ABSTRACT #1

SEQUENCING AND CHARACTERIZATION OF THE CANINE TELOMERASE REVERSE TRANSCRIPTASE (TERT) PROMOTER. S.N. Long, E. Gault, L. Nasir, Institute for Comparative Medicine, University of Glasgow Vet School, Glasgow, Scotland, UK.

Telomeres are specialized DNA-protein complexes that cap the ends of linear chromosomes and protect them from degradation and end to end fusions. Due to the "end replication problem," telomeres undergo progressive shortening with each cell division. Shortening to a critical length triggers cell growth arrest and cellular senescence pathways, thereby acting as a means for regulating cellular lifespan. The enzyme telomerase is a ribonucleoprotein complex that adds nucleotides to the 3' end of telomeres, thus maintaining telomere length and bypassing cellular senescence. Telomerase activity is absent from most adult somatic cells, with expression limited to activated lymphocytes, germ cells and stem cells. In contrast, up to 90% of cancers possess telomerase activity, suggesting that telomerase activity within cells represents the acquisition of an immortal phenotype. Telomerase is composed of an RNA component, TR, a reverse transcriptase catalytic subunit, TERT, and associated proteins. Whilst TR is present in some somatic cells, the finding that TERT is only present in those cells possessing telomerase activity and that telomerase activity can be induced in telomerase-negative cells through the addition of TERT alone suggests that telomerase activity is regulated primarily through regulation of the TERT catalytic subunit. Our group has recently sequenced and characterized the canine TERT gene and identified approximately 5Kb of the upstream regulatory region. The purpose of this study was to sequence the promoter of the canine TERT gene and to identify the core region of the promoter essential for activity.

PCR amplification and cloning of selected regions of the canine TERT promoter followed by luciferase assays revealed that core promoter activity is contained within a region extending approximately 300bp upstream of the ATG codon. Transient transfections in telomerase-positive canine cell lines and telomerase negative fibroblasts showed that the promoter is only active in telomerase positive cell lines. Sequence analysis demonstrated that the 5' regulatory region is GC-rich and contains no TATA or CAAT box, similar to the human TERT promoter. Motif searches revealed the presence of multiple transcription factor binding sites common to both the human and canine TERT promoters, including a single E-box, Sp1, AP1, MZF-2 and ER/Sp1 binding sites. These findings suggest that the canine TERT gene shares similar transcriptional control to the human TERT gene. Identifying the core promoter necessary for activity will enable the development of telomerase-targeted therapies in canine cancer patients similar to those investigated in human patients.

ABSTRACT #2

N-ACETYLCYSTEINE DECREASES VASCULAR ENDOTHELIAL GROWTH FACTOR PRODUCTION IN CANINE HEMANGIOSARCOMA. Douglas H. Thamm, Ann M. Mitzey, Ilene D. Kurzman, David M. Vail. The Animal Cancer Center, Colorado State University (DHT, DMV) and Department of Medical Sciences, University of Wisconsin-Madison (AMM, IDK)

Canine hemangiosarcoma (HSA) is a common disease, with very aggressive biologic behavior and short survival times with standard treatments. Novel therapies are desperately needed. As a tumor derived from vascular endothelium, antiangiogenic therapies may be uniquely efficacious for HSA. Our group and others have demonstrated the abundant expression of vascular endothelial growth factor (VEGF) and other angiogenic growth factors, as well as the receptors for these growth factors, in canine HSA cells, suggesting the possibility of autocrine signaling through these growth factor receptors.

N-acetylcysteine (NAC) is a potent free radical scavenger that possesses multiple antineoplastic activities in vitro and in murine models, including inhibition of tumor and endothelial cell migration and invasion, matrix metalloproteinase activity, and VEGF production. We hypothesized that NAC would be capable of downregulating VEGF production under both normoxic and hypoxic conditions, and that this might be accompanied by decreased cell proliferation and survival as a result of attenuation of putative autocrine signaling through the VEGF receptor.

Two canine HSA cell lines were cultured under normoxic conditions, or cobalt chloride ($CoCl_2$) was added to simulate hypoxia, and varying concentrations of NAC were added. Cell supernatants and lysates were then collected and assayed for VEGF concentration using a commercially available ELISA kit. In separate experiments, HSA cells were incubated with varying concentrations of NAC and/or doxorubicin for 72 hours, followed by determination of relative viable cell number using a commercially available ATP-based kit.

One of two cell lines tested responded to simulated hypoxia with stimulation of VEGF production, suggesting that dysregulation of the hypoxia response pathways could be implicated in the pathogenesis of the disease. NAC reduced VEGF production in a dose-dependent fashion, however a higher concentration of NAC was required to inhibit $CoCl_2$-stimulated VEGF production. NAC inhibited HSA proliferation and enhanced chemosensitivity in a dose-dependent fashion, however these effects were seen only at suprapharmacologic doses.

These results provide proof of principle that redox balance is an important mediator of VEGF production in canine HSA cells, and imply that autocrine stimulation through a VEGF-VEGF receptor loop could contribute to HSA pathogenesis. It is possible that more potent antioxidants could prove clinically useful for the treatment of canine HSA in the future.

ABSTRACT #3

RISK OF OSTEOSARCOMA IN RETIRED RACING GREYHOUNDS. Cynda Crawford, Julie Rosenberger, Norma Pablo. University of Florida, Gainesville, FL.

Osteosarcoma (OSA) is the most common primary bone tumor diagnosed in dogs. Previous studies have determined that appendicular OSA is the most frequent form with a site predilection for metaphyseal regions of long bones, particularly the forelimbs. Several studies have identified several risk factors for OSA, including breed, age, height, sex, and neuter status. For the past decade, increasing numbers of retired racing Greyhounds have entered the pet population. Simultaneously, increased numbers of Greyhounds have been diagnosed with OSA, but there are no reports documenting their risk for this malignancy. The purpose of this study was to determine the risk of OSA in Greyhounds compared to other breeds, and to evaluate the association of host factors with this risk.

In a retrospective case-control study, the medical records of all dogs diagnosed with OSA at the Veterinary Medical Teaching Hospital (VMTH) at the University of Florida from January 1996 to December 2004 were examined. Only dogs with OSA confirmed by histopathology were included in the case group. Potential risk factors recorded for each case included breed, age, sex, neuter status, and location of the OSA lesion. Prevalence for each breed was determined as the number of cases with OSA relative to the total number of the same breed seen during the same time period. Crude odds ratio (OR) and 95% confidence limits (CL) for risk in individual breeds were calculated by comparing each breed with mixed breeds, arbitrarily selected as the reference group. The risk for OSA was estimated only for breeds with 10 or more OSA cases. Unadjusted crude OR and 95% CL were estimated for age, sex, neuter status, and location of the primary lesion for the three breeds with the highest risk for OSA. P values < 0.05 were considered significant.

The total number of confirmed OSA cases diagnosed at the UF VMTH from 1996 to 2004 was 172. The Greyhound had the highest breed prevalence of OSA (6.2%), followed by the Rottweiler (6.0%), Irish Wolfhound (5.7%), Irish Setter (5.7%), and Great Dane (5.2%). Compared to mixed breed dogs, the Greyhound had the highest risk for OSA (OR=10.5), followed by the Rottweiler (OR=10.1), Great Dane (OR=8.8), Doberman Pinscher (OR=3.7), Golden Retriever (OR=2.6), and Labrador Retriever (OR=2.2). Potential risk factors for OSA in the Greyhound were compared to those for the Rottweiler and Great Dane. Dogs seven to 10 years of age had the highest risk for all three breeds. There were no sex-related differences for the Greyhound or Great Dane, but spayed female Rottweilers had higher risk than sexually intact females. In all three breeds the appendicular skeleton was more likely to be affected than the axial skeleton, and forelimbs were affected as often as hindlimbs.

Greyhounds have a high risk of appendicular OSA, similar to other large and giant breed dogs.

ABSTRACT #4

A PROSPECTIVE STUDY OF UNFRACTIONATED HEPARIN THERAPY TO PREVENT THROMBOSIS IN CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA. EL Breuhl[1], C Scott-Moncrieff[1], M Brooks[2]. [1]Purdue University, West Lafayette, IN and [2]Cornell University, Ithaca, NY.

Thromboembolic (TE) events are an important cause of mortality in dogs with immune-mediated hemolytic anemia (IMHA). Unfractionated heparin (UFH) is often given empirically to canine IMHA patients, However, few clinical studies have evaluated the efficacy of UFH for preventing thrombosis in this population. The objectives of this study were to develop a dosage regimen of UFH for canine IMHA based on attainment of a predetermined in vitro anticoagulant activity, and to determine whether this protocol improved patient survival. IMHA dogs were prospectively enrolled in the study and their survival was compared to historical controls. A target heparin Factor Xa inhibitory range of 0.35-0.7 anti-Xa U/mL was chosen based on human heparin efficacy studies. An initial UFH dose of 300 IU/kg SQ every six hours was administered. Fifteen dogs with primary IMHA were enrolled in the study. Plasma anti-Xa activity was measured at 0, 4, 16, 28, and 40 hours after initiation of UFH therapy. No dogs had activity in the target range (TR) at 4-hour, five dogs attained TR values at 16 and 28 hours, and four dogs had TR values at 40-hours. One dog died prior to the 40-hour sample. The dose of UFH was increased by 17%-25% in five dogs having values below TR at the 40-hour sample. Two of the five dogs subsequently attained TR values after the increase in their heparin dose. The mean plasma anti-Xa activity was 0.2 U/mL at the 40-hour sample (n=14). The mean UFH dose was 318 IU/kg at the time dogs (n= 8) attained TR values. Four dogs were tapered from heparin based on improvement in anemia and resolving inflammatory leukogram, and one dog died prior to a dose adjustment. The highest heparin dose any dog received was 375 IU/kg SQ every six hours. The highest measured plasma anti-Xa activity was 1.1 U/mL. No complications due to hemorrhage occurred. Necropsies were performed in three of four dogs that died. Thrombi were identified in two of the dogs. One dog had an iliac thrombus and one had micro thrombi noted in alveolar capillaries. The last measured anti-Xa activity in these dogs was 0.33 U/mL (16-hr) and 0.4 U/mL (40-hr) respectively. Thirteen of the 15 dogs survived to be discharged and 11 dogs were alive one month after diagnosis. Patient survival to discharge (P=0.37) and one-month survival (P=0.43) were not statistically significantly different from control data. However, the power of the study to detect a difference was low.

The results of this study indicate that administration of 300 IU/kg UFH q. 6 hr is generally inadequate to attain a target anti-Xa activity > 0.35 U/mL in IMHA dogs. Moreover, a target range of 0.35 to 0.7 U/ml anti-Xa activity may not provide adequate anticoagulant effect to ameliorate the thrombotic tendency in these dogs. Larger treatment trials are needed to determine whether UFH or low molecular weight heparins, given in high dosages, can be effective as the sole anticoagulant agent to reduce morbidity and mortality due to thrombosis in canine IMHA.

ABSTRACT #5

EFFECT OF HEPARIN USE ON SURVIVAL TO DISCHARGE OF DOGS WITH IMMUNE-MEDIATED HEMOLYTIC ANEMIA. Jennifer S. Fryer, Maureen A. McMichael, Margaret R. Slater. Department of Veterinary Small Animal Clinical Sciences, Texas A&M University (TAMU), College Station, TX.

Thrombotic complications are common causes of morbidity and mortality in patients with immune-mediated hemolytic anemia (IMHA). Heparin is often used to prevent or treat thrombotic complications in such patients by amplifying the activity of the remaining anti-thrombin III (ATIII) in circulation. When endogenous heparan sulfate (HS) lining normal endothelium binds with ATIII, anti-inflammatory mediators are released, providing additional benefit to patients with thrombotic disease due to inflammation. However, when exogenous heparin is used to increase ATIII activity, endogenous HS lining the endothelium cannot provide this anti-inflammatory activity. It is surmised that the low activity of ATIII in IMHA is due to a decrease in ATIII, not endogenous HS. IMHA is a highly inflammatory disease process and exogenous heparin administration might be detrimental by blocking anti-inflammatory activity. The purpose of this study was to determine whether dogs who received therapy with heparin or low-molecular weight heparin had reduced survival to discharge compared to those who did not receive heparin.

Retrospective data were compiled from 47 canine IMHA cases presented to TAMU from June 1, 1991 to November 1, 2004. Diagnosis was based on presence of anemia as well as auto-agglutination and/or spherocytosis. Cases with neoplasia, infectious disease, or concurrent thrombocytopenia were excluded. Cases were divided into two groups: those that had received heparin (heparin group) and those that had not (non-heparin group). Cases were subdivided based on index of suspicion of disseminated intravascular coagulation (DIC; presence of compatible clinical signs [acute dyspnea compatible with pulmonary thromboembolism] and/or the presence of at least three of the following: prolonged prothrombin time, prolonged activated partial thromboplastin time, decreased ATIII, elevated d-Dimers, increased fibrin degradation products), or confirmed DIC (presence of thromboemboli at necropsy). Statistical analysis was conducted using Chi-square test, Fisher's exact test, two sample t-test and Wilcoxan rank sum test.

In the heparin group, 14 dogs survived to discharge and 17 died or were euthanized. In the non-heparin group, 14 dogs survived to discharge and two dogs died or were euthanized. There was a statistically significant difference in survival to discharge in the non-heparin group (p=0.0051). Evidence of DIC (p=0.0076) and suspicion of DIC (p=0.0176) were also both positively associated with death and not associated with heparin use. Confounding factors such as initial packed cell volume, total bilirubin, neutrophil count/toxicity were not significantly different between the groups. Potential explanations for this difference include the detrimental effects from blocking endogenous HS binding with ATIII, early euthanasia or cessation of treatment due to owner concerns, or other confounding factors.

ABSTRACT #6

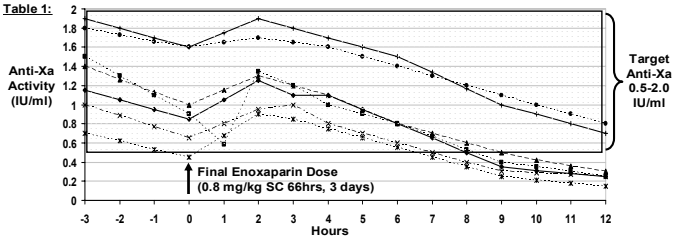
PHARMACOKINETICS OF THE BIOLOGICAL EFFECTS OF SUBCUTANEOUS ENOXAPARIN IN DOGS. Kari Lunsford, Andrew Mackin, Cory Langston, *Marjory Brooks. Mississippi State University, Starkville, MS and *Cornell University, Ithaca, NY.

Unfractionated heparin has been the standard heparin used in human and veterinary medicine, however, it has a highly variable efficacy and requires close monitoring of coagulation parameters. Low-molecular-weight heparins (LMWHs) such as enoxaparin overcome many of these problems, and have become the 'standard of care' for heparinization in people. A subcutaneous (SC) enoxaparin dose derived from the human literature, 1 mg/kg bid, has been recommended in dogs. The pharmacokinetic properties of SC enoxaparin, however, may vary between species. Our project was designed to determine the optimum dose and dosing interval for enoxaparin in dogs.

The biologic effect of enoxaparin was measured by determining plasma anti-factor Xa levels via chromogenic assay. Single SC dose response curves were generated in two normal dogs at, above, and below the recommended dose of 1 mg/kg, and used to determine the dose and dose interval to be used in a steady-state study. In the subsequent steady-state study, seven normal dogs were given SC enoxaparin at 0.8 mg/kg q6hrs, and anti-factor Xa levels were measured at regular intervals after the 1st dose, and again after steady state was attained on day three.

The disposition of enoxaparin was best described by a one-compartment open non-intravenous model. At a dose of 0.8 mg/kg SC, the mean (n=7) apparent specific volume of distribution over the fraction absorbed (V/F) was 90 ml/kg, and the proximal and terminal half-lives were 0.69 hrs and 5.10 hrs respectively. At steady state anti-factor Xa activity remained within accepted human ranges (0.5-2.0 IU/ml), and no dogs dropped to subtherapeutic levels (Table1).

Table 1:



Prothrombin and partial thromboplastin times remained normal, and there was no clinical bleeding.

Enoxaparin at a dose of 0.8 mg/kg SC every six hours appears to effectively and consistently maintain therapeutic levels of anti-Xa activity in normal dogs.

ABSTRACT #7

A NEW BLOOD GROUP ANTIGEN IN DOMESTIC SHORTHAIR CATS: THE FELINE MIK RED CELL ANTIGEN. NM Weinstein, MC Blais, K Greiner, DA Oakley, A Hyson, and U Giger. Section of Medical Genetics, University of Pennsylvania, Philadelphia, PA.

The feline blood group system is currently defined by blood types A, B, and AB, and, thus far is the only system recognized in cats. Naturally-occurring alloantibodies have been well documented in type A and type B cats and require that blood typing be performed prior to both blood transfusion and breeding to assure appropriate blood compatibility. Blood incompatibilities, unrelated to the AB blood group system, have also been recognized following blood transfusion through crossmatching cats or as a result of acute hemolytic transfusion reactions. Utilizing standard tube and novel gel column crossmatching techniques, the presence of a clinically relevant alloantibody, formed against a newly discovered feline red blood cell antigen, has been identified.

Evidence of this alloantibody was demonstrated through routine crossmatch testing in a healthy, previously never transfused, type A, DSH, blood donor cat, named Mike. Mike's plasma was incompatible with more than 50 type A, B, and AB feline patients and donors, with the exception of two, likely related, type A, DSH, blood donor cats. It was, therefore, concluded that Mike lacks the red cell antigen, referred to as Mik, as do these two additional blood donor cats. The anti-Mik alloantibody formed by Mike and one of the other Mik red cell-negative cats resulted in equivalent incompatible crossmatch results with over 30 additional type A, B, and AB cats. Furthermore, the anti-Mik plasma titers were 1:64 and 1:32, respectively, for Mike and for the second Mik red cell-negative blood donor cat. Plasma from the remaining Mik- negative blood donor cat did not react or only reacted weakly with red blood cells from over 30 cats.

Further evidence documenting the clinical relevance of the anti-Mik alloantibody was seen in a DSH, feline renal transplant candidate with blood type A and a prior transfusion history. Following an AB-matched blood transfusion, this cat experienced an acute hemolytic transfusion reaction. Subsequent crossmatching results revealed that patient plasma, obtained both pre- and post transfusion, was incompatible with the blood donor used, as well as with numerous other cats, but not with any of the three Mik red cell antigen-negative blood donors. Plasma from both blood donors with a documented anti-Mik alloantibody was crossmatch-compatible with this renal transplant patient, suggesting this patient's red blood cells also lack the Mik red cell antigen.

The absence of a Mik red cell antigen in some type A, DSH cats may result in formation of anti-Mik alloantibodies which can occur naturally. The presence of these alloantibodies may elicit an acute hemolytic transfusion reaction following an AB-matched blood transfusion. Additional studies to determine both the frequency of Mik red cell antigen-negative cats and the presence of anti-Mik alloantibodies in the general feline population are needed as is molecular characterization of the Mik-red cell antigen. Screening feline blood donors and patients for the presence of this apparently common red cell antigen and corresponding alloantibody may prove necessary in clinical practice.

ABSTRACT #8

VALIDATION OF TISSUE FACTOR ACTIVATED THROMBOELASTOGRAPHY ON CANINE CITRATED WHOLE BLOOD FROM CLINICALLY HEALTHY DOGS –ANALYTICAL VARIATION AND STABILITY OF BLOOD SAMPLES. B Wiinberg[1], AL Jensen[1], R Røjkjær[2], P Johansson[3], M Kjelgaard-Hansen[1], AT Kristensen[1]. [1]Small Animal Clinical Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark, [2]Novo Nordisk A/S, [3]Copenhagen University Hospital.

Thromboelastography (TEG) enables global assessment of hemostatic function in whole blood with evaluation of both plasma and cellular components during initiation, amplification and propagation of clot formation. TEG has a largely unused potential in the diagnostic workup and monitoring of dogs with hemostatic disorders and it may be a valuable supplement to the traditional coagulation parameters such as platelet count, PT, APTT, fibrinogen and D-dimer currently used in most clinical pathology laboratories.

It was the objective of this study to validate a human recombinant tissue factor (TF) activated TEG assay on citrated whole blood (WB) from clinically healthy dogs, with the aim of estimating a reference range for physiological hemostasis in healthy dogs. Further, assessment of analytical variation of reaction time (R), clotting time (K), angle (α), and maximum amplitude (MA) of clot formation and evaluation of effect of storage of citrated blood samples at room temperature (RT) for 30 (T30) and 120 (T120) minutes was evaluated.

Citrated WB was collected from 18 clinically healthy dogs and stored at RT for subsequent analyses. Duplicate TEG analyses with TF as activator at a concentration of 1:50,000 were performed 30 and 120 minutes after blood sampling. R, K, α and MA were analyzed.

Distribution of the data was assessed with the D'Agostino and Pearson omnibus normality test. A paired t-test was applied to identify any significant change of R, K, α and MA between 30 and 120 minutes. Statistical significance was set at $p < 0.05$. The analytical variations (CVs) of R, K, α and MA were assessed by the arithmetic mean and the pooled variance estimate based on the difference of duplicate TEG measurements.

The observed mean TEG parameters for T30/T120 were: R = 5.61/4.91min, K = 4.20/3.34min, α = 45.33/50.90° and MA= 47.96/50.19mm. Significant differences were observed between the mean TEG parameters measured after storage for 30 and 120 minutes at RT: R (t = 2.225, 2-tailed p = 0.0399), K (t = 3.682, 2-tailed p = 0.0019), α (t = 4.015, 2-tailed p = 0.0009) and MA (t = 4.375 2-tailed p = 0.0004), with a tendency towards hypercoagulability at T 120. The observed mean CVs were: CV_R = 6.46% CV_K = 15.71%, CV_α = 7.86% and CV_{MA} = 4.30%.

