response curves to ET-1 were generated. The contractile response to cumulative concentrations of ET-1 (10<sup>-9</sup> to 10<sup>-6</sup>M) was quantified by measuring change in area under the curve (AUC) for the 3-minute period following each ET-1 dose. The EC<sub>50</sub> of ET-1 for the control, BQ-123, IRL-1038, and BQ-123 + IRL-1038 were determined for CH and LH at each blocker concentration. Endothelin-1 caused concentration-dependent contraction of CH and LH. Response to BQ-123 and IRL-1038 supports the existence of ET<sub>A</sub> and ET<sub>B</sub> receptors within the equine myometrium. The combined response to BQ-123 + IRL-1038 suggests the presence of another receptor or receptor subtype. These findings support a receptor-mediated physiologic role for ET-1 in uterine contractility.

## ABSTRACT #286

PHARMACOKINETICS OF OMEPRAZOLE IN *CAMELUS DROMEDARIUS*. F. Van-Gool<sup>1</sup>, L. Sun<sup>2</sup>, M. Alvinerie<sup>3</sup>, M. Murray<sup>4</sup>. <sup>1</sup> Merial Limited, Lyon, France; <sup>2</sup> Merial Limited, North Brunswick, NJ, USA; <sup>3</sup> Laboratoire de Pharmacologie-Toxicologie, Toulouse, France; <sup>4</sup> Merial Limited, Duluth, GA, USA.

The camelid "stomach" consists of 3 compartments, the first of which (C1) holds the largest volume and is responsible for primary fermentation of cellulose. The third compartment (C3) is a tubular structure which has a parietal component that secretes hydrochloric acid. Ulceration of the C3 compartment has been reported in several species of Camelidae. Pathogenesis is not completely understood, but mucosal injury from hydrochloric acid is considered to have a role. It has been suggested that clinical signs improved after orally-administered omeprazole, but absorption of drug after oral administration of the veterinary product Gastrogard<sup>®</sup> has not been demonstrated in camelids. The pharmacokinetics of omeprazole after oral administration of Gastrogard<sup>®</sup> to dromedaries at a dose of 4 mg/kg were characterized in this study. Five adult dromedaries were used. Animals were fed 3 kg of wheat straw, 2 kg of alfalfa hay, and 2 kg barley, then feed was withheld 19 hours before Gastrogard<sup>®</sup> was given. Blood samples were taken at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 10 hours after administration. Omeprazole in plasma was measured by high-pressure liquid chromatography. Omeprazole appeared in plasma within 30 minutes. Elimination half-life ( $t_{1/2}$ ) was  $1.8 \pm 0.5$  hours. Two peaks were present in the plasma concentration-time profiles for each of the five animals ( $T_{max1} = 0.7$  hr,  $T_{max2} = 4.0$  hr;  $C_{max1} = 60.6 \pm 25.7$  ng/ml,  $C_{max2} = 40.5 \pm 21.3$  ng/ml). The fraction of omeprazole that experienced delayed (or secondary) absorption was 57%. These results demonstrate that orally administered omeprazole in the approved product Gastrogard<sup>®</sup> is absorbed in the stomach of *Camelus Dromedarius*. On the basis of the plasma profile, we speculate that initial omeprazole absorption occurs in C1 and that the remainder of absorption is in C3 or the small intestine.

## ABSTRACT #287

CLINICAL ASPECTS AND MORTALITY AND PREVALENCE RATES OF THE HEPATOGENOUS PHOTOSENSITIZATION IN CATTLE GRAZING *Brachiaria decumbens* PASTURES, IN BRAZIL. JJ Fagliari, HT Okuda, M Passipieri, GT Pereira. College of Veterinary Medicine, Sao Paulo State University, Jaboticabal Campus, Brazil.

Bovine hepatogenous photosensitization has been related in Brazil since 1975. Hepatotoxic saponins and/or sporidesmin, mycotoxin present in *Pithomyces chartarum* spores, have been incriminated as primary causes of the disease. This study aimed to determine clinical characteristics and mortality and prevalence rates of the hepatogenous photosensitization in cattle grazing lush *Brachiaria decumbens* pastures. Two hundred-eighty weaned calves grazing lush *B. decumbens* naturally contaminated with at least 50,000 *Pithomyces chartarum* spores/gram, amount sufficient to induce bovine photosensitization, were evaluated. Sick animals were apart from the group and placed into *B. decumbens* pasture free of *P. chartarum* spore. Then, they were submitted to experimental protocol. Clinical photosensitization was classified as moderate or severe form. Animals with moderate photosensitization exhibited mild skin lesions which healed within 30 days following the onset of the disease. Animals with severe photosensitization had generalized skin lesions and died 18 to 47 days after initial symptoms. Animals that had no skin lesion but showed serum gamma-glutamyltransferase (GGT) activity increased ( $>60$  U/L) were submitted to liver biopsy and if the histologic evaluation showed cholangitis, they were referred as subclinical photosensitization animals. This information allowed to form 4 groups: healthy bovine (Group [G] 1); subclinical photosensitization (G2); moderate photosensitization (G3); and severe photosensitization (G4). Any time since the onset of the

disease or at day 90<sup>th</sup> following the beginning of *B. decumbens* grazing if animal had no photosensitization, was performed liver biopsy for histology.