In conclusion, canine citrated WB can be used for TEG analysis with TF as activator when stored at RT for either 30 or 120 minutes. At both T30 and T120 the observed analytical variation was low with a narrow range of measurements in healthy dogs, suggesting that this assay may be of value in evaluating dogs with hemostatic disorders. A statistically significant trend towards hypercoagulability was observed at T 120 vs. T 30, suggesting a fixed time point should be chosen for serial measurements. Further studies on dogs with pathologic hemostasis are in progress.

ABSTRACT #9

CANINE VOLUNTEER BLOOD DONOR PROGRAMS IMPLEMENTED VIA COMMUNITY BLOOD DRIVES ARE A SAFE AND EFFECTIVE MEANS OF ANIMAL BLOOD BANKING.

Lawrence A. DeLuca, Sharon G. Glass, Richard E. Johnson, Jeffrey Frohock; Sun States Animal Blood Bank, Wilton Manors, FL.

Human volunteer blood donor programs are commonplace, yet the concept of animal blood banking is a relatively new one. Few studies exist on the safety and efficacy of animal volunteer donor programs. We evaluate a non-profit, community-based canine volunteer donor program utilizing community blood drives with regard to donor screening, phlebotomy, and adverse events.

A retrospective study design was used. All potential donors presenting to community blood drives during the period from October 2003 to December 2004 were included in the study. Recruitment methods included referrals, print advertising, and direct appeals during blood drives. Informed consent was obtained prior to donation, and donors were assessed for cooperativity. Guardians were present to reassure donors during donation unless they requested otherwise. Donors were not sedated. Blood was collected with commercially available equipment using standard protocols. Information on adverse events and donor screening was abstracted from donor files kept by the blood bank. The study was approved by the Institutional Review Board.

Of 98 potential donors, three did not have adequate jugular vessels, two signed up to donate but left before phlebotomy, and one was accompanied by a minor and could not be consented. Four were uncooperative and deemed ineligible to volunteer. Four tested positive for blood-borne pathogens. Of the 84 donors, 45 were DEA 1.1 positive and 39 were DEA 1.1 negative. A total of 143 donations were made, with 29 repeat donors (35%). There were no serious adverse events. Four dogs (2.8%) experienced an acute donor reaction and were treated with a 100-200cc fluid bolus (3 IV, 1 PO). Six dogs (4.2%) developed a hematoma that resolved in several days. Three dogs (2.1%) experienced rebleeding from the venipuncture site which responded to direct pressure. One patient (0.70%) developed a hot spot at the venipuncture site. All guardians received follow-up phone calls. Donor ID tags were provided as an incentive after successful blood donation.

We conclude that non-profit, community-based canine volunteer donor programs for animal blood banks can be successfully implemented. Few dogs were recruited that did not pass screening tests. No serious adverse events occurred during or after phlebotomy. Rates of minor incidents were comparable to those published for human donors. Discrepancies may be due to increased movement of some donor animals, overdraws, shaving prior to blood collection, or small sample size. A substantial fraction of donors donated multiple times, suggesting that volunteer donors and their guardians also perceived the donation process as safe and effective.

ABSTRACT #10

IMMUNOMODULATORY ACTIVITY OF MYCOBACTERIAL CELL WALL-DNA COMPLEX (MCC) IN VITRO AND FOLLOWING INTRAVENOUS ADMINISTRATION TO DOGS.

Nathalie Saha, Isabelle Voccia, Mario C.Filion and Nigel C. Phillips; Bioniche Therapeutics Division, Bioniche Life Sciences Inc, Montréal, Québec, Canada.

Mycobacterial cell wall-DNA complex (MCC), a mycobacterial cell wall composition prepared from the non-pathogenic microorganism Mycobacterium phlei, has a direct anticancer activity towards canine cancer cells (inhibition of proliferation and induction of apoptosis). In the present study we have evaluated the cytokine-inducing activity of MCC towards a canine monocyte/macrophage cell line (DH82 cells), canine peripheral blood mononuclear cells (PBMC) and following intravenous injections to Beagle dogs. Our results showed that MCC in the concentration range 0.1 to 100 µg/ml induced the synthesis of IL-10 by DH-82 cells, and of IL-6, IL-10, IL-12, TNF-α, MCP-1 and IFN-γ by canine PBMC as determined by ELISA. In vivo, the intravenous administration of 0.008, 0.08 and 0.8 mg/kg of MCC (6 dogs per group) induced the synthesis of IL-10, IL-12 and IFN-γ, 0.008 mg/kg of MCC being the most active dose for the synthesis of IL-12 and 0.8 mg/kg of MCC for IL-10 and IFN-γ. The highest level of IL-10 or IL-12 was reached three hours after the administration of MCC, while the highest level of IFN-γ was detected six hours post-treatment in the majority of dogs. IL-6, TNF-α and MCP-1 were not detected following the injection of MCC. Our data indicate that MCC may serve as an immunopotentiating agent in dogs. The combination of antiproliferative/apoptosis-inducing activities of MCC towards canine cancer cells and the immunomodulatory activity towards canine immune effector cells may be important contributing factors to the overall anticancer activity of MCC.

ABSTRACT #11

SYSTEMIC IMMUNE EFFECTS OF AN INHALANT GLUCOCORTICOID IN CATS.

CR Reinero,[1] L Brownlee, [2] B Seguin, [2] K Decile,[2] LJ Gershwin[2] . [1]University of Missouri, Columbia [2]University of California, Davis.

Therapy of feline bronchial disease has traditionally relied on oral or injectable glucocorticoids (GC); however, not all cats tolerate high levels of GC. Delivery of metered dose inhalant steroids in human asthmatics maximizes local efficacy and minimizes systemic bioavailability. The current study evaluated systemic immune effects of inhaled flunisolide (IFP; 250mcg/puff) versus oral prednisone at high doses (10 mg/day) and placebo (an empty metered dose inhalant). We hypothesized that IFP would have minimal systemic effects on a variety of immunologic parameters in healthy pet cats.

A randomized crossover design was used. Six cats received each treatment for two weeks followed by a one month washout. Immune effects were determined using flow cytometry to evaluate percentages of peripheral lymphocyte classes (pan T cell marker, CD4+ T cell marker, CD8+ T cell marker, and pan B cell marker), lymphocyte proliferation assays using mitogenic stimulation *in vitro*, serum total

IgA and IgM levels, and cytokine profiles (IL-2, IL-4, IL-10 and IFN-gamma) using RT TaqMan PCR.

Flow cytometric evaluation of markers on peripheral lymphocytes revealed decreases in the group mean % of total T cells, cytotoxic T cells, and total B cells in cats receiving oral GC compared with placebo, but not with IFP. Lymphocytes had diminished proliferative responses to a mitogen from cats receiving oral GC, but not IFP, in comparison to placebo (7±1.3; 24±2.6; 19±5.6, respectively). Serum total IgA and IgM showed no significant differences across treatment groups. There was no significant suppression of IL-2, IL-4, or IFN-gamma mRNA transcription with oral GC or IFP compared with placebo. However, there was a significant increase in IL-10 when cats were administered either oral GC or IFP compared with placebo.

In summary, while oral GC decreased the percentages of total T cells, total B cells and cytotoxic T cells, and decreased proliferative response to mitogenic stimulation, IFP did not produce these systemic immunologic effects. Additionally, neither the inhaled nor the oral GC decreased serum levels of IgA or IgM in the serum, perhaps due to the comparatively long half-lives of these antibodies in relation to the short course of drug therapy. Further studies would be necessary to determine the effects of long term GC on serum antibody levels. Interestingly, compatible with recent studies, GC can increase IL-10 (an immunosuppressive cytokine) production. In conclusion, high dose IFP administered to cats do not appear to have significant systemic immune effects.

ABSTRACT #12

THE USE OF AN IN-CLINIC DOT-ELISA TEST FOR THE ASSESSMENT OF THE IMMUNIZATION STATUS TO CANINE PARVOVIRUS AND DISTEMPER VIRUS IN ADULT DOGS. Trevor Waner[1], Shlomit Mazar[2], Ephraim Keren-Kornblatt[3]. 1. Veterinary Clinic, Rehovot, Israel; 2. Biogal Gal'ed Laboratories, Kibbutz Gal'ed, Israel. 3. Veterinary Clinic, Hashmonaim, Israel.

A growing body of literature has been published indicating that the current historic approach of annual vaccination may not be beneficial and in some cases may even be harmful. A number of publications have proposed assessing the immunization status of dogs prior to annual revaccination. In this study we tested the usefulness of a dot-ELISA kit to evaluate the duration IgG antibody titers to canine parvovirus (CPV) and canine distemper virus (CDV) in dogs vaccinated for at least one year previously. To the best knowledge of the authors this is first study in which an in-clinic dot-ELISA test kit has been used for this purpose.

Serum samples from 158 dogs, which had not been vaccinated for at least one year previously, were assayed for serum IgG antibody titers to CPV and CDV using an in-clinic dot-ELISA kit (ImmunoComb®, Biogal Laboratories, Kibbutz Gal'ed, Israel). An antibody titer equivalent to 1:80 was regarded as a protective for both CPV and CDV. Mean antibody titers and their standard deviations for the dogs were arranged by 6-monthly intervals since their last vaccination.

Overall, the percentage of dogs with protective antibody titers to both CPV and CDV was 84%. The percentage of dogs with borderline antibody titers was 11% for CPV and 10% for CDV. Four percent of the dogs had no detectable antibody to CPV and 6% had no antibody to CDV. There were no statistically significant differences in the mean CPV and CDV antibody titers respectively for any of the categorized 6-monthly intervals after vaccination. There did not appear to be any pattern regarding the incidence of low or borderline titers to either CPV or CDV with increasing time after the last vaccination.

The results reported here are in good agreement with other laboratory IgG antibody assayed studies which indicate that a large percentage of healthy dogs have protective serum antibody to CPV and CDV for many years after their preceding vaccination, and to the conclusion that the majority of dogs do not require annual vaccination. Use of the dot-ELISA kit in this study was able to identify dogs retaining protective antibody titers to CPV and CDV after a number of years following their last vaccination. Quantification of specific IgG antibody levels by the dot-ELISA kit offers the veterinarian the opportunity of following antibody titers and detecting trends over time which can be used for early detection of dogs which may have lost their immunity to vaccinatable diseases and revaccinating only those whose antibody titer to specific diseases has waned.

ABSTRACT #13

ACID-BASE IN DOMESTIC PIGEONS: CALCULATION OF PLASMA ATOT AND KA VALUES FOR USE IN THE QUANTITATIVE STRONG ION MODEL. Henry Stämpfli[1], Michael Taylor[1], Ady Gancz[1], Carl McNicoll[1], and Peter D. Constable[2]. [1]Dept. of Clinical Studies, University of Guelph, Guelph Ontario, Canada; [2]Dept. of Veterinary Clinical Medicine, University of Illinois, Urbana-Champaign, IL.

Acid-base abnormalities are frequently encountered in sick birds and basic information on evaluating acid-base disturbances is unavailable for many species of birds. The quantitative mechanistic acid-base approach to clinical assessment of acid-base status requires 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), but values for Atot and Ka are not available for avian species. The aim of this study was to determine Atot and Ka values for plasma in domestic pigeons.

Plasma was harvested from eight healthy commercial domestic pigeons and tonometered with 20%CO_2 at 37 ^oC. Plasma pH, Pco_2, and plasma concentrations of quantitatively important strong cations (Na, K, Ca), strong anions (Cl, L-lactate), and non-volatile buffer ions (total protein, albumin, phosphate) were measured over a pH range of 6.8 to 7.7. Strong ion difference (SIDest=Na + K + Ca - Cl - lactate) was estimated from the measured strong ion concentrations and nonlinear regression was used to calculate Atot and Ka from the measured pH and Pco_2 and SIDest. Anion gap was calculated as: Anion gap = $([Na^+]+[K^+]) - ([Cl^-] + [Lactate]+[HCO_3^-])$.

Table 1: Venous blood values in 8 pigeons

Variable	Mean	SD
pH	7.41	0.04
Pco_2 (mm Hg)	42.4	4.6
$[Na^+]$ (mmol/L)	148.8	1.9
$[K^+]$ (mmol/L)	3.74	0.58
$[Ca^{2+}]$ (mmol/L)	1.29	0.04
$[Cl^-]$ (mmol/L)	112.8	0.99
[Lactate] (mmol/L)	8.35	3.6
SIDest (mEq/L)	31.2	3.4
$[HCO_3]$ (mmol/L)	25.5	3.6
[Plasma protein] (g/L)	23.9	3.4
[Albumin] (g/L)	15.3	1.4
[Phosphate] (mmol/L)	1.0	0.30
Anion gap (mEq/L)	5.79	1.8
[Glucose](mmol/L)	18.4	1.53

Mean (± SD) values for bird plasma were: Atot = (8.09 ± 2.45) mmol/l (equivalent to 0.35 mmol/g of total protein or 0.54 mmol/g of albumin); Ka = (1.74 ± 0.74) x 10^{-7}; pKa = 6.76. The calculated SID for bird plasma (Table 1) was 31.2 mEq/l. The net negative charge of plasma non-volatile buffers (A^-) at pH 7.41 is 6.6 mEq/L; this value is consistent with a low anion gap value after adjusting for high mean lactate concentrations induced by restraint during blood sampling. (Table 1) The calculated value for A^- indicates that plasma proteins in birds have a lower net anion charge due to lower concentrations of total proteins when compared to mammals.

These results can be used to help identify the mechanisms involved in acid-base disturbances in birds, because acid-base changes are caused by a strong ion acidosis or alkalosis (change in SID), respiratory acidosis or alkalosis (change in P_{CO2}), or a non-volatile buffer ion acidosis or alkalosis (change in Atot).

ABSTRACT #14

RISK FACTORS AFFECTING THE OCCURRENCE OF ANTIMICROBIAL RESISTANCE OR INTERMEDIATE SUSCEPTIBILITY IN FECAL *E. COLI* ISOLATED FROM HEALTHY DOGS AND CATS FROM PRIVATE VETERINARY CLINICS IN SOUTHERN ONTARIO. Colleen Murphy[1], Richard Reid-Smith[1,2], John Prescott[3], Brenda Bonnett[1], and Scott McEwen[1]; [1]Department of Population Medicine, Ontario Veterinary College University of Guelph, Guelph Ontario, [2]Laboratory for Foodborne Zoonoses, Health Canada, Guelph Ontario, [3]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph Ontario, [4]Department of Clinical Studies, Large Animal Medicine, Ontario Veterinary College, University of Guelph, Guelph Ontario.

Risk factors associated with the occurrence antimicrobial resistance in companion animals have not been described. Identification of these risk factors is important for a better understanding of the general epidemiology of antimicrobial resistance, in addition to the identification modifiable risk factors. The objective of this study was to determine risk factors for antimicrobial resistance or intermediate susceptibility in commensal *E. coli* isolates from healthy dogs and cats exposed to antimicrobials.

Healthy dogs and cats from private veterinary hospitals in Southern Ontario without a recent exposure to antimicrobials were enrolled into the study. Fecal samples were collected using a rectal swab, a questionnaire was administered to pet owners and prescription history was obtained from the pets' medical records.

Unconditional analysis and multivariable logistic modelling revealed associations between antimicrobial resistance/intermediate susceptibility and dietary exposures, specific water sources, associations with livestock and historical antimicrobial exposure (exposure greater than six weeks in the past). There were strong associations between the consumption of pig ears treats and trimethoprim-sulfamethoxazole resistance/intermediate susceptibility, consumption of raw beef products, and ampicillin or cephalothin resistance/intermediate susceptibility. Additionally, there were strong associations between historical enrofloxacin treatment and ampicillin, amoxicillin-clavulanic acid or cephalothin resistance/intermediate susceptibility, and historical short-acting penicillin treatment and resistance/intermediate susceptibility to amoxicillin-clavulanic acid and cephalothin.

Although some of the risk factors for resistance/intermediate susceptibility are not modifiable, the exposure of pets to food items such as pig ears and raw beef can be changed. Pet owners should be aware of these risks when selecting foods for their pet and veterinarians can use this information to guide owners to select food alternatives for their pet that are less of a risk in terms of antimicrobial resistance and zoonotic disease. The associations demonstrated between historical antimicrobial therapy and resistance/intermediate susceptibility to commonly prescribed antimicrobials illustrates that veterinarian have an important role to play. Veterinarians impact the dynamics and epidemiology of antimicrobial resistance through their antimicrobial practices.

ABSTRACT #15

SILDENAFIL (VIAGRA®) AS A THERAPY FOR PULMONARY HYPERTENSION IN DOGS. J Bach, EA Rozanski, J MacGregor, JM Betkowski and JE Rush. Tufts University, Grafton, MA.

Severe pulmonary hypertension (PHTN) is associated with a poor outcome in dogs. Oral sildenafil is a phosphodiesterase type V inhibitor which results in increasing concentrations of cyclic GMP, which subsequently results in NO-mediated vasodilation. Sildenafil has been used as an oral agent for therapy of people with PHTN. The purpose of this study was to describe the clinical characteristics and outcome of dogs with PHTN treated with sildenafil.

The cardiology database was searched for dogs identified with PHTH and treated with sildenafil. PHTN was defined as a systolic pulmonary arterial pressure (PAP_s) of >30 mmHg at rest. The medical records were reviewed for the following information: signalment, duration and type of clinical signs prior to treatment, underlying disease (if identified), estimated (Tricuspid regurgitation velocity + estimated right atrial pressure) or measured PAP_s, dose and dosing interval of sildenafil, effect of treatment on clinical signs and pulmonary arterial pressure and survival time.

Nine dogs were included in the study. Seven dogs were spayed females and two were castrated males. Three dogs were Shih-tzus, two were Chihuahuas and one each Shetland Sheepdog, Cairn terrier, Labrador retriever, and Golden retriever. The median age was 13, with a range of four to 15 years. The most common clinical signs recorded include collapse (n=6), dyspnea with exertion (n=5) and cough (n=4). The duration of clinical signs ranged from three days to five months. The underlying cause was identified in six dogs and included chronic valvular disease (n=2), suspected pulmonary fibrosis (n=2), right-to-left PDA (n=1) and pulmonary thromboembolism (n=1). The PAP_s prior to therapy was directly measured in two dogs at 102 and 89 mmHg and estimated in 6 dogs at a median of 98.8 mmHg with a range 61-136 mmHg. An accurate Doppler estimate of PAP_s was impossible in the dog with the right-to-left PDA. Sildenafil was administered orally at a median dose of 1.7 mg/kg (range 0.5 to 2.7 mg/kg) at a dosing interval of 8 to 24 hours. Six dogs were treated with concurrent medications including ACE inhibitor (n=3), furosemide (n=3), amlodipine (n=2) and diltiazem (n=1). PAP_s was measured or estimated by Doppler in 7 dogs following therapy. PAP_s decreased in 6/7 dogs, with a median decrease of 19 mmHg (range -1 to 37 mmHg) or 18 % over pre-treatment values (range -1 to 56%). Systemic blood pressure (sBP) was measured in all dogs before treatment and in five dogs after treatment. Following treatment sBP decreased by a median 36 mm Hg (range +10 to -60 mmHg). Subjective improvement in clinical signs occurred in six dogs. Three dogs were euthanized within one week of starting therapy due to on-going severe clinical signs. An additional dog died one month later following inadvertent concurrent therapy with a nitrate. The five remaining dogs had a median survival of 12 months (range six to 13.5 months)

Sildenafil is well tolerated in dogs with PHTN, resulting in a decrease in PAP_s in all dogs and an amelioration of clinical signs in 6/9 of treated dogs. Sildenafil represents a viable treatment in dogs with pulmonary hypertension.

ABSTRACT #16

THE EFFECT OF BODY POSITION, SEDATION AND THORACIC BANDAGING ON FUNCTIONAL RESIDUAL CAPACITY IN HEALTHY DEEP-CHESTED DOGS. EA Rozanski, J Lofgren, D Bedenice, J Abrams, J Bach, AM Hoffman. Lung Function Testing Laboratory, Tufts University, North Grafton, MA.

Functional residual capacity (FRC) represents the resting end-expiratory intra-thoracic gas volume. FRC is a dynamic value. Decreases in FRC are associated with ventilation-perfusion mismatch, and may contribute to hypoxemia. In dogs with pulmonary disease, thoracotomies are performed for diagnostic or therapeutic purposes. Post-operatively, dogs are often in lateral recumbency with thoracic bandages and sedation/analgesia are administered for patient comfort. The purpose of this study was to examine the effect of body position, sedation and bandage placement on FRC in healthy dogs.

Six healthy dogs were enrolled in the study. FRC was measured by helium dilution. FRC was measured in duplicate at the following time points: baseline, after chest bandage placement, after sedation with butorphanol (0.1 mg/kg IV) and acepromazine (0.03mg/kg IV) with bandage and with sedation without bandage. At each time point, dogs were measured standing or sternal and after 10 minutes in lateral recumbency. All bandages were placed by a single investigator (ER)

to a set tension (5 cmH20). Results were compared using a paired sample t-test with a p value of <0.05 considered significant. Results are shown as mean ± SEM.

Group/time period	Standing /Sternal	Lateral Recumbency
Baseline	75.3 ± 10.1 ml/kg	50.8 ± 5.6 ml/kg
Bandage	55.2 ± 8.1 ml/kg	38.2 ± 3.9 ml/kg
Sedation only	50.5 ± 13.0 ml/kg	32.5 ± 4.3 ml/kg
Bandage and sedation	38.2 ± 6.4 ml/kg	29.4 ± 3.9 ml/kg

All interventions resulted in a significant decrease in FRC from baseline values. Sedation resulted in lower FRC in all groups. Lateral recumbency resulted in significantly lower FRCs than while sternal or standing at all times except during sedation. Placement of a chest wrap significantly lowered FRC in unsedated dogs.

In conclusion, common clinical interventions lowered FRC in healthy deep-chested dogs. These data may be applicable to dogs undergoing thoracotomy for thoracic disease and should be further investigated.

ABSTRACT #17

NON-INVASIVE FORCED EXPIRATORY FLOW-VOLUME CURVES TO MEASURE LUNG FUNCTION IN CATS. H. Bark, A. Epstein School of Veterinary Medicine, Hebrew University of Jerusalem; E. Bar-Yishay, A. Putilov, S.Godfrey, Hadassah University Hospital, Jerusalem, Israel.

The relation between expiratory flow rate and expired volume during forced expiration (forced expiratory flow-volume curve) provides important insight into the severity and nature of lung diseases and especially of small airway obstruction and is the basic test of lung function in humans. More than 20 years ago a thoracic compression technique was developed for use in human infants who cannot actively cooperate with breathing tests, in which forced expiratory flow is produced by the brief application of pressure from a thoracic compression jacket. The purpose of the present study was to determine whether this technique could be applied to measure lung function non-invasively in small animals.

Thirteen healthy adult cats (mean weight 4.78 kg) were used in this study. They were sedated with xylazine and ketamine at a dose such that they retained their swallowing and palpebral reflexes. The apparatus and computer program used for the study were adapted from those used for human infants. The cat reclined in sternal recumbency and breathed through a face mask connected to a pneumotachygraph from which flow and volume were obtained after appropriate calibration. The total dead space of the equipment was ±3 ml. Vaseline was smeared around the mouth and nose and silicone putty was used to reduce the dead space and seal the mask to the face of the cat so that the animal breathed naturally through its nose with the mouth closed. Thoracic compression was applied from an inflatable bag extending over the sternal surface of the chest and abdomen held in place by a non-expandable canvas jacket totally surrounding the trunk of the animal. The bag was suddenly inflated at end-inspiration by a computer initiated pulse sent to a solenoid controlling the connection of the inflation bag to either room air or an appropriately pressurized chamber. From the signals recorded from the pneumotachygraph we determined the maximum forced expiratory flow at a lung volume equivalent to the resting lung volume (end-expiration), which is termed V'maxFRC. The test was repeated with different compression pressures and the highest value obtained from a technically satisfactory flow-volume loop was taken as the result in conformity with human infant practice.

For the 13 cats studied the mean (± 95% CI) for V'maxFRC was 397 ml/sec (334-460). Due to a complete lack of previous studies of V'maxFRC in cats, we cannot compare our results with those of others but compared with the human infant of similar weight (V'maxFRC approximately 180 ml/sec), cats had a much higher V'maxFRC. In 10 of the cats the test was repeated on another day and the mean (± 95% CI) percentage difference between paired tests was –2.0% (-13.7 - +9.7). In conclusion, we showed that non-invasive forced expiratory lung function testing is easy and practical to perform in sedated cats using techniques developed for human infants.

ABSTRACT #18

NON-INVASIVE MEASUREMENTS OF COMPLIANCE AND RESISTANCE USING PASSIVE EXPIRATION IN CATS. A. Epstein, H. Bark School of Veterinary Medicine,Hebrew University of Jerusalem; E. Bar-Yishay, A. Putilov, S.Godfrey, Hadassah University Hospital, Jerusalem, Israel.

Measurements of compliance (a measure of stiffness) and resistance are basic tests used to evaluate lung function in humans and to some extent in veterinary practice, although in animals the measurements have almost always been performed in anesthetized and intubated subjects. In lightly sedated human infants the compliance of the total respiratory system (Crs) can be determined by measuring the airway pressure during brief occlusions which inducing the Hering-Breuer inflation reflex and relax the expiratory muscles at different expired volumes. Furthermore, a plot of expired volume against flow during the relaxed expiration after release of the occlusion yields the time constant (τrs) of the respiratory system from which total respiratory resistance (Rrs) can be calculated since τrs = Crs x Rrs. The purpose of the present study was to determine whether this technique could be applied to measure lung function non-invasively in small animals.

Fourteen healthy adult cats (mean weight 4.71 kg) were used in this study. They were sedated with xylazine and ketamine at a dose such that they retained their swallowing and palpebral reflexes. The apparatus and computer program used for the study were adapted from those used for human infants. The cat reclined in sternal recumbency and breathed through face mask connected to a pneumotachygraph from which flow and volume were obtained after appropriate calibration. Vaseline smeared around the mouth and silicone putty were used to reduce the dead space and seal the mask to the face of the cat. The tidal breathing of the cat was observed in real time on the computer screen and brief occlusions of expiration from the pneumotachygraph were performed manually at different lung volumes from end inspiration to as near as possible end expiration. The slope of the plot of lung volume above resting lung volume against the airway pressure during the occlusion for all the breaths yielded Crs. The lungs were briefly inflated manually above normal end inspiration to induce relaxation for each of 3-4 breaths. Passive expiration on release from inflated lung volume was recoded and τrs was determined by the computer from the slope (volume / flow) of the linear portion of each passive expiration. Rrs was calculated as above.

For the 14 cats studied the mean (± 95% CI) for Crs was 7.9 ml/cmH_2O (6.9-8.8), τrs was 0.35 sec (0.31-0.39) and Rrs was 0.45 cmH_2O/l/sec (0.40-0.50). There are few comparable data in the literature however in anesthetized and intubated cats compliance has been reported as approximately 7.2 ml/cmH_2O, and resistance as 0.34 cmH_2O/l/sec. In 10 of the cats the test was repeated on another day and the mean (± 95% CI) percentage difference between paired tests was –1.7% (-9.0 - +5.7) for Crs, 3.2% (-8.5 - +14.8) for τrs and 4.7% (-11.2 - +20.5) for Rrs. In conclusion, we showed that the non-invasive measurement of compliance and resistance is easy and practical to perform in sedated cats using techniques developed for human infants.

ABSTRACT #19

TRACHEOSTOMY IN CATS: 16 CASES (1998-2004). C Yenke and EA Rozanski, School of Veterinary Medicine, Tufts University, North Grafton, MA.

Upper airway obstructions may be life-threatening. Tracheostomy in cats is purported to be more difficult to manage than in dogs or other larger animals. The purpose of this study was to describe the indications, complications and outcome of cats undergoing tracheostomy.

The medical records of cats undergoing tracheostomies for management of upper airway obstruction were retrospectively evaluated. Records were evaluated for signalment, indications for tracheostomy, duration of tracheostomy tube placement, number of complications and outcome. Complications were defined as either major which included tube occlusions requiring emergent suctioning or replacement, unplanned extubations or asphyxiation or minor, which included tracheostomy site infection or fever, stay suture dislodgement, or subcutaneous emphysema. All cats were monitored in the ICU.

Sixteen cats were included in the study. Cats ranged in age from nine months to 18 years with a median of nine years. Eight cats were castrated males, six were spayed females and two were intact males. One cat was a brachycephalic. Indications for tracheostomy included a laryngeal mass (n=9), trauma (n=4), and upper airway swelling (n=3). Fifteen cats had a temporary tracheostomy performed and one cat had only a permanent tracheostomy performed. Five additional cats had permanent tracheostomies performed and one cat had revision of permanent tracheostomy performed. Cats had tracheostomy tubes in place from one to 11 days, with a median of three days. Major complications were recorded in six cats and minor complications were observed in 13 cats. Thirteen cats were discharged home, two cats were euthanized when biopsy results were obtained and one cat died due to causes unassociated with the tracheostomy tube. In the 11 cats with only temporary tracheostomies performed, the healing of the stoma was uneventful. In the six cats with permanent tracheostomies; one cat with benign laryngeal disease had the permanent tracheostomy reversed and is alive (36 months) and five cats had squamous cell carcinoma with one cat alive (104 days), four cats surviving at home two days, seven days and six and eight weeks after discharge. Two cats died of tracheostomy site occlusion and two were euthanized due to anorexia/dysphagia.

Tracheostomy represents a viable option for management of upper airway obstruction in cats. Major complications were uncommon, and no fatalities resulted. Minor complications were more common but easily managed. Permanent tracheostomies may provide for a good short-term solution for some cats with severe upper airway disease.

ABSTRACT #20

MMP-9 ACTIVITY AND 8-ISO-PGF_{2A} ARE INCREASED IN BRONCHOALVEOLAR LAVAGE FLUID IN A MODEL OF FELINE ASTHMA. Kirschvink N.[1], Leemans J.[1], Delvaux F.[1], Snaps F.[2], Clercx C.[2], Gustin P.[1] [1]Department for Functional Sciences, [2]Department for Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium.

Activation of matrix metalloprotease 9 (MMP-9) and the lipid peroxidation marker 8-iso-PGF2α are implied in the pathophysiology of asthma in human patients and are considered as important markers of airway remodeling and pulmonary oxidative stress, respectively. The aim of our study was to determine whether MMP-9 and 8-iso-PGF2α were increased in bronchoalveolar lavage fluid (BALF) in a feline model of asthma.

Eight cats sensitized with *Ascaris suum* (AS) antigen and eight age-matched healthy control cats (C) were used. AS-cats were nebulized for five minutes with 0.01% AS antigen whilst C-cats were exposed to placebo (NaCl 0.9%). Respiratory function in response to AS-antigen or placebo inhalation challenge was assessed using barometric whole body plethysmography (BWBP) and allowed the assessment of the enhanced pause (Penh, an index of airflow limitation). Bronchial reactivity towards inhaled carbachol was investigated 48 hours after challenge and allowed the calculation of the carbachol concentration inducing a 300% increase of Penh (%carb-Penh300). Bronchoscopy and collection of BALF were performed 72 hours after challenge under anesthesia. BALF was analyzed cytologically, gelatin zymography was used for quantification of MMP-9 activity (densitometric arbitrary units) and EIA was used for determination of 8-iso-PGF2α.

Table 1. Results.

			BALF			
	Penh (-)	**%carb-Penh 300**	PMN (%)	Eosinos (%)	MMP-9 (AU)	8-iso-PGF2α (pg/ml)
C	1.02±0.21	0.038±0.015	12 ± 7	7±6	195 ± 102	3.33 ± 2.05
AS	1.62±0.61	0.025±0.006*	26 ± 21	26±16*	1360 ± 697*	12.73 ± 8.39*

* Significantly different from Controls, $p<0.05$

Correlation analyses revealed that BALF MMP-9 was correlated with BALF 8-iso-PGF2α ($r=0.78$, $p<0.0005$), with BALF neutrophil% ($r=0.52$, $p<0.05$) and BALF eosinophil%. BALF 8-iso-PGF2α was correlated with BALF eosinophil% ($r=0.62$, $p<0.01$).

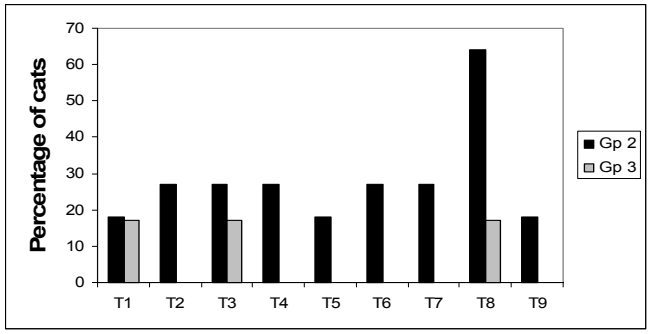
These results show that the inhalation challenge with AS induced a significant eosinophilic airway inflammation and increased bronchial reactivity in AS-sensitized cats. The increase of MMP-9 activity and of 8-iso-PGF2α in BALF suggests that matrix remodeling and oxidative stress, which are both tightly correlated with bronchial inflammation, occur in feline asthma. These biomarkers are potentially interesting for diagnosis and monitoring the response to treatment in asthmatic felines.

ABSTRACT #21

DISSOCIATION BETWEEN AIRWAY INFLAMMATION AND CLINICAL SIGNS OF BRONCHOCONSTRICTION WITH CpG MOTIFS IN EXPERIMENTAL FELINE ASTHMA. CR Reinero,[1] JR Byerly,[2] LJ Berghaus,[2] DM Hyde, [2] ES Schelegle, [2] LJ Gershwin[2]. [1]University of Missouri, Columbia [2]University of California, Davis.

Asthma is an allergen-specific Th2 driven response against aeroallergens. Strategies to diminish the Th2 response would be desirable therapeutically. CpG motifs, components of the bacterial genome that signal the mammalian immune system that a foreign pathogen is present, result in alterations in host dendritic and T cells, and their cytokines. CpG motifs in experimental models have dampened the asthmatic phenotype. We hypothesized that different CpG motif protocols would decrease the bronchoalveolar lavage fluid (BALF) eosinophil % and clinical signs of bronchoconstriction in experimental feline asthma using Bermuda grass allergen (BGA).

Three groups of cats were studied. Group 1 (Gp1, n=6 cats) had placebo sensitization & received "placebo" motifs; group 2 (Gp2, n=11) had BGA sensitization & received CpG motifs; group 3 (Gp3, n=6) had BGA sensitization & received no CpG motifs. The protocols for CpG administration were altered monthly for nine months (treatment 1-9; T1-T9), to evaluate three different sequences of CpG motifs, three different routes (subcutaneous, intranasal, and aerosol), and either a low or high dose (0.1 mg/kg vs 1.0 mg/kg). BALF was collected monthly and clinical signs after allergen challenge were graded from 0-5 (absent to severe). Results showed the average BALF eosinophil % across all treatments was: Gp 1, 5%; Gp 2, 35%; Gp 3, 48%. The percentage of Gp2 and Gp3 cats that had fewer than 20% eosinophils in their BALF for each treatment period is shown below: *(see next page)*



No significant differences in clinical scores between Gp2 and Gp3 cats were noted. Gp1 cats had scores of 0 over all treatments.

In conclusion, certain protocols of CpG motif administration in experimental feline asthma appear to dampen eosinophilic airway inflammation without affecting clinical signs of bronchoconstriction.

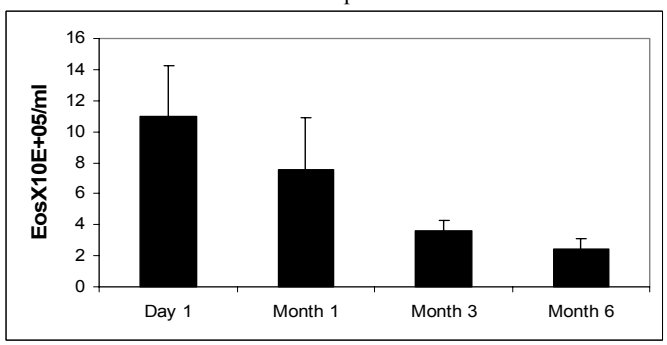
ABSTRACT #22

RUSH IMMUNOTHERAPY IN EXPERIMENTAL FELINE ASTHMA. CR Reinero,[1] JR Byerly,[2] RD Berghaus,[2] DM Hyde, [2] ES Schelegle, [2] LJ Gershwin[2]. [1]University of Missouri, Columbia [2]University of California, Davis.

Specific allergen immunotherapy represents the only curative treatment of allergy. However, immunotherapy has not been systematically evaluated in cats with allergic asthma. To elucidate mechanisms of immunotherapy we used an experimental feline model of chronic asthma using Bermuda grass allergen (BGA) that results in a variety of immunologic, physiologic, and pathologic changes. We hypothesized that an abbreviated course of immunotherapy (rush immunotherapy, RIT) would blunt eosinophilic airways inflammation and be associated with beneficial immunomodulatory changes.

Experimental asthma was induced in seven cats using BGA. Serially increasing doses (10ug-200ug) of BGA were given parenterally over two days. Numbers of eosinophils in bronchoalveolar lavage fluid (BALF), cytokines in blood and BALF, serum and BALF immunoglobulins, and lymphocyte blastogenesis assays were evaluated over 6 months.

All cats had at least one adverse clinical sign, the most common being localized swelling around the injection site (n=7), and the most severe being anaphylaxis (n=1). RIT led to significantly (P=0.048) decreased numbers of BALF eosinophils over time:



Restimulated peripheral blood mononuclear cells, which at baseline produced a mixture of Th1 and Th2 cytokines, showed a global depression of these cytokines early after RIT (day 2 and week 1), which rebounded by month 1. In BALF, cytokine profiles favored a Th2 response at baseline but shifted to increased IFN-g and IL-10 at months 1, 3, and 6. Significant (P<0.001) increases in serum BGA-specific IgG were noted. The lymphocyte proliferative response to BGA was significantly decreased at 6 months (SI<1).

In conclusion, RIT dampens eosinophilic airway inflammation in cats with experimental asthma. RIT may work by altering cytokine profiles, forming allergen-specific IgG blocking antibodies, or inducing hyporesponsive lymphocytes.

ABSTRACT #23

ISOLATED FRONTAL SINUS ASPERGILLOSIS IN 8 DOGS: 2001-2004. LR Johnson, TL Drazenovich, MA Herrera. University of California, Davis, CA.

Diagnosis of nasal aspergillus is typically confirmed when two of the three following criteria are met: characteristic findings on computed tomography, fungal plaques on rhinoscopy, and fungal hyphae on histology. However, because of the time delay in obtaining histopathology results and the need to instill topical medication under anesthesia, dogs are often treated prior to obtaining results of histopathology. The purpose of this study is to report the number of dogs in which the diagnosis of aspergillosis could be confirmed prior to treatment and to address the utility of sinus trephination in the diagnosis and management of nasal aspergillosis in dogs.

Medical records from the University of California-Davis were searched from January 2001 through December 2004 to identify dogs that had received topical treatment for nasal aspergillosis. Fifty-eight cases were identified and medical records were reviewed for imaging characteristics, rhinoscopic findings, and biopsy results.

All dogs had characteristic imaging findings, and in 40 of 58 dogs, definitive diagnosis of nasal aspergillosis was made based on two of the three diagnostic criteria. Thirty-eight of 40 dogs had fungal plaques visualized during rhinoscopic examination of the rostral nasal cavity, and fungal hyphae were present in nasal biopsies of 22 of 40 dogs. In the remaining 18 dogs, fungal plaques were not detected during the rhinoscopic procedure and were not found on histopathology. Of these 18 dogs, trephination of the frontal sinus was performed in 8 dogs, and *Aspergillus* infection was confirmed by visualization of fungal plaques in all 8 dogs. Biopsies from the frontal sinus were submitted in two dogs and both contained intralesional fungal hyphae. Eleven of the original 40 cases with aspergillosis confirmed in the nasal cavity were also treated with trephination of one frontal sinus, and fungal plaques were seen in eight of 11 cases. Biopsies of the frontal sinus were positive for fungal elements in two of three samples submitted. The final 10 dogs that received intranasal clotrimazole treatment did not have confirmation of the diagnosis by visualization of fungal plaques during rostral rhinoscopy or sinuscopy, and did not have histopathologic evidence of fungal hyphae. Computed tomography in at least two of 10 dogs suggested involvement of the frontal sinus, however trephination and sinuscopy were not performed.

Trephination of an affected frontal sinus and visualization or biopsy of fungal plaques aids in the diagnosis of aspergillosis in dogs. In this study, trephination was particularly useful in dogs with destructive rhinitis detected on rostral rhinoscopy that did not have detectable fungal plaques. In addition, we believe that trephination allows better debridement of fungus within the sinus, as well as direct placement of drug delivery catheters into the frontal sinus for topical therapy. Therefore, sinus trephination and sinuscopy should be considered as a diagnostic and therapeutic method in dogs suspected of aspergillosis.

ABSTRACT #24

UTILITY OF *ASPERGILLUS* SEROLOGY AND TISSUE FUNGAL CULTURE IN CANINE NASAL DISEASE. JS Pomrantz, LR Johnson. School of Veterinary Medicine, University of California, Davis, CA.

Diagnosis of nasal aspergillosis relies on a combination of imaging abnormalities (turbinate destruction, hyperostotic bone lesions, frontal sinus involvement), detection of fungal plaques on rhinoscopy, and histologic evidence of fungal hyphae. Dogs are often diagnosed late in the disease process due to the need for invasive and expensive tests. *Aspergillus* serology and nasal fungal culture have

not been considered useful in the diagnosis of nasal aspergillosis due to the presumption that they have low sensitivity and specificity, however, no recent studies have investigated the utility of these tests. The aim of this prospective study was to determine the sensitivity and specificity of *Aspergillus* serology (agar gel immunodiffusion technique) and tissue fungal culture in the diagnosis of nasal aspergillosis.

All dogs completing CT and rhinoscopic evaluation for nasal discharge at UC Davis between November 2003 and December 2004 were eligible for the study. *Aspergillus* serology and fungal culture of plaque lesions or nasal mucosa were performed on all dogs. Definitive diagnosis of nasal aspergillosis was made in dogs with at least two of three criteria: histopathologic evidence of infiltrating tissue hyphae on nasal biopsies, characteristic changes on computed tomography, and visualization of destructive rhinitis with fungal plaques on rhinoscopy. Dogs were separated into three groups based on their disease processes: aspergillosis, neoplasia, and rhinitis.

Forty-six dogs were entered into the study: 13 dogs with nasal aspergillosis, 11 dogs with rhinitis, six dogs with nasal neoplasia, and 16 healthy dogs (negative controls for *Aspergillus* serology). *Aspergillus* serology was positive in nine of 13 (69%) dogs with aspergillosis, 0 of 11 dogs with rhinitis, 0 of six6 dogs with neoplasia, and 0 of 16 healthy dogs. Fungal culture of nasal tissue was positive in eight of 13 (62%) dogs with aspergillosis, 0 of 11 dogs with rhinitis, and 0 of six dogs with neoplasia. The sensitivity, specificity, positive and negative predictive values were 69%, 100%, 100%, and 89%, respectively, for *Aspergillus* serology, and 62%, 100%, 100%, and 77% for tissue fungal culture.