Results indicate that 181 (64,64%) showed symptoms of photosensitization. Of all sick bovines, 151 (83,42%) survived to the disease and 30 (16,58%) died. Death occurred 18 to 47 days after onset of clinical signs. Eighty-six (47,52%) animals developed subclinical disease, 65 (35,91%) had the moderate clinical form, and 30 (16,58%) presented severe photosensitization. Seventy-one (39,22%) became sick in the first 30 days following the *B. decumbens* grazing, 63 (34,80%) showed photosensitization within 31 to 60 days, and 47 (25,96%) between 61 to 90 days.

In conclusion, the high prevalence and mortality rates of photosensitization verified in weaned calves suggest that lush *B. decumbens* pasture must be avoid just after weaning. Initially, these pastures could be used by adult animals, which are less prone to the development of the photosensitization. It is important a good management of *B. decumbens* pastures to avoid debris excess on the soil, which is necessary for the development of *Pithomyces chartarum*.

## ABSTRACT #288

LONG-TERM TRILOSTANE TREATMENT FOR EQUINE METABOLIC SYNDROME. C.M. McGowan, Schools of Animal Studies and Veterinary Science, University of Queensland, Gatton, QLD, Australia.

Equine metabolic syndrome (EMS) is a condition of hypercortisolaemia and insulin resistance. The purpose of this study was to determine clinical, biochemical and endocrinological effects of trilostane, a 3-beta-hydroxysteroid dehydrogenase inhibitor, in EMS horses treated over 12-18 months. Blood samples were obtained at 4 hourly intervals over 24 hours for measurement of blood [glucose], serum [insulin] and serum [cortisol] at Time 0, prior to treatment, Time 1, 1-6 months and Time 2, 12-18 months after commencing treatment with trilostane. Results were analysed using 2-way ANOVA and T-tests.

21 horses (14  $\pm$  5 years) comprising 14 Welsh ponies or crosses, 5 Shetland ponies and 2 cobs were included. Mean estimated body weight was 300  $\pm$  96 kg; 9 were mares and 12 geldings. All horses presented for recurrent or chronic laminitis despite good management and hyperinsulinaemia (mean equivalent 94.4, 95%CI 70-128 uIU/L). Basal cortisol was elevated in 20/21 (mean 187  $\pm$  8.69 nmol/L) and hyperglycaemia was present in 6/14 horses. Equine Cushing's syndrome was excluded by lack of hirsutism (21/21), negative dexamethasone suppression test (DST) (15/21), combined DST and thyrotropin releasing hormone (TRH) stimulation (6/21), both DST and DST/TRH (3/21) or basal ACTH (3/21). Absence of pituitary pathology was confirmed by post mortem examination in 3 horses. Trilostane therapy was initiated at 0.9  $\pm$  0.3 mg/kg, but increased during the trial to 1.2  $\pm$  0.7 mg/kg.

All horses started the trial with laminitis as the major clinical sign, 14 (67%) responding to the medication being clinically sound and available for final follow up at Time 2. Of these, 4 horses had recurrent bouts of laminitis throughout the trial and were considered partially responsive. 7 horses did not respond long term. 1 horse was euthanased due to liver disease and continued laminitis 2 months after starting the trial, a second responded for only 1 month, developed a severe bout of laminitis and was euthanased 2 months after starting. 5 horses responded initially for a period of 4-5 months and of these 2 withdrew and 3 were euthanased due to recurrent laminitis and perceived lack of response.

Following trilostane therapy, there was no change in serum [insulin], although horses that failed to respond clinically showed a trend for an increased serum [insulin] compared to ones that did (P=0.07). Serum [cortisol] decreased from Time 0 (187  $\pm$  8.69 nmol/L) to Time 1 (151  $\pm$  10.3 nmol/L) (P=0.01), but did not

decrease further at Time 2 ( $175.5 \pm 13.6$  nmol/L). Serum [glucose] was significantly higher at Time 2 ( $6.01 \pm 0.30$  mmol/L) than Time 0 ( $5.02 \pm 0.21$  mmol/L) or Time 1 ( $4.85 \pm 0.22$  mmol/L) ( $P < 0.02$ ).

Following long-term trilostane treatment, 67% horses showed reduced signs of laminitis and there was a significant reduction in serum [cortisol]. Further research is needed to study the relationships between hypercortisolaemia and insulin resistance in horses with EMS.