Results of this study suggest that *Aspergillus* serology and tissue fungal culture are specific for the diagnosis of aspergillosis in dogs with nasal disease and *Aspergillus* serology may be useful as a non-invasive screening test for canine nasal aspergillosis.

ABSTRACT #25

AIRWAY RESPONSIVENESS TO HISTAMINE, CARBACHOL AND ADENOSINE IN HEALTHY DOGS BEFORE AND AFTER CADMIUM CHLORIDE INHALATION. R.A. Hirt[1], K. Vondrakowa[1], A. Guija de Arespacochaga[2], A. Guetl[1], R. Van den Hoven[2]; [1]1st Medical Clinic, [2]Central Laboratory, Veterinary University Vienna, Austria.

We investigated the value of barometric whole body plethysmography (BWBP) in assessing the effects of low dose cadmium chloride inhalation on bronchoprovocation testing with directly (histamine, carbachol) and indirectly (adenosine 5´-monophosphate, AMP) acting agonists, in seven healthy one- to four-year-old Miniature pinschers. Airway responsiveness was measured before (BCC) and one day after (ACC) airway challenge with cadmium chloride. Dogs were placed in a BWBP chamber, and respiratory variables derived from changes in box pressure were calculated at baseline and after nebulization of increasing concentrations of histamine, carbachol and AMP. Airway responsiveness was determinated by increases in enhanced pause (PENH), a unitless variable measuring bronchoconstriction, derived from concentration-response curves. The endpoint chosen was the agonist concentration that increased PENH to 300% of baseline ("PCPENH300"). To demonstrate cadmium-induced airway inflammation, bronchoalveolar lavage fluid (BALF) cytology was analyzed before and after cadmium chloride inhalation.

BWBP was well tolerated in all dogs. Bronchoprovocation with all three agonists, and measurements of respiratory variables could be performed. After inhalation of cadmium chloride, PC PENH300 for all 3 agonists significantly decreased. Mean (±SD) PCPENH300 for histamine was 0.72±0.28mg/ml BCC, and 0.35±0.31mg/ml ACC ($p<0.02$), for carbachol 0.34±0.16mg/ml BCC, and 0.064±0.032mg/mlACC ($p<0.02$), for AMP 1000mg/ml BCC), and 415±398mg/ml ACC ($p<0.03$), BALF leukocyte counts increased from 728±104 BCC to 3255±1407 ACC; the BALF neutrophil percentage from 6.7±7.3% to 77.9±8.6% ($p<0.02$).

Low dose cadmium chloride challenge results in acute airway inflammation in dogs, as demonstrated by increased BALF leukocytes. Airway hyperresponsiveness, induced with cadmium chloride, can be detected with BWBP in conscious unrestrained dogs. Not only direct, but also indirectly acting agonists can elicit a response in acute non-allergic inflammation. Cadmium chloride challenge may be a valuable tool for investigating airway inflammation, as it partially mimics exposure to sidestream tobacco smoke.

ABSTRACT #26

CLINICAL OUTCOME OF FELINE HYPERTROPHIC CARDIOMYOPATHY WITH AND WITHOUT CONCURRENT LEFT VENTRICULAR OUTFLOW TRACT OBSTRUCTION. TC DeFrancesco, K Gebhardt, CE Atkins, DT Moore[1], BW Keene. College of Veterinary Medicine, North Carolina State University, Raleigh, NC and [1]University of North Carolina, Chapel Hill, NC.

Hypertrophic cardiomyopathy (HCM) is the most common feline heart abnormality. The pathology of HCM results in the thickening of the left ventricular wall (LVPWd) and interventricular septum (IVSd), which in some cats results in the narrowing of the LV outflow tract (OT). LVOT obstruction in humans has been shown to be a negative predictor of outcome, however, the effects of this narrowing in cats is unclear. This retrospective study compares the clinical outcome and survival times of cats diagnosed with asymptomatic HCM ± LVOT obstruction at NCSU Veterinary Teaching Hospital from 1984 to May 2003. Cats were included if they had been diagnosed with asymptomatic HCM or hypertrophy obstructive cardiomyopathy, complete echocardiographic data (ECHO), and IVSd or LVPWd ≥0.60 cm (n=110). Cats were excluded if they had congestive heart failure, saddle thrombus, cardiogenic pulmonary edema or pleural effusion, hyperthyroidism, hypertension, >7 years old without blood pressure or thyroid measurements, incomplete ECHO data, and no follow up information (n=375). The overall median age at diagnosis was 5.5 years. Of cats included in this study, 84/110 (76%) did not have LVOT obstruction, while 26/110 (24%) did have LVOT obstruction. The median age at diagnosis for cats without LVOT obstruction was 5.6 years, and the cats with LVOT obstruction was 4.9 years. This difference was not statistically significant. Thirty-six of 110 cats (33%) have died and 74 (67%) were still alive. Six cats were still alive more than eight years out from the date of diagnosis. The median follow-up for survivors was 3.2 years. The median survival time was 7.8 years (95% CI, 6.2 – 10.2 years). Of the 36 deaths, 13 (36%) were known to have been cardiac deaths, 18 (50%) were known to have been not cardiac related, and five (14%) deaths were of unknown origin. Within the unobstructed LVOT group 31/84 (37%) cats have died, while 5/26 (19%) of the obstructed group have died. This difference was not statistically significant. When examining covariates of interest (LVOT, IVSd, LVPWd, LV internal dimension at systole and diastole, fractional shortening %, left atrial dimension, gender and age at diagnosis) one at a time by Cox regression, only age at diagnosis showed to be a significant prognostic covariate, (the older the cat, the greater the probability of death, $p=0.0002$). While this is a large cohort, the number of events is fairly small. Continued follow-up will be of interest. In summary, we have failed to find evidence that LVOT status significantly affected the survival time of cats diagnosed with asymptomatic HCM.

ABSTRACT #27

OCCULT DILATED CARDIOMYOPATHY IN THE DOBERMAN PINSCHER: A RETROSPECTIVE STUDY OF PROGNOSIS IN 163 CASES. M.L. O'Sullivan, M.R. O'Grady, S.L. Minors, K.M.T. Kean, R. Horne. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Dilated cardiomyopathy (DCM) is the most common myocardial disease in dogs and a clinically important cause of congestive heart failure and sudden death in this species. DCM in Doberman pinschers, a breed experiencing a particularly high prevalence of the disease, has been the subject of investigation for a number of years at the Ontario Veterinary College.

The medical records of Doberman pinschers diagnosed with occult DCM were examined with the purpose of identifying prognostic indicators. Dogs were included if left ventricular (LV) eccentric hypertrophy was present on echocardiographic examination (LV internal dimension in diastole [LVIDd] > 49 mm or in systole [LVIDs] > 42mm) in the absence of mitral valve disease or other cardiac disease, and if clinical signs of cardiac disease were historically absent. 163 dogs were identified meeting these criteria. Cardiac endpoints included the onset of congestive heart failure (CHF) or sudden death (SD). Data collected from the records included gender, weight, age, auscultation findings, degree of ventricular arrhythmia, echocardiographic data including LVIDd, LVIDs, fractional shortening, E-point to septal separation (EPSS), and medication information. The influence of these variables on time to endpoint was examined by constructing a multivariate model using Cox proportional hazards analysis. Dogs were censored if they did not meet one of the cardiac endpoints.

Of the 163 Dobermans, 100 (61%) were male (68 neutered, 32 intact) and 63 (39%) were female (46 spayed, 17 intact). Average age at the time of diagnosis was 7.3 years (range 1.9-12.8 years). 126/163 (77%) reached a cardiac endpoint of CHF or SD, and the remaining 37/163 (23%) were censored. Reasons for censoring included euthanasia or death from non-cardiac disease (27), lost to follow-up (3), and still occult at the time of analysis (7). For the 126 non-censored dogs, mean and median times to cardiac endpoint were 435 days and 350 days, respectively. 92/126 (73%) developed CHF and 34/126 (27%) experienced SD. Mean and median times to CHF were 427 days and 326 days, respectively. Mean and median times to SD were 379 days and 344 days, respectively. The following variables were included in the multivariate model for time to CHF or SD, with the nature of the relationship indicated (+ for increased risk with presence of or increase in variable, and – for decreased risk with presence of or increase in variable): number of VPCs on a 3-minute ECG (+), presence of a left-sided heart murmur (+), LV free wall thickness in systole (-), number of days on an ACE-inhibitor (-), and LVIDs index (+) representing the % that the LVIDs exceeded the upper limit of normal LVIDs predicted by body weight.

A number of parameters routinely collected during clinical evaluation may provide insight into time to CHF or SD in occult DCM.

ABSTRACT #28

TOXICITY IN DOBERMAN PINSCHER DOGS WITH VENTRICULAR ARRHYTHMIAS TREATED WITH AMIODARONE. Marc S. Kraus[1], Laura G. Ridge[2], Anna R.M. Gelzer[1], Romain Pariaut[1], Sydney Moïse[1], Clay Calvert[2], [1]Cornell University, Ithaca, NY, [2]University of Georgia, Athens, GA.

Ventricular arrhythmias (VA), syncope and sudden death associated with cardiomyopathy are common in Doberman pinschers (DP). Amiodarone (AM) is a potent antiarrhythmic drug used for refractory cases of VA. However AM use is controversial due to its numerous side effects. The purpose of this study was to report adverse drug effects of AM treatment and outcome in DP at high risk of sudden death due to severe VA. Between 1996-2004 we evaluated 20 client-owned DP (12 males, eight females, mean age 7.58 years). All dogs displayed abnormal myocardial function by echocardiography (LVIDd > 50mm, LVIDs >40mm, FS <25%, EPSS ≥ 10mm). AM therapy was initiated when rapid ventricular tachycardia and or syncope either persisted or re-occurred while DP were receiving other antiarrhythmic drugs. Oral AM treatment consisted of a one- or two-week loading schedule followed by a once daily maintenance schedule with dosages listed in the table below. Calculated for bodyweight, the 400 mg maintenance schedule produced a dose range of 9.0-12.1 mg/kg and the 200 mg maintenance schedule produced a dose range of 4.3-6.3 mg/kg. Serum AM concentrations were acquired in nine of twenty dogs at one to 12 weeks after initiation of AM administration.

Serial CBC and serum chemistries were obtained in all dogs receiving AM. Six of 20 dogs (30%) experienced adverse effects attributed to AM.

Number of Dogs	Loading doses	Maintenance dose
1	400 mg q12h for 1st week	400 mg q24h
4	400mg q 12h for 1st week 300mg q12h for 2nd week	400 mg q24h
3	400 mg q12h for 1st week 400mg q24h for 2nd week	200 mg q24h
2	400 mg q12h for 1st week 200mg q12h for 2nd week	200 mg q24h
10	400 mg q12h for 1st week	200 mg q24h

AM toxicity consisted of vomiting, anorexia, thrombocytopenia, bilirubinemia, and elevation of hepatic enzyme activities that were detected from six weeks to eight months after initiation of treatment. Five of these six dogs were receiving 400mg q 24 hours and one dog was receiving 200 mg q 24 hours. Clinical signs and laboratory abnormalities resolved after AM was either discontinued or the dosage was reduced. Fourteen of 20 dogs died suddenly while receiving AM. The median survival time after initiation of AM was 114 (range 27-360) days. In conclusion, the incidence of AM toxicity in DP is high and in fact might be underestimated due to the large number of patients that died suddenly while receiving AM, possibly before developing signs of toxicity. AM therapy in DP should therefore be reserved for refractory cases of VA. Furthermore, AM did not prevent sudden death.

ABSTRACT #29

A COMPARISON OF THE CARDIOVASCULAR EFFECTS OF HYPERTONIC SALINE VERSUS PENTASTARCH IN HYPOVOLEMIC COLIC PATIENTS. Hallowell, GD and Corley, KTT. Royal Veterinary College, Hertfordshire, London, UK.

The aims of this clinical trial were to investigate the cardiovascular effects of pre-anesthetic administration of either hypertonic saline or pentastarch in hypovolemic colic patients under anesthesia.

Thirty horses requiring surgery for acute abdominal pain were enrolled in the study. Inclusion criteria were owner consent, and meeting two of the following three parameters: packed cell volume (PCV) was greater than 45%, total solids (TS) greater than 80 g/L and/or serum lactate concentrations greater than 2.5mmol/L. Study horses were randomly assigned to receive either 4ml/kg of hypertonic saline (7.2% NaCl; n=14) or pentastarch (a 10% solution of a 200kD mean molecular weight hydroxyethylstarch; n=16) as a bolus given 45 to 60 minutes prior to induction of anesthesia. All horses also received a bolus of 20ml/kg lactated Ringer's solution.

All horses were sedated with intravenous xylazine, and anesthetized with ketamine and diazepam and maintained with either halothane or isoflurane in 100% oxygen. Parameters measured every five minutes included direct blood pressure from the facial artery, heart rate, respiratory rate, end-tidal carbon dioxide and inhalation agent. In addition cardiac output (CO), stroke volume and systemic vascular resistance were measured using lithium dilution indicator method of

cardiac output monitoring every 30 minutes (LiDCOplus, LiDCO, Cambridge, England, UK). Dobutamine was used to maintain mean arterial blood pressure >70mmHg (9.3KPa). If horses remained hypotensive with dobutamine treatment, pentastarch and low dose norepinephrine were administered.

The mean arterial blood pressure (MABP) at 90 minutes from induction was significantly greater for those horses that received pentastarch compared to hypertonic saline. There was a trend towards higher MABP at 30 and 60 minutes. Cardiac index (CO/kg bwt) was significantly greater at 60 and 120 minutes in the horses that received pentastarch compared to hypertonic saline, with a trend towards higher cardiac indices 30 and 90 minutes from induction. There were no significant differences between the two groups regarding PCV, TS, lactate at admission, type of surgical colic (strangulating versus non-strangulating and large intestine versus small intestinal), MAC multiples, peri-operative fluid administration (including the requirement for extra pentastarch), heart rate, stroke volume, systemic vascular resistance, stroke volume index, amount of dobutamine, norepinephrine or other drugs administered.

This study suggests that pentastarch provides more favourable hemodynamics during anaesthesia when administered to hypovolemic patients when compared to hypertonic saline. This effect lasts for up to three hours post- administration.

ABSTRACT #30

ERYTHROCYTOSIS IN EQUINE LIVER DISEASE. Alessandra Pellegrini-Masini[1] and Brett A. Dolente[2]. [1] College of Veterinary Medicine, Auburn University, Auburn, Al. [2] New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.

In human medicine, the occurrence of erythrocytosis has been documented in the presence of liver disease of different nature (including hepatocellular carcinoma, hepatic adenoma, liver fibrosis, liver cirrhosis, focal nodular hyperplasia and hepatopulmonary syndrome). In the veterinary literature, erythrocytosis was reported with hepatic neoplasia in two cows (hepatocellular carcinoma), a sheep (colangiocellular carcinoma) and three horses (hepatocellular carcinoma and hepatoblastoma). The purpose of this study was to evaluate the possibility of occurrence of erythrocytosis in equine liver disease.

The medical records of horses diagnosed with liver disease at the Widener Large Animal Hospital of the University of Pennsylvania in the years 1980 to 2004 were evaluated in a retrospective study. Criteria of inclusion in the study included: age of one year or older, primary diagnosis of liver disease confirmed by histopathology, hospitalization time of one day or longer, and administration of fluid therapy. The records of 29 horses met these conditions. Packed cell volume (PCV) and total protein were evaluated on admission, and at 12, 24, 36 and 48 hours after admission. The volume of fluids administered in the first 48 hours in the hospital, and the body weight (when recorded) were noted. On admission, plasma activity of gamma-glutamyl transferase, aspartate amino-tranferase, sorbitol dehydrogenase, as well as bile acids, creatinine, total and direct bilirubin, and ammonia levels were evaluated. Based on the PCV after fluid therapy, the horses were divided in two groups. Group 1 (n=7) included horses whose PCV remained above 50% despite fluid therapy. Group 2 (n=22) included horses with PCV lower than 50% after fluid therapy. Statistical analysis consisted of Student's T test.

The average PCV of Group 1 on admission was 55 % (± 4.9 %) and after fluid therapy 51 % (±1.89 %). The average PCV of Group 2 on admission was 42 % (± 7.6 %) and after fluid therapy 39 % (± 6.9 %). Group 1 had a significantly higher PCV, but no significant difference in total protein, compared to Group 2, at all the considered time points. Plasma creatinine on admission was not significantly different among the two groups. No significant difference in any of the above mentioned biochemical parameters was observed between the two groups. The volume of fluids received was higher for the horses in Group 1, but the difference was not significative.

These results support the occurrence of an abnormally elevated PCV unresponsive to fluid therapy, in the absence of clinical features of dehydration, in a limited number of horses affected by liver disease.

ABSTRACT #31

PERIPHERAL-BLOOD MONONUCLEAR CELL mRNA CYTOKINE EXPRESSION IN HORSES TREATED WITH DEXAMETHASONE. Monteiro FG, Buechner-Maxwell VA, Witonsky SG, Huckle WR, Ward D. Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg VA.

Glucocorticoids are widely used in horses for a variety of autoimmune and inflammatory conditions. Their potent antiinflammatory properties have been associated with the suppression of a number of different inflammatory cytokines. The purpose of the study was to evaluate the effect of dexamethasone treatment in horses on mRNA cytokine expression, including interleukin-1β, interferon-γ, interleukin-4 and interleukin-6, during a five day treatment period and a five day post treatment period.

A randomized complete block design was performed on 14 healthy horses. Group I (7 horses) received 0.1 mg/kg of dexamethasone sodium phosphate by intravenous injection once daily for five days. Group II (7 horses) received an equivalent volume of sterile saline by intravenous injection daily for five days. A sample of 5x10 mililiters of blood in acid citrate dextrose was obtained prior to initial treatment. Thirty minutes after each treatment injection (placebo or dexamethasone) a sample of blood was obtained during the 5 day treatment period and 24, 48, 72, 96 and 120 hours after the last treatment injection was administered. Peripheral-blood mononuclear cells were isolated from the blood samples and stimulated with Concavalin A. RNA was isolated using the QIAGEN RNeasy kit. cDNA first strand synthesis was achieved using QIAGEN's OMMISCRIPT RT KIT. cDNA was also constructed for the house keeping gene β actin. Primer pairs specific for each cytokine were designed using equine cytokine sequences available on Genbank. cDNA for each cytokine and β actin was amplified using Real Time PCR technique

Interleukin-4, interleukin-6 and interferon-γ mRNA expression was significantly suppressed in horses treated with dexamethasone when compared to control horses. Interleukin-1β was only significantly suppressed on day 5. Interleukin-4, interleukin-6 and interferon-γ mRNA expression suppression was initially observed on day 2 and lasted 24 hours after the last dose of dexamethasone was administered. Interleukin-6 mRNA expression was significantly higher when compared to the control group on day 10.

Our results suggest that dexamethasone treatment of healthy horses suppresses mRNA expression of several cytokines, including interleukin-4, interleukin-6 and interferon-γ. This effect could explain part of corticosteroids' mechanism of action for controlling inflammation in a variety of disease conditions. The time-course effect of dexamethasone showed that the effect on mRNA cytokine expression suppression is only observed on day 2 of treatment and mRNA suppression is maintained for 24 hours after discontinuation of treatment.

ABSTRACT #32

THE EFFECT OF SODIUM AND POTASSIUM CHLORIDE SUPPLEMENTATION ON SERUM ELECTROLYTE CONCENTRATIONS, WATER INTAKE, BODY WEIGHT AND PERFORMANCE OF ENDURANCE HORSES. F. Sampieri, H.C. Schott, K.W. Hinchcliff, R.J. Geor. The Ohio State University, Columbus, OH: Michigan State University, East Lansing, MI; and University of Guelph, Guelph, Ontario.

Horses lose large quantities of sodium, potassium and water during endurance racing and are often supplemented with electrolytes orally. We speculated that supplementation with higher doses of sodium chloride and potassium chloride would increase water intake and decrease body weight loss of horses competing in an endurance ride. In a randomized, blinded, cross-over study eight Arabian or mixed breed horses (age range: 9 to 17 years, average 11.8 years; weight average: 441.5 kg, range: 383 - 529 kg) participated in two 50 mile endurance races. Four horses received 0.22 g/kg NaCl and 0.074 g/kg KCl (high dose, approximately equal to losses in 30 L sweat, HD) and the other four received 0.07 g/kg NaCl and 0.02 g/kg KCl (low dose, approximately equal to losses in 10 L sweat, LD) orally before and at 25 miles of the first ride. Horses received the alternate treatment in the second ride 28 days later. Rides were run over the same course. Body weight, blood for measurement of serum electrolyte concentrations, and estimated water intake were measured, collected or recorded with an interview form within 30 minutes of the start, 25 miles into the ride, within 30 min of finishing and 2 hours after finishing. Data were analyzed by 2-way repeated measures analysis of variance ($P \leq 0.05$, mean ± SEM). There was a significant effect of treatment on serum sodium and chloride concentrations and water intake, but not body weight. Serum sodium concentrations were significantly higher at 50 miles in horses receiving HD (149 ± 1 vs 143 ± 1 mEq/l), but were not different before or during recovery. Serum chloride concentrations were significantly higher at 50 miles in horses receiving HD (110 ± 1 vs 102 ± 1 mEq/l), but were not different before or during recovery. Serum potassium concentration was not affected by treatment ($P = 0.053$) and was 2.9 ± 0.1 vs 2.4 ± 0.1 mEq/l for HD and LD at the end of the ride. Water intake was significantly greater at 25 and 50 miles in horses receiving HD (47.3 ± 2.8 l vs 24.6 ± 2.8 l at 25 miles, and 36 ± 2.8 vs 30.3 ± 2.8 l at 50 miles). Overall water consumption was different between treatments (100.3 ± 9.5 vs 75 ± 8.5 for HD and LD, respectively). There was no difference in percentage of body weight lost during the race by HD or LD treated horses ($p = 0.36$, -3.8 ± 0.3 vs -4.0 ± 0.3% immediately after racing). Time to complete the ride was not significantly different between treatments (534 ± 24 and 540 ± 22 min, HD and LD, respectively). HD caused hypernatremia, hyperchloremia and increased water intake, but did not attenuate body weight losses or improve performance compared to a lower dose of electrolyte supplement. Based on the results of this study, supplementation of horses during endurance racing with dosages of NaCl of 0.22 g/kg is not warranted, as this dose may induce abnormalities in electrolyte concentrations without providing any apparent competitive advantage.

ABSTRACT #33

THE EFFECT OF DETOMIDINE SEDATION ON RESPIRATORY SYSTEM RESISTANCE AND HISTAMINE BRONCHOPROVOCATION MEASURED WITH FORCED. OSCILLATORY MECHANICS. Rose Nolen-Walston, Melissa Mazan, Daniela Bedenice, Andrew Hoffman. Tufts University School of Veterinary Medicine, Pulmonary Function Laboratory, N. Grafton, MA.

With pulmonary disease second only to lameness as a cause of decreased performance in saddle horses, accurate measurements of respiratory function are essential for thorough evaluation of the underachieving equine athlete. Forced oscillatory mechanics (FOM) is a non-invasive, sensitive, and user-friendly technique to measure baseline respiratory system resistance (R_{RS}), and can easily be coupled with nebulized histamine bronchoprovocation to evaluate airway hyperreactivity in the horse with suspected Inflammatory Airway Disease. Although the testing is non-invasive, sedation is essential to ensure the compliance of most patients who undergo evaluation. *In vitro* data indicates that α-2 agonists have a bronchodilatory effect on pre-constricted airways. The purpose of this study was to evaluate the effect of intravenous detomidine on R_{RS} at baseline and after histamine bronchoprovocation in horses without Recurrent Airway Obstruction (RAO).

Out of 19 enrollees, nine adult horses (with no evidence of RAO) successfully completed a randomized crossover study of FOM using detomidine at 0.01mg/kg IV (D+) or no sedation (D-) with a 48 hour washout period between tests. R_{RS} was measured at 1, 2, and 3 Hz at baseline and with exponentially increasing concentrations of histamine nebulization (see chart). The data were analyzed using a modified Bland and Altman's technique of measuring agreement (*Lancet* 1986).

	1Hz (cmH_2O/L/sec)			2Hz (cmH_2O/L/sec)			3Hz (cmH_2O/L/sec)		
	n	Mean Δ [95% CI]	%Δ	n	Mean Δ [95% CI]	%Δ	n	Mean Δ [95% CI]	%Δ
Baseline	4	0.06 [-0.07– 0.20]	-15	5	0.07 [-0.08– 0.21]	-12	**9**	**0.10[-0.002– 0.21]**	**-14**
0.9% saline	5	0.13 [-0.01– 0.27]	-19	**7**	**0.05 [-0.07– 0.18]**	**-9**	**9**	**0.01[-0.11– 0.13]**	**1**
Hist. 4mg/ml	5	-0.25 [-0.47– -0.03]	36	**7**	**-0.06 [-0.28– 0.16]**	**7**	**9**	**0.03[-0.04– 0.11]**	**-4**
Hist. 8mg/ml	5	-0.13 [-0.57– 0.31]	18	**6**	**-0.05 [-0.34– 0.24]**	**7**	**9**	**-0.02[-0.18– 0.14]**	**7**
Hist. 16mg/ml	3	0.12 [-0.17– 0.41]	0.4	4	0.02 [-0.33– 0.36]	10	**6**	**0.01[-0.25– 0.27]**	**6**
Hist. 32mg/ml	3	-0.16 [-0.54– 0.22]	3	2	0.12 [-0.15– 0.39]	-9	4	0.24[0.02 – 0.46]	-13

In groups of >5 horses (bold), the mean intra-horse difference [Δ = (D-)-(D+)] in R_{RS} at a given dose of histamine was less than 0.1 cmH_2O/L/s (<15% variation), and the 95% confidence intervals of standard error were narrow and included 0. However, due to the inherent difficulty in testing un-sedated horses, many incomplete data sets made the evaluation of dose-response curves and the comparison of some lower frequency data (1 and 2Hz) unrewarding. Detomidine was found to have small, non-directional effects on R_{RS} and bronchoprovocation, and evidence of detomidine-induced bronchodilation was not observed.

ABSTRACT #34

CONCENTRATION OF NITRIC OXIDE IN BRONCHOALVEOLAR FLUID IN HORSES WITH HEAVES. Susana Macieira, Daniel Jean, Jean-Pierre Lavoie. Faculté de Médecine Vétérinaire, Université de Montréal, Qc, Canada.

Nitric oxide (NO) has been shown to modulate the immune response in various inflammatory diseases. NO is synthesized by numerous cells normally present in the respiratory tract and could therefore be implicated in amplification and perpetuation of airway inflammation in heaves. The purpose of this study was to determine the concentration of nitric oxide in bronchoalveolar lavage fluid of heaves-affected horses and normal horses.

Ten adult horses weighing approximately 450 to 650 kg were studied. Horses had normal CBC results and endoscopy of the upper respiratory tract did not reveal any abnormalities. Horses were diagnosed with heaves (n=5), or were considered to be free of respiratory disease (control horses; n=5) on the basis of history, clinical examination, and pulmonary function measurements. All horses were stabled in the same barn and were exposed to a dusty environment and moldy hay for several weeks before the beginning of the study. BAL was performed under endoscopic guidance in the right lung using two 250 ml bolus of warm isotonic saline and aspirated with a suction pump. The same procedure was then

repeated in the left lung. BAL fluid was centrifuged and the supernatant was frozen at –80ºC until analyzed. Concentration of NO in each BAL sample was indirectly measured by determining the concentrations of reactive nitrogen intermediate (nitrate and bound NO) using a chemiluminescent method.

Heaves-affected horses had significantly ($p < 0.05$) higher transpulmonary pressure (ΔPpl, 17-67 cm of H2O), pulmonary resistance (RL, 1.307-3.079 cm of H2O/l/s). and dynamic elastance (EL, 1.313-4.995 cm of H2O/l) at baseline compared with control horses. The percentage of neutrophils in BAL was also significantly greater in horses with heaves (mean + standard error, 34.5 + 9.374 %) compared to controls (11.8 + 2.692 %). BAL fluid concentration of nitric oxide in heaves-affected horses was slightly but not significantly greater in the right lung (7.090 + 1.397 µmol/ml) than in the left lung (5.694 + 0.917 µmol/ml). However, nitric oxide concentration in BAL fluid did not differ between the two groups of horses and was not correlated with BAL neutrophilia.

In conclusion, the results of this study suggest that NO is not likely to be an indicator of chronic airway inflammation in horses with heaves.

ABSTRACT #35

EFFICACY OF THEOPHYLLINE COMBINED WITH A LOW DOSE OF DEXAMETHASONE FOR THE TREATMENT OF HORSES WITH HEAVES. Carla Cesarini, Emma Hamilton, Valérie Picandet, Jean-Pierre Lavoie. Faculty of Veterinary Medicine, University of Montreal, Quebec.

Corticosteroids are currently the most effective drugs for the treatment of heaves, but their long-term administration is often hampered because of a concern of the development of severe side effects. Theophylline is a bronchodilator with anti-inflammatory effects but its narrow therapeutic index has limited its use in the management of equine heaves. Several recent clinical studies demonstrate that the administration of low dosages of theophylline have a steroid-sparing effect in human asthmatics. The aim of the present study was to evaluate if theophylline administration potentiates the effects of a low dose of dexamethasone in horses with heaves.

Ten heaves affected horses were randomly allocated to four treatment groups (n=6) during three experimental periods, using an incomplete cross-over design. Horses were administered daily, for 7 days, either dexamethasone at 0.05 mg/kg IV SID (group A), 0.02 mg/kg SID PO alone (group B) or combined with 5 mg/kg BID of theophylline (group C), or the same dose of theophylline alone (group D). All horses were stabled together, fed hay and bedded on straw. A 4-week washout period separated each experimental treatment. Respiratory mechanics measurements were performed before drug administration, on days 3 and 7 and after atropine administration at the end of the study. Serum concentration of theophylline (groups C and D), dexamethasone (group C) and cortisol (group C) were measured on days 2 and 5. Horses were evaluated daily during the study.

A significant improvement ($p<0.05$) in airway function was observed only in horses from group A and following atropine administration. Peak and trough serum levels of theophylline measured on days 2 and 5 were within the expected therapeutic range. In group C, serum levels of dexamethasone were detectable in all horses and combined with a suppression of serum cortisol concentrations.

In summary, the oral administration of theophylline for seven days did not improve the airway function of horses with heaves and did not potentiate the effects of a low dose of dexamethasone.

ABSTRACT #36

MODEL TO ESTIMATE THE PROBABILITY OF SURVIVAL, WITHIN TWO HOURS OF HOSPITALIZATION, IN FOALS ≤ 7 DAYS OF AGE. B. Rohrbach[1], B. Buchanan[1], J. Drake[1], F. Andrews[1], F. Bain[2], D. Byars[2], B. Bernard[3], M. Furr[4], M. Paradis[5], J. Lawler[6], S. Giguere[7], B. Dunkel[8] [1]University of Tennessee, Knoxville TN [2]Hagyard, Davidson, McGee Equine Hospital, Lexington KY, [3]Rood & Riddle Equine Hospital, Lexington KY, [4]Marion Dupont Equine Hospital, Leesburg, VA, [5]Tufts University, Boston MA, [6]Peterson Smith Equine Hospital, Ocala FL, [7]University of Florida, Gainesville FL, [8]University of Pennsylvania, Kennett Square PA.

The purpose of the study was to identify a combination of characteristics that can be measured within two hours of admission and used as a diagnostic test for survival in hospitalized foals ≤ 7 days of age. The results of the diagnostic test (model) when combined with the clinician's initial assessment can provide clients with an adjusted probability of survival. Medical records of foals from one university and two private equine hospitals for years 2000-2002 were reviewed, and 1,026 of these included a foal ≤ 7 days of age and had an outcome (died, euthanized or discharged alive) recorded. Historical, physical and laboratory information were extracted from each foal's medical record along with historical information for the mare. Foals that were euthanized were excluded from the study leaving 910/1,026 (89%) for further analyses. Univariate analyses were used to identify those characteristics of the mare and foal that were associated with foal survival. Using the p-value associated with the univariate comparison, combined with the biological plausibility of the association of the characteristic with outcome, individual characteristics were entered into a logistic regression model in a forward stepwise manner. The final logistic regression equation included six variables; presence or absence of a suckle reflex, ability to stand, temperature, white blood count, serum creatinine and anion gap. The ability of the model to predict survival in hospitalized foals ≤ 7 days was validated on a group of foals (n=145) admitted to five equine hospitals, other than those used to generate the original model, during 2004. Sensitivity, specificity, positive and negative predictive values for the retrospective model to predict survival in foals were 90%, 74%, 95% and 60%, respectively. Likelihood ratios were also generated from this model. When applied to foals in 2004 the sensitivity and specificity, positive and negative predictive values to predict survival were 87%, 54%, 91% and 45%, respectively. A positive predictive value of 91% in the population of foals on which the model was validated indicates that the model is useful to predict survival; however, a 45% probability of non-survival, given a negative test result, implies that the owner be should be cautioned as to a substantial risk of non-survival. This model can be used to assist clinicians in providing quantitative information on the probability of foal survival to the client prior to referring the foal to a hospital or within a short time after hospitalization.

ABSTRACT #37

GASTROCNEMIUS RUPTURE IN NEONATAL FOALS: 6 CASES (2000-2003). Sophy A. Jesty, Jonathan E. Palmer, Eric J. Parente, Thomas P. Schaer, Pamela A. Wilkins. University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square PA.

Gastrocnemius rupture is uncommon in the foal but should be suspected in any neonatal foal with an inability to rise or with abnormal hindlimb conformation or gait. This retrospective study was undertaken to characterize the clinical presentation and course of neonatal foals diagnosed with ruptured gastrocnemius muscles. Records from six cases presenting between 2000-2003 with a primary or secondary diagnosis of gastrocnemius disruption were reviewed.

Dystocia and assisted delivery was reported in 5/6 foals. Many of the foals had significant additional medical problems, including

gastrointestinal disease (4/6), other musculoskeletal abnormalities (4/6), hypoxic ischemic encephalopathy (2/6), sepsis (2/6), renal disease (1/6), and congenital cardiac malformation (1/6). Treatment specific for gastrocnemius rupture was attempted in 3/6 foals, and consisted of limb stabilization using splints, bandaging, and exercise restriction. In one foal, splinting was associated with significant additional morbidity. Four foals were euthanized, two due solely to the gastrocnemius rupture, and two due to the combination of this and other significant problems. For the two survivors, treatment of the gastrocnemius rupture necessitated protracted hospitalization beyond that predicted for the other problems, resulting in considerable additional cost to the owner. Both of these foals were broken to ride. One of these foals has a mechanical lameness associated with damage from the splint (four years later) and is kept at pasture. The other foal raced twice as a 2-year-old, but has not raced as a 3-year-old.

The outcome of neonates with a ruptured gastrocnemius is often complicated by the presence of other significant abnormalities. Mild tears may be an incidental finding and may heal well without intervention. Moderate to severe tears require specific therapy, including placement of appropriate splints and exercise restriction. In our experience the best method of splinting for this problem is a custom fit hemicircumferential fiberglass splint placed on the dorsal aspect of the leg. Pressure sores can develop rapidly and any splinting regimen requires diligent adjustment and careful monitoring of the underlying soft tissues. The prognosis for elite athletic use in surviving foals appears poor.

ABSTRACT #38

LACTATE CONCENTRATION IN FOALS PRESENTING TO A NEONATAL INTENSIVE CARE UNIT: ASSOCIATION WITH OUTCOME. Wotman K, Palmer JE, Boston RC, Wilkins PA. University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square PA.

Interest in lactate concentrations ([LAC]) as a predictor of survival or indicator of response to therapy in equine neonates admitted to Equine Neonatal Intensive Care Units (NICU) has increased with the availability of blood gas (BG) analyzers that report lactate concentrations. [LAC] increases under anaerobic conditions, with sepsis associated hypermetabolism, cytokine induced pyruvate dehydrogenase blockade, inflammatory cell metabolism and sepsis induced epinephrine surge. Lactate also serves as a carbohydrate substrate and energy source. Lactate clearance occurs primarily in the liver and muscle. The sensitivity and specificity of a single lactate measurement as a predictor of outcome is debated, while evidence is accumulating that persistence (>24hr) of increased [LAC] is associated with increased mortality rates in foals and humans. Conversely, early lactate clearance is associated with improved outcome. We hypothesized that failure to resolve increased [LAC] within 48 hours of admission to a NICU is associated with poor outcome. [LAC] was measured at admission using a commercial BG analyzer in ~200 neonatal foals admitted to the Graham French Neonatal Intensive Care Unit between 2002 and 2004. [LAC] was also determined in foals surviving at 24 and 48 post admission that had additional BG. Foals were categorized by condition as either alive (A), died (D) or euthanized (E) for each sampling period. Ninety-seven surviving foals did not have additional BG at 24 hours and an additional 36 survivors did not have BG at 48 hours, all due to good clinical progress not requiring further BG determinations. Foals euthanized for purely economic reasons or due to poor perceived prognosis on the part of the clinician or owner have been included in this data set.

In a preliminary evaluation of the data set Kruskall-Wallace testing revealed that lactate concentration was significantly different between outcome groups at all testing times. Using logistic regression it was determined that odds of poor outcome (D or E) increased by 23% (Admission), 43% (24 hours) and 68% (48 hours) for each 1.0 mmol/L increase in [LAC] at sequential time periods, suggesting that foals failing to clear lactate, or having increased lactate production and accumulation, have a poorer prognosis. These data suggest that [LAC] may be clinically useful in foals presenting to NICU and that sequential monitoring of [LAC] may provide reliable prognostic information. Foals that rapidly clear lactate may have an improved prognosis. Additional investigation is warranted.

ABSTRACT #39

JUVENILE HYPER-REACTIVE AIRWAY DISEASE IN THE FOAL: A DESCRIPTION OF THE SYNDROME AND IDENTIFICATION OF RISK FACTORS. P Heidmann[1], JLWatson[1], NM Slovis[2], IA Gardner[1], WD Wilson[1]. [1]University of California, Davis, CA, [2]Hagyard-Davidson-McGee Assoc, Lexington KY.

The purpose of this study was twofold: to characterize the syndrome of hyper-reactive airway disease in foals, and to describe farm-related environmental risk factors associated with development of the clinical syndrome.

Medical records of foals presenting for respiratory disease to the UC Davis Veterinary Medical Teaching Hospital (UCD) and Hagyard-Davidson-McGee Associates (HDM) were reviewed. Foals age three to 12 months, presenting between 1/1/1999 and 12/31/2003, were considered eligible for inclusion. In order to limit cases to those with clinically significant bronchoconstriction but without primary bacterial pneumonia, all foals with large numbers of pathogenic bacteria, or any number of *Rhodococcus equi*, noted on cytology or culture of trans-tracheal wash were excluded. Foals with plasma fibrinogen levels greater than 600 mg/dl were excluded. Clinicopathologic, microbiologic, and imaging data were collected for each patient when available, and descriptive statistics were applied. A survey was created and sent to each farm with affected foal(s) to identify potential management-related environmental risk factors for farms in each geographic region. This survey included questions regarding animal housing, care, and husbandry, and was intended to elicit information related to theoretical risk factors such as air quality, stocking density, preventative care strategies (including insect control and vaccination protocols), and the effects of local agricultural practices. Meteorological data for each case at the time of presentation were obtained, including local ambient temperature, solar radiation, humidity, barometric pressure, and precipitation.

A total of 73 cases (37 from HDM and 36 from UCD) were identified from 49 farms in California and Kentucky. Presentation was seasonal in both regions, with 71% of cases occurring during the months of June through September. The farm-related factors positively associated with development of hyper-reactive airway disease included housing of foals on straw, previous outbreaks of respiratory disease on the premises, and proximity to local agriculture.

In this study we describe the syndrome of Juvenile Hyper-reactive Airway Disease, distinct from known causes of infectious pneumonia, which occurs in foals ages three to 12 months, and conclude that specific farm-related and environmental risk factors play a role in the development of the disease.

ABSTRACT #40

BILE ACID CONCENTRATION IN HORSES WITH DIARRHEA. Pamela A Wilkins, Imogen Johns, Brett Dolente, Robert Poppenga, University of Pennsylvania School of Veterinary Medicine.

Abnormalities in liver function and liver enzyme activity have been recognized in horses with gastrointestinal diseases, most recently proximal enteritis.[1] Clinically, we have recognized significant hyperammonemia in some horses with diarrhea and horses with diarrhea are known to be at risk for development of DIC, perhaps associated with decreased liver origin clotting factors.[2] We hypothesized that horses with diarrhea have hepatic dysfunction.

As a preliminary investigation, bile acid concentrations were measured at admission and 24 and 48 hours post-admission in 21 mature horses presenting to the George D. Widener Hospital for Large Animals at New Bolton Center with a complaint of diarrhea of less than five days duration between November 2002 and July 2003. Seven of 21 horses were euthanized prior to sampling completion due to severity of clinical signs, including unresponsive DIC (6), hypotensive shock (5), laminitis (3) and unresponsive hyperammonemia (3). Diagnoses included Clostridiosis (7), Salmonellosis (2), Potomac Horse Fever (2) and unknown (10). Mean ± SE (N) bile acid concentrations were 8.2 ± 1.3 μmol/L at admission (21), 6.4 ± 1.4 μmol/L at 24 hours (15), and 19.2 ± 5.3 μmol/L at 48 hours (12) and were outside the normal range (3-11 μmol/L) for our laboratory only at 48 hours post-admission. However, 13/21 horses had at least one bile acid determination outside the normal range during at least one sample period, range 11.0-55.8 μmol/L. Bile acid concentration significantly increased between 24 and 48 hours (p= 0.032). During this period, bile acid concentration decreased for the two non-survivors still in the study and increased in survivors. Although not significant, probably due to low statistical power, non-survivors tended to have larger bile acid concentrations at admission when compared to survivors (11.4 ± 2.5 μmol/L *vs.* 6.6 ± 1.5 μmol/L, two-sample T test, p= 0.074).

Bile acid metabolism appears to be abnormal in horses with diarrhea. Bile acid concentration may increase with anorexia, as with bilirubin. However, many of the measured concentrations were increased above those expected for anorexia alone. Potential mechanisms of hepatic injury include ascending infection, absorption of endotoxin or inflammatory mediators from the portal circulation, or hepatic hypoxia associated with poor cardiac output, shock or abdominal compartment syndrome. Further investigation of liver injury and function in horses with diarrhea is warranted.

[1] Davis JL, Blikslager AT, Catto K, et al. A retrospective analysis of hepatic injury in horses with proximal enteritis (1984-2002). J Vet Intern Med. 2003; 17:896-901.

[2] Dolente BA, Wilkins PA, Boston RC. Clinicopathologic evidence of disseminated intravascular coagulation in horses with acute colitis. J Am Vet Med Assoc. 2002; 220:1034-8.

ABSTRACT #41

EXPERIMENTAL HYPERCALCEMIA IN HORSES RESULTS IN HYPOMAGNESEMIA, HYPOKALEMIA, AND HYPERPHOSPHATEMIA WITH INCREASED URINARY EXCRETION OF ELECTROLYTES. Toribio RE,[1] Kohn CW,[2] Rourke KM,[2] Levine AL,[1] Rosol TJ[1]. [1]Departments of Veterinary Biosciences and [2]Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Electrolyte disturbances are common in critically ill humans and animals. In critically ill horses, low serum ionized calcium (Ca^{2+}) and ionized magnesium (Mg^{2+}) concentrations are frequent findings. High serum Ca^{2+} is common in horses with chronic renal failure, hypercalcemia of malignancy, hyperparathyroidism, and vitamin D intoxication. There is evidence that the renal reabsorption of Ca^{2+} is also regulated by parathyroid hormone (PTH)-independent mechanisms, primarily by the calcium-sensing receptor (CaR) that affects the transepithelial transport of Ca^{2+} and Mg^{2+}. Because extracellular Ca^{2+} can affect other electrolytes, the goals of this study were to evaluate the effects of experimental hypercalcemia on the serum concentrations of electrolytes and their urinary excretion in healthy horses. By the direct effects of Ca^{2+} on CaR, we speculated that hypercalcemia will result in hypomagnesemia and increase the urinary excretion of electrolytes.

Hypercalcemia was induced in twelve healthy mares; six were infused with 23% calcium gluconate (G) for 120 min and six mares were infused with 10% calcium chloride ($CaCl_2$) for 120 min. Blood was collected to measure serum electrolytes, PTH, and insulin concentrations, and urine was collected to determine the fractional excretions of Ca^{2+} (FCa), Mg^{2+} (FMg), Na^+ (FNa), Pi (FP), K^+ (FK), and Cl^- (FCl).

In hypercalcemic mares, serum Ca^{2+} increased 6.6±0.1 to 9.7±0.3 (G) and 6.4±0.1 to 10.2±0.10 mg/dL ($CaCl_2$); Mg^{2+} decreased 0.52±0.02 to 0.33±0.02 (G) and 0.51±0.02 to 0.32±0.02 mmol/L ($CaCl_2$); K^+ decreased from 4.3±0.1 to 3.4±0.1 (G) and 4.2±0.2 to 3.6±0.2 mEq/L ($CaCl_2$), and Pi increased from 3.4±0.2 to 4.8±0.3 (G) and 2.9±0.2 to 4.1±0.4 mg/dL ($CaCl_2$) (all $P<0.05$). PTH decreased to very low concentrations. No changes in insulin concentrations were detected. FCa increased from 5.4±1.0 to 56.2±6.9% (G) and 5.4±1.1 to 47.7±6.4 % ($CaCl_2$); FMg from 23.5±2.4 to 55.0±4.5 (G) and 28.5±4.3 to 54.4±7.3 % ($CaCl_2$); FNa from 0.09±0.04 to 4.3±0.8 (G) and 0.03±0.003 to 4.8±0.8 % ($CaCl_2$); FK from 45.4±7.6 to 116.6±19 (G) and 38.4±1.5 to 89±8.3% ($CaCl_2$); FCl from 0.65±0.1 to 5.6±1.5% (G) and 0.7±0.1 to 9.3±1.6% ($CaCl_2$), and FP from 0.04±0.02 to 0.5±0.2 (G) and 0.14±0.06 to 0.81±0.3 % ($CaCl_2$). Urine specific gravity and osmolality decreased and urine output increased (all $P<0.05$).

In conclusion, hypercalcemia results in hypomagnesemia, hypokalemia, and hyperphosphatemia, increases the urinary excretion of Ca^{2+}, Mg^{2+}, K^+, Na^+, Pi, and Cl^-, and has diuretic effects. This study has clinical implications as excessive administration of Ca^{2+} salts can further increase the waste of electrolytes, in particular of Mg^{2+}.

ABSTRACT #42

ALTERATIONS IN SERUM PARATHYROID HORMONE AND ELECTROLYTE CONCENTRATIONS AND URINARY EXCRETION OF ELECTROLYTES IN HORSES WITH EXPERIMENTAL ENDOTOXEMIA. Toribio RE,[1] Kohn CW,[2] Hardy J,[1] Rosol TJ[1]. [1]Departments of Veterinary Biosciences and [2]Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

We have reported that hypocalcemia and hypomagnesemia are frequent findings in horses with clinical evidence of sepsis and endotoxemia. We hypothesized that in horses, endotoxemia triggers a systemic inflammatory response that results in hypocalcemia and hypomagnesemia. The goal of this study was to determine the effect of endotoxin (LPS) administration to healthy horses on serum parathyroid hormone (PTH), ionized (Ca^{2+}) and total calcium (tCa), ionized (Mg^{2+}) and total magnesium (tMg), phosphate (Pi), potassium (K^+), sodium (Na^+), chloride (Cl^-) and insulin concentrations, and on the urinary excretion of these electrolytes.

Twelve mares were infused with *E. coli* LPS (30 ng/kg/hr/IV) for one hour. Six mares were infused with saline (controls). In LPS-infused horses, heart rate increased significantly from 40.0±1.3 to 70.0±9.0 beats/min, respiratory rate from 12.7±1.0 to 21.1±3.0 breaths/min, body temperature from 37.4±.03 to 38.9±0.6 °C, and TNF-∀ concentrations from 6.6±3.5 to 507±260 pg/mL ($P < 0.05$). White blood cell count decreased significantly from 7,570±600 to 1,960±560 cells/μL. Serum concentrations of Ca^{2+} decreased from 6.5±0.3 to 6.0±0.3 mg/dL, Mg^{2+} from 0.53±0.06 to 0.43±0.04 mmol/L, tMg from 0.78±0.05 to 0.62±0.08 mmol/L, K^+ from 4.3±0.4 to 3.0±0.5 mEq/L, and Pi from 3.4±0.5 to 1.7±0.5 mg/dL ($P < 0.05$). PTH increased significantly from 1.3±0.4 to 6.0±5.2 pmol/L; however, in some horses (n=2) PTH did not increase despite hypocalcemia. Insulin increased significantly from 9.4±3.6 to 50.5±9.6 μIU/ml (n=3). Urinary fractional excretion of Ca^{2+} (FCa) decreased significantly from 4.7±1.4 to 1.7±1.2%, of Mg^{2+} (FMg) from 36.6±6.5 to 11.7±7.3%, and K^+ (FK) from 37.9±11.3 to 17.7±6.2%. Fractional excretion of Pi (FP) increased from 0.02±0.02 to 0.14±0.07% and Na^+ (FNa) from 0.26±0.13 to 1.2±0.5%. There were no changes in serum Na^+ and Cl^- concentrations. No changes in any of the variables evaluated were detected in control horses.

Based on our results, we believe that endotoxemic horses

developed hypomagnesemia and hypokalemia due to shifting of Mg^{2+} and K^{+} to the intracellular compartment and hypocalcemia from movement of Ca^{2+} to the interstitial compartment and intracellular subcompartments. The decrease in FCa, FMg, and FK makes urinary loses of these electrolytes an unlikely cause of their serum disturbances, but rather a compensatory mechanism. Some of our findings can be explained by the increased serum PTH and insulin concentrations, and activity of the Ca^{2+}-sensing system. In conclusion, endotoxemia in horses resulted in electrolyte abnormalities that included hypocalcemia, hypomagnesemia, hypokalemia, hypophosphatemia.

ABSTRACT #43

VENTRICULAR SEPTAL DEFECT IN CATTLE: 25 CASES (1987-2003). Sébastien MC Buczinski, Gilles Fecteau, Rocky DiFruscia. Département des sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.

Ventricular septal defect (VSD) is the most common congenital heart defect in large animals. In bovine, little is known on precise etiology of the defect, classical presentation, results of ancillary tests and long term prognosis for VSD affected animals.

Clinical and complementary examinations, pathological findings and outcome were reviewed for cattle diagnosed with a VSD at the Centre Hospitalier Universitaire Vétérinaire of the Université de Montréal between 1987 and 2003. Twenty five medical records were reviewed. All affected animals were female. Holstein (n=22) and Ayrshire (n=3) were the only two breeds represented. Age at presentation ranged from 36 hours to 21 months (median=2 months). The most common reasons for consultation were chronic respiratory problems (n=11, 44%) and cardiac problems (n=5, 20%). A pansystolic murmur audible on the right thoracic side was audible in 20 cases (80%). Compatible findings with pneumonia were identified in 15 cases (60%) which may interfere with adequate cardiac auscultation in field conditions. Chemistry panel (n=23) findings were unremarkable. The most common changes in the complete blood count (n=23) were neutrophilia identified in 13 cases (range 4.8 to 42.9 x 10^9 cells/L; mean=14.7 x 10^9 cells/L) and hyperfibrinogenemia in seven cases (range: 6 to 8g/L; mean=6.9g/L). Arterial blood gases (n=11) results showed mild to severe hypoxemia (range: 78.1 to 25.6 mmHg; mean=53.5 mmHg) in 8 cases, and hypercapnia (range: 45.9 to 52.4 mmHg; mean=49.1 mmHg) in 5 cases. Echocardiography was useful to establish the final diagnosis of VSD in 16 cases out of 17 (sensitivity of 94% in this study). In two cases an Eisenmenger's complex with a reversal of the shunt was diagnosed by ultrasonography and confirmed by necropsy. Thoracic radiographs (n=17) were useful to identify cardiomegaly in only 2 cases. However, thoracic radiographs revealed lesions compatible with pneumonia in 15 patients (88%). VSD was associated with other cardiac anomalies in 13 calves. Other anomalies detected by necropsy and ultrasonography were hypertrophy of the right ventricle (n=6), dextroposition of the aorta (n=3), patent ductus arteriosus (n=3), pulmonary trunk dilation (n=3) and atrial septal defect (n=1). Clinical signs of heart failure were associated with rapid death or euthanasia in 10 of 11 cases (92%). Prognosis was poor and not associated with the size of the defect (mean=2.8cm). Only 10 calves were discharged from the hospital and none had a productive life in the herd. Inadequate performance in reproduction was reported by the owner in four out of five cases. Inadequate growth was also noted.

Further studies are needed to better understand the true clinical implication of VSD in calves. Analysis of pedigrees in a sufficient number of cases may help to confirm the hereditary component of the defect. Prediction of outcome based on ultrasonographic measurements (such as blood velocity through the defect) could also be helpful.

ABSTRACT #44

ROLE OF *CLOSTRIDIUM DIFFICILE* IN NEONATAL CALF DIARRHEA: A BLINDED PAIRED CASE-CONTROL STUDY IN DAIRY CALVES. A Rodriguez[1]; H Staempfli[1]; T Duffield[2]; A Peregrine[3]; L Trotz-Williams[2]; L. Arroyo[1] & JS Weese[1]. Departments of Clinical Studies[1], Population Medicine[2] & Pathobiology[3], Ontario Veterinary College, University of Guelph, Canada.

Clostridium difficile is a spore-forming anaerobic bacterium that has been associated with the development of pseudomembranous ulcerative colitis and with antibiotic-associated diarrhea in humans. In addition, this organism has also been associated with enteric disease in other species, including dogs and horses. Diarrhea develops secondarily to cellular damage of the intestinal epithelium induced by two major *C. difficile* toxins, toxins A and B.

Although *C. difficile* and its toxins can be detected in fecal specimens of diarrheic calves; to date, there is lack of scientific evidence addressing the significance of this pathogen in cattle, the prevalence in non-diarrheic calves, and the potential zoonotic implications of bovine-derived isolates. The primary objective of this study was to investigate the degree of association between the prevalence of fecal *C. difficile* and its toxins and fecal consistency in young dairy calves with diarrhea. Diarrheic and non-diarrheic calves less than one month of age from the same farms were matched for a blinded paired case-control study. A second objective was to explore genetic similarities between bovine and pathogenic human and other animal isolates. 102 dairy farms in Southern Ontario were visited during May-September of 2004 to obtain single fecal samples from calves between four and 28 days of age. Surveys were used to identify basic farm management practices. Samples were code-blinded and processed for presence of toxins A/B using a commercial immunoassay, and cultured using a *C. difficile*-selective protocol. PCR-based ribotyping analyses were conducted on the *C. difficile* isolates. The presence of the toxin genes was also investigated. The resulting ribotyping patterns were compared with known pathogenic human and canine isolates.

The prevalence of farms with at least one animal positive for fecal toxin A/B was 56.8% (58/102). A total of 230 calves, sampled from 79 farms, were paired as case-controls based on fecal consistency; the average number of pairs per farm was 1.4. Statistical analysis of this paired data revealed a significant association (p=0.004) between the presence of fecal toxins and diarrhea (OR=2.38; 95% CI= 1.30-4.48). *Clostridium difficile* was isolated from 32 calves: 11 diarrheic, and 21 controls, representing 25.5% of the farms (26/102). Ribotyping was performed on 22 of the 32 isolates. At least five different ribotype patterns were present. Two of these patterns, identified in calves from at least six different farms, were indistinguishable from common human and canine isolates. The results of this study suggest that *C. difficile* might be an important pathogen in neonatal calf diarrhea and that a potential risk of zoonotic transmission might exist.

ABSTRACT #45

COLOSTRAL LACTOFERRIN (LF) MODULATES INFLAMMATORY MEDIATOR EXPRESSION DURING *IN VITRO* LPS-STIMULATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC). M Dawes, J Lakritz, A Marsh, M Cockrell and JW Tyler.

Cytokines are soluble polypeptides and are produced by cells of both the innate and adaptive arms of the host immune system following antigenic stimulation. They regulate and mediate immune and inflammatory reactions and their role as effectors of the immune system is widely described. In this study we evaluated the effects of lactoferrin (LF) on cytokine and prostanoid expression by peripheral blood mononuclear cells (PBMC). Lactoferrin is an iron-binding protein, a characteristic that bears specific implications to lymphocyte proliferation and subsequent effector mechanisms following antigenic exposure. It also has a high affinity for LPS.

High concentrations of LF exist in colostrum and in the secondary granules of neutrophils. Lactoferrin plays protective and regulatory roles in mammary gland health and superoxide production in neonatal bovine neutrophils. We hypothesized that LF inhibits cytokine expression by bovine PBMC through prevention of lipopolysaccharide (LPS) binding. Since lymphocyte proliferation is iron dependent we evaluated cytokine and mediator expression in the presence and absence of supplemental iron (Fe).

Peripheral blood mononuclear cells were isolated from healthy Holstein steers. Cell viability and differential were determined by trypan blue exclusion and counting 200 Wright stained cells, respectively. Cells (5 x 10^6/well) were cultured in RPMI supplemented with bovine fetal serum for 24 h after which the media was replaced and viability was again determined (90 – 100%). Affinity purified bovine colostral LF was either used as is (non-extracted), or ethanol-extracted (extracted) to remove bound LPS. Cellular treatments included LF prior to iron, LPS or media alone; negative controls included no treatment (media alone). Cells were stimulated for 24 h, after which RNA was isolated by TRIzol extraction. Semi-quantitative Reverse Transcriptase-PCR was performed to evaluate the expression of inducible Cox-2, IL-1β, TNFα and MMP-9. PCR products were analyzed on a 2 % agarose gel after ethidium bromide staining. GAPDH was used as control for loading and PCR mix components devoid of cDNA product served as the negative control.

Lactoferrin inhibited LPS-induced bovine PBMC IL-1β, COX-2 and TNFα expression. These findings support previously reported cytokine/mediator expression in human PBMC and the role of LF in the prevention of LPS binding and induction of acute responses by leukocytes. It is believed that the mechanism here is one of iron sequestration as well as inhibition of LPS-cellular interaction. Differences were noted between the extracted and non-extracted LF treatments. Cytokine expression in the latter may be related to LF reagent contamination with LPS.

ABSTRACT #46

EFFECTS OF DEXAMETHASONE AND ISOFLUPREDONE ACETATE ON MILK PRODUCTION AND SERUM BIOCHEMISTRY VALUES IN DAIRY COWS. Natalie Coffer, Nicholas Frank, Sarah Elliott, Sarel van Amstel; University of Tennessee, Knoxville, TN.

Corticosteroids are commonly administered to dairy cows with ketosis to lower milk production and enhance gluconeogenesis, but their use may be complicated by the development of hypokalemia and profound weakness. The purpose of this study was to assess the effects of isoflupredone acetate (ISO) and dexamethasone (DEX) on milk production and serum biochemistry values in lactating dairy cows. Thirty-three healthy dairy cows 20 to 25 days in milk were randomly allocated to 5 treatment groups and received two intramuscular injections of 10-ml sterile saline (control) 48h apart (days 0 and 2), 20 mg ISO or DEX followed by saline 48h later, or two injections of 20 mg ISO or DEX 48h apart. Milk production was measured and blood samples were collected daily for eight days. Group, time, and group x time effects were examined by ANOVA for repeated measures. Least squares means were compared to the baseline (day 0) mean value within each group using Bonferroni tests.

Weakness was not detected in any treated cows and groups did not differ with respect to measured physical examination parameters. However, atrial fibrillation developed on day 4 in one cow that received two injections of ISO. Significant group x time effects were detected and means differed significantly from the baseline mean value for potassium, phosphorus (PO_4), total carbon dioxide (TCO_2), and glucose variables. Corticosteroid administration was also associated with a significant reduction in milk production on day 1 when treated cows were grouped together. Hypokalemia (< 3.9 mEq/L) developed and mean plasma potassium concentrations were significantly lower than baseline mean values on day 2 and days 1 to 5 in cows that received one or two doses of ISO, respectively. Mean plasma TCO_2 concentration rose significantly on days 2 to 5 in cows treated with ISO twice. Administration of either corticosteroid raised mean plasma glucose concentrations significantly above baseline on day 1, and significantly higher concentrations were also detected on day 3 when a second injection of ISO or DEX was administered. Mean plasma PO_4 concentration significantly decreased on day 1 in two of the four corticosteroid groups.

We conclude that corticosteroid administration lowers milk production in early lactation cows. Both DEX and ISO exhibited glucocorticoid activity, but only ISO demonstrated a mineralocorticoid-like effect on plasma potassium concentrations, and this effect was influenced by dosage. Results also suggest that ISO administration and subsequent hypokalemia promotes metabolic alkalosis in early lactation dairy cows.

ABSTRACT #47

DIETARY CATION ANION BALANCING FOR THE REDUCTION OF URINE PH IN GOATS. Meredyth Jones, Robert Streeter. Center for Veterinary Health Sciences, Oklahoma State University, Stillwater OK.

Objective data is limited regarding the correlation of dietary cation anion difference (DCAD) and urine pH in goats. This information would be useful in the development of feeding programs for the prevention of urolithiasis. The purposes of this study were to determine urinary pH values as they correlate to DCAD levels in goats, to determine which DCAD level produces urine pH in the range of 6.0 - 6.5, and to determine the urine dilution effect of DCAD level.

Twenty four adult, crossbred goat wethers were utilized in a completely randomized design. Each goat was assigned to one of four treatment groups of DCAD levels -150, -75, 0 or +75 mEq/kg on a dry matter basis. Goats were limit-fed a basal ration of pelleted feed and ground hay, which had a DCAD of 126 mEq/kg, during a seven day acclimation period. During the seven day study period, ammonium chloride was administered in addition to the basal ration to attain the DCAD level assigned. Free-catch urine samples were obtained for each goat at the time of voluntary urination in three hour increments prior to feeding, and hours 1-3, 5-7, 9-11 and 13-15 after the morning feeding and salt administration. Each urine sample was analyzed for pH and specific gravity. Venous blood gas analysis was performed on days 1, 3, 5 and 7 of the study period at the time of the hour 5-7 urine sampling.

Urine pH differed significantly among treatment groups after the second day of the treatment phase. At DCAD levels of -150 mEq/kg and -75mEq/kg, a urine pH of 6.0 – 6.5 was achieved two days after initiation of the treatment diet at the time of the 5-7 hour urine sampling. DCAD level 0 mEq/kg resulted in urine pH levels between 6.0 – 6.5 on day 5 of the treatment period, while urine pH levels at DCAD level +75 mEq/kg remained above 6.5 during the seven day trial period. By the end of the trial period, treatment levels -150 mEq/kg and -75 mEq/kg resulted in urine pH levels below the target range. Analysis of covariance and least squares means procedures were used for data analysis of urine pH, urine specific gravity and blood pH values. For analysis of urine pH values, a baseline covariate of 8.0 was used. Urine specific gravity did not differ significantly among treatment group pairs with the exception that there was a significant difference between the -150 mEq/kg and the -75 mEq/kg groups, with 1.030 as a baseline. Blood pH for the -150 mEq/kg level was significantly lower than that for the other treatment levels, while blood pH did not differ significantly among the -75, 0 and +75 mEq/kg treatment groups.

In conclusion, based upon the DCAD levels tested here, a DCAD level of 0 mEq/kg results in a urine pH of 6.0 – 6.5 in a reasonable period of time, without significant reduction in blood pH.

ABSTRACT #48

BIOAVAILABILITY AND PHARMACOKINETICS OF ORAL OMEPRAZOLE IN LLAMAS. KP Poulsen, GW Smith, JL Davis, MG Papich. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Third compartment (stomach) ulcers are a common cause of sickness and death in camelids of all ages. Although the exact pathogenesis of third compartment ulceration in camelids is not completely understood, stress appears to be the most common predisposing factor. Unfortunately, many of the accepted medications for treatment of gastroduodenal ulcers in other species have proven ineffective in llamas, alpacas, and camels. Intravenous administration of omeprazole has been shown to significantly increase third compartment pH in llamas, but is impractical for routine use by owners. Following IV administration, plasma concentrations between 25 and 60 ng/ml are associated with suppression of gastric acid production. Omeprazole is an acid labile drug and oral absorption is often poor due to degradation of the drug in the acid environment of the stomach. The purpose of this study was to examine the systemic absorption and pharmacokinetics of a commercially available oral omeprazole paste (Gastrogard®, Merial, Duluth GA) in llamas.

The registered equine dose of 4 mg/kg was given orally to 6 llamas once a day for six days. Plasma samples were collected at 0, 15, 30, 45, and 60 minutes and 2, 3, 4, 6, 8, 12, and 24 hours on days 1 and 6. Concentrations of omeprazole in the plasma were subsequently determined by HPLC with ultraviolet detection. Pharmacokinetic parameters calculated included the area under the concentration-time curve ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), and the half-life ($t_{1/2}$).

On day 1 there was minimal absorption of omeprazole with a C_{max} of 0.08 µg/ml (T_{max} = 1.4 hr). The $t_{1/2}$ of omeprazole was 57 minutes and the $AUC_{0-\infty}$ was 0.20 hr/µg/ml. Plasma omeprazole concentrations were significantly higher on day 6 with a C_{max} of 0.12 µg/ml (T_{max} = 45 min). The $t_{1/2}$ of omeprazole on day 6 was 2.3 hours and the $AUC_{0-\infty}$ was 0.38 hr/µg/ml. Plasma concentrations of omeprazole remained above the minimum plasma concentration for inhibition of gastric acid secretion projected from other studies (25 ng/ml) on day 6 in all the llamas for approximately 6 hours. Using data previously published on the IV use of omeprazole in llamas, oral absorption was estimated to be only 2.95%.

These results indicate that after 6 days of treatment, oral omeprazole produced plasma concentrations that would not be predicted to suppress acid secretion throughout a 24 hour dose interval. Higher doses should be considered for future studies. These results also suggest that because an increase in plasma C_{max}, AUC, and $t_{1/2}$ were observed following multiple administrations, oral absorption is improved with repeated doses.

ABSTRACT #49

IDIOPATHIC HEMORRHAGIC PERICARDIAL EFFUSION IN 5 COWS. AM Firshman, AM Sage, SJ Valberg, HJ Kaese, LM Hunt, MJ Murphy, LC Sharkey, M Bouljihad. College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

In cattle, pericardial disease is most frequently caused by traumatic penetration of the pericardium by a foreign body originating from the gastrointestinal tract and prognosis is poor. Clinical signs include anorexia, pyrexia, reluctance to move, decreased milk production and signs of congestive heart failure. Cytological analysis of the pericardial fluid reveals elevated white blood cell counts and protein concentrations. This study describes a further cause of pericardial effusion in five Friesian cows that we have termed idiopathic hemorrhagic pericardial effusion. The five cows from four different farms presented with a history of decreased milk yield (4/5 cows; one cow was dry) and/or fever (3/5) between Feb 2003 to Nov 2004. All cows had tachycardia (103 ± 13 bpm), tachypnea (42 ± 8 bpm), muffled heart sounds, signs of congestive heart failure and diarrhea. CBCs were normal and serum chemistry revealed decreased albumin and Mg and increased CK, GGT and SDH. Thoracic radiographs of three cows revealed no evidence of traumatic reticulopericarditis. Ultrasound examination of all cows revealed marked fibrinous pericardial effusion, cardiac tamponade, pleural effusion, ascites, hepatomegaly and edema of the intestinal wall. Pericardiocentesis resulted in large quantities of red fluid (up to 30 liters) with a PCV of 12.6 ± 5.2% and TPP of 3.3 ± 1.2g/dl and no cytological evidence of infection or neoplasia. Bacterial and mycoplasma cultures and virus isolation were negative from pericardial fluid. Coagulation profiles and toxicological analysis for ionophores, mycotoxins, anticoagulants and selenium showed no abnormalities. Two cows were negative for BVD, and 2/4 cows tested for BLV were positive. No cows showed lymph node enlargement consistent with lymphosarcoma. Transtracheal wash in two cows showed intracellular cocci but cultures were negative. Four cows were treated with pericardiocentesis, pericardial lavage, antibiotics and NSAIDs and clinical signs resolved. Two cows died six months and one year later from unknown causes and two cows are still alive (3m and 2 yr after discharge). One cow was euthanized at presentation after a diagnosis was established. Necropsy revealed myocardial necrosis, epicarditis and pericarditis and *Streptococcus bovis* was isolated from lung, spleen, and myocardium from the euthanized cow. No growth was obtained from her pericardial fluid.

To the authors knowledge this is the first report of idiopathic hemorrhagic pericarditis in cows. In dogs and humans hemorrhagic pericardial effusion can be "idiopathic" or can result from neoplasia, trauma, bleeding disorders or vascular abnormalities. The cause of this syndrome in the cow is currently unknown. But, more importantly, it should be differentiated from traumatic reticulopericarditis due to the potential for a successful outcome with treatment.

ABSTRACT #50

FACTORS INFLUENCING COLOSTRAL IGG CONCENTRATION IN DAIRY COWS HOUSED UNDER DIFFERENT PHOTOPERIOD CONDITIONS DURING THE DRY PERIOD. D. Morin, S. Nelson , P. Constable, E. Reid, and G. Dahl, University of Illinois, Urbana, IL.

Manipulation of photoperiod during the dry period impacts milk production and immune function in dairy cows. Cows housed under short-day (SD; 8 h light, 16 h dark) conditions produce more milk in the subsequent lactation and have enhanced cellular immune responses at calving compared to cows housed under long-day (LD; 16 h light, 8 h dark) conditions. One aim of the study reported here was to determine the effect of photoperiod during the dry period on the volume and IgG concentration of colostrum harvested at the first milking after calving. A second aim was to determine if serum protein concentrations at dry-off or calving are associated with colostral IgG concentration or IgG mass.

Eighty-one multiparous Holstein cows were housed under SD (n=19), LD (n=20), or ambient (AMB; n=21) lighting conditions for the entirety of the dry period, or under AMB until the last 21 d of the dry period and then switched to SD (n=17). Blood was collected from the coccygeal vein on the day of dry off and at the time of first milking after calving. Serum was harvested and frozen for quantification of total protein, total globulin, IgG (RID method), non-IgG globulin, and albumin. The time of calving was recorded for 56 cows. Cows were milked with a portable milking machine at a consistent time of day on the day of calving and colostral weight recorded. An aliquot of bulk colostrum was frozen for IgG testing.

Photoperiod had no effect on colostral yield (weight), IgG concentration ([IgG]), or IgG mass, or on any of the measured serum parameters. Serum concentrations of total protein, albumin, total globulin, and non-IgG globulin were lower at calving than at dry-off but serum [IgG] did not change. Therefore, it appears that the cows were able produce sufficient IgG to prevent a drop in serum concentration despite translocation of a large quantity of IgG from serum to colostrum prepartum. Colostral [IgG] was correlated with serum total protein, globulin, and non-IgG globulin concentrations at dry-off, but not with any of the serum parameters at calving.

Stepwise regression indicated that colostral [IgG] was associated with the weight of the colostrum and the interval between calving and milking. Colostral [IgG] decreased by 0.11 g/dl per hour and 0.14 g/dl per kg colostrum. The interaction between colostral weight and the interval between calving and milking was more important than either factor alone. If calves are routinely fed 4 L of colostrum after birth, results of this study indicate that a 12-h delay in milking after calving would result in 53g less IgG than if colostrum were harvested immediately. The difference in [IgG] between 4 L of colostrum harvested from a cow producing 5 L and one producing 15 L would be 60 g. These findings demonstrate that cows should be milked as soon as possible after calving. Failure to do so will increase the risk of failure of passive transfer in calves.

ABSTRACT #51

ABILITY OF A POLYVALENT BACTERIN AND ANTIBIOTIC THERAPY TO ELIMINATE CHRONIC INTRAMAMMARY *STAPHYLOCOCCUS AUREUS* INFECTIONS IN DAIRY CATTLE. GW Smith, RL Lyman, KL Anderson. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Staphylococcus aureus mastitis continues to be a major problem for the dairy industry of the United States. Cows with chronic *S. aureus* intramammary infections are often culled from the herd. However, many producers are either unwilling to cull these cattle or have so many animals infected that it becomes impossible or uneconomical to cull them all. Therefore, it is important to develop treatment methods to eliminate chronic intramammary *S. aureus* infections. Both vaccination and antibiotic therapy have been used with limited success in an attempt to eliminate these infections. However, it is possible that the use of a combined therapeutic approach using both vaccination and intramammary antibiotic treatment would be successful.

Cows were initially chosen as *S. aureus* suspects based on herd records. Cows with one or more quarters having at least three positive cultures at two- to four-week intervals were considered eligible for the study. Fifty dairy cows were identified from three herds. Cows were paired within herd based on days in milk, lactation number, milk production, and numbers of quarters infected. Treated cows (n = 25) received three doses of a polyvalent *S. aureus* bacterin on days 1, 15, and 21 of the study along with intramammary pirlimycin in all 4 quarters once a day on days 16-20 (5 total treatments). Control cows (n = 25) received no treatment Follow up cultures were taken every 30 days until at least 3 samples could be obtained (90 days or into the next lactation if the cow was turned dry). A cow that cultured negative at all time points following treatment was considered a success and any positive culture in a quarter that was infected with *S. aureus* at the start of the study period was considered a failure.

There were 20 treated cows (28 total quarters) and 23 control cows (41 total quarters) that finished the study (some cows died for reasons unrelated to mastitis or were culled during the trial). Significantly more treated cows (8/20 or 40%) were classified as a treatment success as compared to control cows (2/23 or 8%). The number of actual infected quarters that cultured negative throughout the follow up period was also significantly higher in treated cows (13/28 or 46% in the treated group and 2/41 or 5% in the controls). There was no significant difference between treated and control groups in the number of cows that went through dry periods during the study.

These data indicate that a combination of vaccination and intramammary antibiotic treatment can be successful in eliminating some cases of chronic *S. aureus* infections in dairy cattle. However it is important to consider cows for treatment carefully as there is a high probablility that many cows will remain positive despite therapy.

ABSTRACT #52

EFFECT OF CONTINUOUS IV DEXTROSE INFUSION ON PHOSPHORUS HOMEOSTASIS IN DAIRY COWS. W. Gruenberg, D. Morin, P. Constable, and J. Drackley. University of Illinois, Urbana, IL.

Plasma phosphorus concentration ([P]) can decrease at calving time in dairy cows due to a sudden loss of phosphorus (P) in milk. Reductions in plasma [P] also accompany periparturient hypocalcemia and other periparturient disorders that result in reduced feed intake. Dairy producers and veterinarians frequently administer IV dextrose to periparturient cows, particularly those with ketosis or hepatic lipidosis. Intravenous administration of glucose-containing solutions has been associated with hypophosphatemia in monogastric species. The purpose of the study reported here was to determine the effects of IV dextrose administration on P homeostasis in dairy cows.

Four multiparous lactating Jersey cows were administered 50% dextrose by continuous IV infusion at a rate of 300 mg/kg/hour for five days. This rate caused a sustained increase in plasma glucose concentration while avoiding glucosuria. As a control treatment, the same cows were instrumented but not administered dextrose. A washout period of $\geq$ 5 d was allowed between dextrose and control treatments, which were performed in random order. Plasma [P] was measured before, during, and after each treatment. Dietary P intake was calculated and daily P loss in urine, feces, and milk determined. Salivary [P] was measured twice daily.

Continuous IV dextrose administration decreased plasma [P] by approximately 40%, from 4.1 $\pm$ 0.8 mg/dl (mean $\pm$ SD, 1.32 $\pm$ 0.26 mmol/l) at baseline to 2.0 $\pm$ 0.5 mg/dl (0.65 $\pm$ 0.16 mmol/l) 24 h after the start of the infusion. Plasma [P] remained below baseline until dextrose administration was stopped. Plasma [P] then increased rapidly as plasma glucose concentration declined, peaking 6 hours after the end of the infusion. Neither plasma [P] nor plasma glucose concentration changed significantly during the control treatment. The reduction in plasma [P] associated with IV dextrose administration was not a result of increased P loss in urine, feces, or milk. Salivary [P] did not differ between dextrose and control treatments. Decreased dietary P intake, due to decreased feed intake during dextrose administration, may have contributed to the reduction in plasma [P]. However, dietary P intake did not appear to be the main factor influencing plasma [P], since the changes in plasma [P] after beginning and ending the dextrose infusion occurred more rapidly than can be attributed to a change in feed intake.

Results of our study indicate that dairy cows experience a large and sustained drop in plasma [P] when 50% dextrose is administered by continuous IV infusion. The cause of the drop in plasma [P] is probably a rapid intracellular shift of P compounded by a decrease in dietary P intake, rather than an increase in P excretion in urine or saliva. Dairy producers and veterinarians should be cautious when administering IV dextrose to periparturient dairy cows, particularly those at increased risk for hypophosphatemia. Monitoring of plasma [P] may therefore be indicated in cattle receiving continuous IV dextrose.

ABSTRACT #53

THIN SOLES IN DAIRY CATTLE: CHARACTERIZATION OF THE PROBLEM. S R van Amstel[1], J K Shearer[2] and F L Palin[3]. University of Tennessee, Knoxville, TN[1], University of Florida, Gainesville, FL [2], Georgia State University, Atlanta, GA[3].

Thin soles and resulting lameness have become a major economic problem in large dairy operations. Very limited research data exists on the characterization of the syndrome. For that reason the purpose of this study was to investigate the incidence of the condition in comparison to other claw horn lesions and its occurrence in relation to parity (lactation groups 1-3), days in milk (DIM) (0-60; 61-200; 201-350; 350+) and season (month of year).

Preliminary data collected from the study herd of 3,221 lactating cows during April 2003 through March 2004 will be presented. Cows were housed in free stalls and walking surfaces consisted of grooved concrete. Cows were cooled during the summer months with fans and sprinklers.

Thin-soled cows were identified during daily examination of animals presented with clinical lameness. The diagnosis was based on the presence of a short dorsal wall (< 7.5cm/3 inches) and a soft flexible sole on thumb pressure. These criteria had been established in a previous study. On farm hoof trimmers who had previously attended the Master Hoof Care Program (UFL&UT), were responsible for the diagnosis and electronic recording of data. They were periodically supervised by one of the authors (J S).

The incidence of thin soles for cows presented to the farm trimmer with clinical lameness for the period was 30.1%. This was higher than any other claw horn lesion. Second lactation cows had a significantly higher (p=0.040) incidence (13.3%) of thin soles compared to the 1st (11.4%) and 3rd (8.1%) lactation groups. The incidence of thin soles was similar between the 61-200 and 201-350 DIM groups (11.95% & 12.23% respectively), which were significantly higher (p=0.000) than the other 2 groups (0-60 & 350+ DIM; 2.9% & 5.6% respectively). Results also showed that across the 3 lactation groups, the highest incidence occurred between August and December. The incidence during this period was significantly higher (p=0.000) compared to the rest of the year.

This study emphasizes the importance of thin soles in large herds. The high incidence in second lactation cows may be a carry over from excessive wear in the first lactation with insufficient sole re-growth during the dry period. The abrasive walking surface resulting in a faster rate of sole horn wear as compared to growth could be responsible for the significantly higher incidence of thin soles between 60 and 350DIM. The higher incidence of thin soles in all three lactation groups during the summer could be associated with a higher claw horn moisture content resulting in softer and more flexible horn with a more rapid rate of wear. The seasonality observed also suggests that heat stress contributed to lameness either through alterations in cow comfort (that is, more standing and less lying time), influences on rates of laminitis (by virtue of increased rates of rumen acidosis) or both. Distance walked and nutritional influences and were not studied but may have also contributed to thin soles as a consequence of increased sole wear rumen acidosis and laminitis.

ABSTRACT #54

CLINICAL AND CLINICOPATHOLOGIC DIAGNOSIS OF SPINAL LYMPHOSARCOMA IN CATTLE. [1]Tracy Williams, [2]Dusty W. Nagy, [2]Peter D. Constable, [2]Dawn E. Morin, [1]Washington, GA, [2]University of Illinois, Urbana, IL.

Approximately 44% of US dairy cattle and 10% of US beef cattle are infected with bovine leukosis virus. Up to 5% of infected animals will die due to lymphosarcoma. Clinical manifestations of tumor formation depend on the affected organ systems. Spinal tumors have been associated with weakness and ataxia, especially in the rear limbs. Downer cows also are commonly associated with this manifestation of lymphosarcoma. Spinal lymphosarcoma tumors are extradural and exfoliation of cells into the cerebrospinal fluid (CSF) is considered to be extremely rare if not nonexistent. Thus, CSF has been considered an ineffective diagnostic tool in spinal lymphosarcoma. The purpose of the study was to document the clinical presentation of cattle with spinal lymphosarcoma and to determine if cerebrospinal fluid analysis could be used in the diagnosis of spinal lymphosarcoma in cattle.

Medical records of bovine cases admitted to the University of Illinois Veterinary Teaching hospital from 1981 to 2004 were searched. Animals with a necropsy diagnosis of multicentric lymphosarcoma and clinical signs consistent with a compressive spinal cord lesion or necropsy documenting the presence of tumor in the spinal column were included in the study. An additional subset of animals that had antemortem CSF analysis completed were utilized for a portion of the analysis.

Eighty-six cases of lymphosarcoma were diagnosed over the study period. Twenty-two of these met the study criteria. Onset of clinical signs at the time of presentation ranged from 1-30 days duration. The most common history included ataxia progressing to a down cow (86%,19/22) and decreased feed intake (23%,5/22). The most common exam findings included ataxia or a down cow (82%,18/22) and lymphadenopathy (41%,9/22). Cerebrospinal fluid analysis results from 10 cases were available for the study. Cerebrospinal fluid was diagnostic in 60% (6/10) of the samples. Abnormal CSF samples had a mean protein of 57.5 mg/dl (range, 20-100) and a mean and median cell count of 124 and 45 respectively (range,14-432). Results of necropsy examinations suggest that in at least three animals, diagnostic CSF was obtained without pithing the tumor present in the spinal canal. In addition, three necropsy exams found tumor infiltrating the dura to a subdural location.

In conclusion, this study suggests that cerebrospinal fluid analysis may be a useful tool in the diagnosis of spinal lymphosarcoma. It also challenges the belief that spinal lymphosarcoma masses are all extradural and as a result can not exfoliate cells into the CSF.

ABSTRACT #55

CLINICAL MANIFESTATIONS OF LIVER ABSCESSES IN DAIRY CATTLE: RETROSPECTIVE STUDY OF 18 CASES (1992-2003). Elizabeth Doré, Gilles Fecteau, David Francoz, Pierre Hélie; Centre Hospitalier Universitaire Vétérinaire, Saint-Hyacinthe, Québec, Canada.

The objective of this study was to describe the clinical manifestations, results of ancillary tests, treatment, and outcome of dairy cattle diagnosed with liver abscess at the Centre Hospitalier Universitaire Vétérinaire (CHUV) of Saint-Hyacinthe.

Medical records from 18 Holstein dairy cows presented at the CHUV between January 1992 and December 2003 with a diagnosis of liver abscess were studied. Ultrasound examination or laparotomy findings were used to confirm the diagnosis. Calves with omphalophlebitis were not included in the study.

Age at presentation range from 1.2 to 7.3 years (median: 4.1). At admission, rectal temperature range from 37.8 to 40.0oC (median: 38.7) and heart rate range from 48 to 120 beats per minute (median: 78). Five animals had fever (> 39.2oC). Seventeen cows out of 18 were anorectic. Eight out of 10 animals were slightly dehydrated (5-7%). Five out of 15 animals had diarrhea and six, few feces. Diagnosis of liver abscess was based on ultrasound examination (n=8) or exploratory laparotomy findings (n=10). Concurrent diseases diagnosed included peritonitis (n=6), chronic indigestion (n=4), traumatic reticuloperitonitis (n=1) and abomasal dilatation (n=1). Complete blood count revealed a neutrophilia in 14 cases (median: 5.95 X 109/L) and an increased fibrinogen concentration in 11 cases (median: 6 g/L). Modifications observed on chemistry panel was elevated globulin concentration in 10 cases (median: 46.10 g/L) and elevated GGT activity in five cases (median: 38 g/L).

Bacteriological culture was performed for aerobic bacteria (n=11) and anaerobic bacteria (n=9). Arcanobacterium pyogenes was isolated in four cases in association with an anaerobic bacteria (Bacteroides oralis/veroralis, Fusobacterium necrophorum/nucleatum, Bacteroides spp. or Peptococcus spp.). Other bacteria isolated were: Escherichia coli, non haemolytic Staphylococcus and Actinomyces spp.. Surgical procedure to drain the abscess was performed in five animals. Necropsy was performed in five cases and thrombosis of the caudal vena cava diagnosed in two cases. For the 13 cows discharged, the antimicrobial therapy duration range from nine to 45 days (median: 21). Antibiotic used was penicillin in all cases. Long-term evolution was obtained by phone for seven cases. Four animals had a normal production life and calved at least once after discharge. Two animals were sent to slaughterhouse for low milk production and one for poor reproductive performance.

Liver abscess affecting dairy cattle can be treated with a relatively good success. Antibiotherapy of long duration appears suitable when possible abscess should be drained through marsupialization.

ABSTRACT #56

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ABSTRACT #57

DIFFERENTIAL EXPRESSION OF THE CARDIAC RYANODINE PROTEIN IN NORMAL DOGS AND BOXER DOGS WITH ARVC. K Meurs[1], VA Lacombe[1], K Dryburgh[1], PR Fox[2], PR Reiser[1], MD Kittleson[3]. [1]The Ohio State University, Columbus, OH; [2]The Animal Medical Center, New York, NY; [3]University of California Davis, CA.

Mutations in the cardiac ryanodine receptor (RYR2) gene have been identified in some human families with arrhythmogenic right ventricular cardiomyopathy (ARVC), a disease that preferentially affects the right ventricle (RV). We hypothesized that the increased impact of the disease on the RV was due to a decreased expression of this protein in the normal RV as compared to the left ventricle (LV). Also, we hypothesized that boxer dogs affected with ARVC would have decreased RYR2 mRNA and protein expression in comparison to normal dogs.

Myocardial samples from the RV, LV and interventricular septum (IVS), harvested immediately postmortem from six affected and eight control dogs, were evaluated by immunoblotting and by real time PCR. Immunoblotting was performed with 25ug total protein/lane and a C3-33 monoclonal anti-RyR2 antibody. To verify that any identified concentration change was not associated with loss of myocardial proteins observed with ARVC, SDS-PAGE to identify β-myosin heavy chain (MYHC) protein was also performed as a control. Quantitative determination of both proteins was performed by densitometry. Reverse transcription and real-time PCR was performed with a RYR2 probe and the canine HPRT gene. Relative quantities (% of each control site) were calculated. Differences of protein quantity between the regions of the heart were evaluated with a one-way ANOVA. Differences between affected and control dogs were evaluated with a t – test. An alpha of 0.05 was considered statistically significant.

In normal dogs, the mean RyR2 protein concentration in RV myocardium was significantly less ($P<0.05$) than IVS and LV Additionally, in affected boxer dogs RYR2s were significantly ($P<0.05$) decreased in all three cardiac chambers in comparison to control dogs. The MYHC protein concentration was not different between normal and affected dogs, or at any location examined in the heart. Real time PCR in normal dogs demonstrated a lower quantity of RYR2 mRNA in the RV compared to the LV and IVS. The RYR2 mRNA was decreased in all chambers of affected dogs compared to controls.

In conclusion, these data suggest that the cardiac RYR2 message and protein expression are differentially expressed across the cardiac chambers in the normal heart. More importantly, both the message and protein expression are reduced in affected dogs. We propose that the increased susceptibility of the RV to this disease is related to its lower concentration of RYR2 in normal heart and that all three cardiac chambers are affected by arrhythmogenic right ventricular cardiomyopathy.

ABSTRACT #58

IDENTIFICATION OF A MISSENSE MUTATION IN THE CARDIAC MYOSIN BINDING PROTEIN C GENE IN A FAMILY OF MAINE COON CATS WITH HYPERTROPHIC CARDIOMYOPATHY. Meurs K, Sanchez X, David R, Bowles NE, Towbin JA, Reiser PJ, Kittleson JA, Munro MJ, Dryburgh K, Boyer M, Mathur D, MacDonald KA, Kittleson MD. The Ohio State University, Columbus, Ohio; Baylor College of Medicine, Houston, TX; University of California, Davis, CA.

A feline model of familial hypertrophic cardiomyopathy (HCM) has been previously identified in Maine Coon cats. The disease is inherited as an autosomal dominant trait, develops during early to mid-adult life, and often causes heart failure, systemic thromboembolism, and sudden death. We hypothesized that a sarcomeric gene mutation is present in this animal model. The objectives were to determine if a sarcomeric mutation is present and to determine its effect on the protein.

Disease status of Maine Coon cats was verified through repeated echocardiographic examinations (affected = 16; unaffected = 7). DNA was extracted from white cells from the 23 Maine Coon and 106 control cats. Myocardium was collected from Maine Coon cats that died (n=8) and from control cats (n=3). Primers were developed for amplification of exonic regions of several feline sarcomeric genes, including the cardiac myosin binding protein C (cMyBP-C) gene, using known human sequences and Primer3 software. Primers were optimized and DNA was amplified, purified and sequenced. Sequences were compared for base pair changes. Base pair alterations were evaluated to determine if they changed a conserved amino acid and the computed structure of the protein. Western blot analysis, using a cMyBP-C antibody, was performed on frozen myocardial tissue from affected and unaffected cats.

A single base pair change (G to C) was identified within exon 3 in all of the Maine Coon cats with HCM but none of the unaffected Maine Coon or control cats. The missense mutation changed a conserved amino acid from alanine to proline and resulted in a computed alteration in the conformation of the molecule. Immunobinding demonstrated a significant reduction in cMyBP-C in the myocardium from affected cats when compared to unaffected cats ($p<.001$). Conclusions: We conclude that we have identified a spontaneous sarcomeric mutation associated with HCM in the cat. It is a missense mutation in the feline cMyBP-C gene, and the mutation changes a conserved amino acid resulting in alteration of and a reduction in cMyBP-C protein.

ABSTRACT #59

INDEX OF MYOCARDIAL PERFORMANCE AND SYSTOLIC TIME INTERVALS IN BOXER DOGS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY. Baumwart RD, Meurs KM. The Ohio State University, Columbus, OH.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a form of cardiomyopathy characterized by ventricular tachyarrhythmias and fibro-fatty infiltrates in the right ventricle (RV) recognized in Boxer dogs. Clinical symptoms include syncope, increased risk of sudden cardiac death, intermittent weakness, and in some cases congestive heart failure. The objective of this study was to determine if Boxer dogs with ARVC have abnormal (RV) function

as measured by a Doppler-derived Index of Myocardial Performance (IMP). Normal RV values for canine IMP were defined and presented previously.

Two groups of Boxer dogs were studied. Ten boxers with ARVC defined by ≥ 1000 ventricular premature complexes (VPC)/24 hours on an 24-hour ambulatory electrocardiography (AECG) and normal left ventricle (LV) echocardiographic parameters and 10 boxers with ≤ 5 VPCs/24 hours and normal left ventricle (LV) echocardiographic parameters.

Echocardiograms images were obtained in right and left lateral recumbency without sedation. Pulsed wave Doppler recordings of tricuspid inflow and pulmonic ejection were acquired with an ECG at 200 mm/s sweep speed. Prejection period (PEP), ejection time (ET), PEP/ET, tricuspid valve closure to opening time (TVC-TVO), and IMP for the RV was determined from five consecutive beats, unless inhibited by ventricular ectopic beats. IMP was calculated as: {(isovolumetric contraction + isovolumetric relaxation) / ET}. Differences in mean variables for the two groups were identified by a t-test. Significance was set at alpha=0.05.

Mean (+/- SD) ages for the two groups were 7.0 (+/-1.4) and 3.6 (+/-1.8) years, respectively. Mean (+/- SD) weights were 29.8 kg (+/-3.8) for ARVC group and 27.3 kg (+/-4.8) for the normal group. Mean (+/-SD) VPCs/24 hours were 9,165 (+/- 9,094) for the ARVC group and 2 (+/- 2) for the normal group. Mean (+/-SD) RV IMP were 0.19 (+/-0.07) for the ARVC group and 0.21 (+/-0.04) for the normal group. RV IMP values for both groups were within or below the 95% confidence intervals previously reported for normal dogs (0.20-0.32) between 15.1-35 kg. A statistically significant difference was not identified between groups for RV IMP, RV PEP, RV PEP/ET, RV ET, TVC-TVO, LV FS%, LVID, aortic velocity, R to R interval during measurement, or weight. A significant difference was observed for the ages and VPC/24 hours for the two groups ($p<0.001$).

We conclude that Boxer dogs with ARVC have normal RV IMP suggesting normal RV function. However, it is possible that the RV IMP may not be a sensitive indicator of RV dysfunction, or that the ARVC group's stage of disease was early in its development. Further long term studies may be warranted.

ABSTRACT #60

COMPARISON OF DIFFERENT HOLTER ANALYSIS METHODS FOR THE DETECTION OF VENTRICULAR ARRHYTHMIA FREQUENCY AND SEVERITY IN DOGS. AW Spier and DM Fine. University of Missouri, Columbia MO.

Twenty-four hour ambulatory electrocardiographic (Holter) monitoring has become a routine procedure in veterinary medicine. Ambulatory monitoring is most often used to identify the presence of arrhythmias in an effort to quantify its severity, or determine a relationship between arrhythmia occurrence and clinical signs. Holter recordings are analyzed by a wide variety of commercial laboratories, academic institutions, and individual veterinary practices. The procedure for interpretation of recordings is primarily directed by computer algorithms, but is also heavily influenced by operator adjustments to identify errors made by these software systems. Consequently, the accuracy of interpretation is dependent not only on software templates but also on the skill and attention of laboratory personnel. The aim of this study was to identify differences in the analysis and interpretation of Holter recordings by commercial and academic facilities.

A total of 10 previously obtained Holter recordings with varying degrees of ventricular premature complexes (VPCs) were evaluated by two commercial labs and one academic institution. Evaluation of recordings from the academic institution was performed by a trained technician under the supervision of a boarded cardiologist. The number of VPCs (VPC#) and grade of arrhythmia were determined from each recording. Grade was defined as follows: grade 0→no VPCs seen; grade 1→single VPCs only; grade 2→bigeminy, trigeminy or couplets; grade 3→triplets; grade 4→runs of VPCs (4 or more), including ventricular tachycardia and idioventricular rhythm.

The VPC# ranged from 0 to >30,000, with all grades represented. Discrepancies in VPC# between the commercial and academic evaluations were expressed as a percent change from the highest (H) value to lowest (L) value across all labs, according to the following formula (H-L)/H. Percent change ranged from 0% to as high as 100%. By definition, cases in which a percent change of 100% was identified occurred only when one recording identified zero VPCs. In these cases, the highest recording was limited to 2 VPCs. None of the differences between laboratories exceeded what was considered clinically significant or within the limits of spontaneous biologic variability. The discrepancy in the grade of arrhythmia between labs was minimal, and never exceeded more than 1 grade, and was usually associated with a grade 0 vs grade 1. These results suggest that evaluation of Holter recordings by trained technicians in an academic institution and those performed by commercial laboratories are comparable. Therefore, in-house Holter analyses performed by academic institutions are a reasonable alternative to analysis by commercial facilities. Because differences in VPC# did occur, repeated evaluation of the same patient is ideally performed using the same laboratory.

ABSTRACT #61

SUPPRESSION OF INHERITED VENTRICULAR ARRHYTHMIAS IN GERMAN SHEPHERDS BY MEXILETINE AND SOTALOL. Anna R.M. Gelzer, Marc S. Kraus, Hollis N. Erb, Mary Ellen Charter, Shari Renaud-Farrell and N. Sydney Moïse; Cornell University, Ithaca, NY.

German shepherd (GS) dogs, affected with inherited ventricular arrhythmias and sudden death have up to 60% ventricular ectopy (VE) during a 24-hour period. Cases have been documented both in the US and abroad, but thus far no effective antiarrhythmic therapy has been established. The purpose of this study was to evaluate different orally available antiarrhythmic drugs for suppression of VE in severely affected GS.

We enrolled 11 affected GS, bred and raised at Cornell University in the study. Five males and six females GS with a median age of 20 weeks (range 17 to 22 weeks) qualified for the study, based on a 24h ECG (Holter) with > 250 ventricular premature beats (VPC) per hour. In a prospective cross-over design, dogs were randomized to receive one of three drug protocols: Mexiletine (M) alone (8 mg/kg TID), Sotalol (S) alone (2.5 mg/kg BID) or a combination therapy of M with S (C, same dosages) for suppression of ventricular arrhythmias. For each of the three drug protocols we acquired a baseline (BL) Holter (pre drug) and a repeat Holter after six days of drug therapy. In between the different drug protocols there was at least a three-day washout period. The 24-hour ECG was analyzed using the Burdick Vision Premier™ Holter analysis software and subsequently edited by a cardiologist or technician. The following variables were statistically analyzed: % ventricular ectopy (%VE) calculated based on total number of ventricular beats out of total number of QRS complexes recorded over 24 hours, as well as absolute numbers of single ventricular beats (VPC), ventricular couplets (VC) and runs of ventricular tachycardia (VT) recorded over 24 hours. The paired data was analyzed using the Wilcoxon's signed-rank test.

Combination therapy of M with S resulted in a statistically significant reduction of median %VE as compared to baseline (BL_C: 17% vs C: 13%, $p<0.005$). This effect was also reflected in a significant reduction of median number of VPC's (BL_C: 23,474 vs C: 16,722, $p=0.05$) and number of runs of VT (BL_C: 299 vs C: 225, $p<0.05$). In contrast, monotherapy with either M or S resulted in an insignificant increase in median % VE as compared to baseline (BL_M: 21% vs M 23.5 %; and BL_S: 24.5% vs S: 28.5%, respectively). Monotherapy with S caused a significant increase median number of runs of VT as compared to baseline (BL_S: 617 vs S: 5203, $p<0.05$).

We conclude that combination of M with S is a potentially effective therapy for suppression of VE in affected GS. However, in the absence of clinical signs other than sudden death in affected GS, the modest VE reduction achieved may not be clinically relevant. Monotherapy with M or S is clearly ineffective, in fact S is proarrhythmic. A possible synergistic mechanism of the combination therapy warrants further investigation.

ABSTRACT #62

SPATIO-TEMPORAL ORGANIZATION OF INDUCED ATRIAL FIBRILLATION IN GERMAN SHEPHERD DOGS WITH INHERITED VENTRICULAR ARRHYTHMIAS. Romain Pariaut, Bethany D. Koetje, Shari Renaud-Farrell, Mary Ellen Charter, Niels Otani, Robert F. Gilmour Jr., James A. Flanders, Anna R. M. Gelzer, Marc S. Kraus, Teresa Gunn, N. Sydney Moïse. Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.

German shepherd dogs (GSD) with inherited ventricular arrhythmias also have infrequent atrial premature complexes. In a pilot study to evaluate atrial repolarization we sought to determine the effective refractory period (ERP) of the atria. These attempts failed because of the unexpected induction of atrial fibrillation (AF). The vulnerability of the GSD to the induction of AF in our specific protocol afforded us the opportunity to determine the spatio-temporal organization of AF. In other species such as the sheep, spatio-temporal organization is higher in the left atrium (LA) than the right atrium (RA). Spectral entropy (SEn) can be used to quantify temporal disorganization. We studied induced AF in GSD to determine if SEn was higher in the RA than the LA. Moreover, we determined if lidocaine slowed the AF frequency and increased temporal organization to similar levels in both atria.

GSD were studied with closed- (n = 5) and opened- (n =7) chest protocols. They were anesthetized with pentobarbital (10 mg/kg) and fentanyl (0.02 mg/kg) induction and fentanyl CRI (0.04mg/kg/hr). Two catheters were placed in the RA: a monophasic action potential (MAP) and a pacing catheter. A pulse train of 20 S1 (350 ms, 500 ms) with S2 and S2-S3 protocol and a dynamic pacedown (350 ms to 100 ms) were used to induce AF. Next, 5 atrial sites (3 left and 2 right) were impaled with unipolar electrodes for pacing and recording of electrograms. MAP recordings were measured from the LA and RA. ERP determination and attempts to induce AF with prematures and pacedown were repeated for each site. Sustained AF was treated with lidocaine (2 mg/kg IV). MAP recordings were analyzed using MATLAB and MAP frequency was plotted as a time frequency spectrum for the duration of the AF for both atria. SEn was used to determine how broadly power (P) was distributed among the various frequencies during AF. $SEn = - \sum [P/Total\ P] \log [P/Total\ P]$

Eighty-one AF episodes were induced (67 with S2/S2-S3, 14 with pacedown). Of these, 26 sustained AF episodes were studied. The AF frequency in the LA was greater than the RA in 15 of 26 AF episodes and the SEn was significantly greater in 20 of 26 AF episodes ($P > 0.05$). Lidocaine decreased the dominant frequency and decreased spectral entropy in all 26 episodes of AF. Lidocaine resulted in conversion to sinus rhythm in all episodes.

In conclusion: (1) AF can be consistently induced in these GSD, (2) the AF in the LA is more organized than in the RA, (3) lidocaine effectively converted the AF to sinus rhythm by decreasing the AF frequency and increasing organization in this unique situation of AF.

ABSTRACT #63

EFFECT OF DOBUTAMINE ON LEFT VENTRICULAR OUTFLOW TRACT DYNAMICS IN BOXERS WITH SOFT EJECTION MURMURS. SL Koplitz, KM Meurs, R Baumwart, Y Nishijima. Ohio State University, Columbus, OH.

In the boxer dog, soft ejection murmurs and mild elevations in aortic velocity (AV) are common, although the genesis of such findings is uncertain. Previously, we identified a smaller left ventricular outflow track (LVOT) area index in boxers, independent of “aortic stenosis”. However, a low correlation coefficient relating LVOT area to AV suggested hemodynamic factors, including increased sympathetic tone, may play a role. In this study, we hypothesized that boxer dogs with soft ejection murmurs would show an exaggerated increase in AV, LVOT pressure gradient (PG), and cardiac output (CO) in response to sympathetic stimulation as compared with nonboxer control dogs.

Eight healthy boxer dogs with soft left basilar ejection murmurs (grade 1-3/6) and a subcostal Doppler echocardiographic (DE) AV of 2 - 3 m/s, and 6 healthy weight-matched control Hound dogs underwent cardiac catheterization. Millar-derived left ventricular and aortic pressures and thermodilution CO were measured. Doppler echocardiography was performed prior to (pre-study) and during the catheterization. Millar catheter (Cath) and DE peak and mean LVOT PG were simultaneously measured at baseline and during dobutamine infusion (5 mcg/kg/min). Data was compared using 2-way RM ANOVA.

For all dogs, the peak and mean AV and the peak and mean DE PG were higher at the pre-study and dobutamine time points compared with baseline. Additionally, during dobutamine infusion, CO was higher than baseline measurements. The average pre-study peak AV (boxers 2.46 m/s, controls 2.18 m/s) and mean AV (boxers 1.54 m/s, controls 1.46 m/s) were not statistically different between the two groups. Boxer dogs developed significantly higher AV and higher DE PG than control dogs during dobutamine infusion. Boxer Cath PG was also higher than controls with dobutamine but was not statistically significant. During dobutamine infusion, boxers developed higher CO than control dogs, while baseline CO was not different between groups.

The results of this study support the role of sympathetic stimulation on AV and LVOT PG, in which boxers may demonstrate an exaggerated response. Such findings may help explain the high prevalence of murmurs and elevated aortic velocity in boxer dogs.

ABSTRACT #64

CHARACTERIZATION OF IMMUNOHISTOCHEMICAL STAINING PATTERNS FOR CARDIAC HEMANGIOSARCOMA, IDIOPATHIC PERICARDITIS, HEART BASE CHEMODECTOMAS, AND PERICARDIAL MESOTHELIOMA IN DOGS. Whit M. Church[1], Anne M. Barger[2], April Paulman[2], D. David Sisson[1], Mark A. Oyama[1]; [1]Department of Veterinary Clinical Medicine and [2]Department Veterinary Pathobiology, University of Illinois, Urbana, IL.

Pericardial effusion (PE) in dogs can be caused by several etiologies including cardiac tumors such as hemangiosarcoma (HSA) and chemodectoma (CH), pericardial mesothelioma (M), and idiopathic pericarditis (IP). The approach to treatment and prognosis of dogs with PE is dependent on accurate identification of the underlying etiology of the effusion. Biopsy specimens obtained during thoracotomy, can provide a definitive diagnosis, but are expensive to obtain and result in substantial patient morbidity. In contrast, pericardiocentesis and cytology of pericardial fluid are readily performed, but are limited by current cytological techniques that do not reliably differentiate between neoplastic and non-neoplastic causes of PE.

We suspect that immunocytochemical stains specific for certain tissue types will greatly improve the diagnostic capability of

pericardial fluid cytology. We performed a preliminary study to evaluate immunohistochemical staining of common neoplasms and IP. Using banked tissue samples, we characterized the staining patterns of cardiac HSA, CH, and M and compared these patterns with normal and reactive pericardial tissue samples using a panel of immunohistochemical stains. Specifically, we evaluated the staining patterns for antibodies specific to CD31, desmin, cytokeratin, vimentin, synaptophysin, chromogranin and endothelin. Our results indicate that common cardiac tumors and pericardial diseases demonstrate differential staining characteristics. The antibody for CD31 was specific for HSA. Synaptophysin and chromogranin demonstrated high specificity but only moderate sensitivity for CH. Vimentin was occasionally useful in differentiating HSA from CH. Cytokeratin was specific for mesothelial tissue, and desmin occasionally differentiated reactive mesothelium from M. This study suggests that immunohistochemical stains may be useful in determining etiology of PE. Further work is required to determine the ability to distinguish M from IP as well as the clinical value of these stains employed during cytological (vs. histological) examination of pericardial effusates.

	CD31	Vimentin	Endothelin	Synaptophysin	Chromogranin	Cytokeratin	Desmin
HSA	+	+	+	-	-	-	-
Chemodectoma	-	+/-	+	+/-	+/-	-	-
Mesothelioma	-	+	+	-	-	+	+/-
Reactive Pericardium	-	+	+	-	-	+	+
Normal Pericardium	-	+	+	-	-	+	-

ABSTRACT #65

ECHOCARDIOGRAPHIC EVALUATION OF DIASTOLIC FUNCTION IN NORMAL, MATURE SMALL BREED DOGS AND DOGS WITH ASYMPTOMATIC, SPONTANEOUS CHRONIC DEGENERATIVE VALVULAR DISEASE. A.B. Saunders, S.G. Gordon, M.W. Miller, Department of Small Animal Clinical Sciences and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Chronic degenerative valve disease (CVD) is the most common form of canine heart disease and the leading cause of heart failure in the dog. Valvular regurgitation resulting in myocardial stress and remodeling may lead to diastolic dysfunction. Echocardiography (Echo) is a noninvasive method of evaluating diastolic dysfunction in dogs.

The purpose of this study was to collect and compare Doppler Echo measurements in normal (NL), mature small breed dogs (n=29) and dogs with various degrees of spontaneous CVD (n=20). All dogs were evaluated with a complete history, physical examination, Echo, ECG, Doppler blood pressure, thoracic radiographs, biochemistry panel, complete blood count and urinalysis. Blood work was used to document normovolemia and radiographs confirmed the absence of pulmonary edema. Measured Echo parameters included mitral inflow peak E and A wave and velocity time integral (VTI_E, VTI_A), deceleration time of E wave (DT_E), ratio of E to A wave (E:A), as well as lateral mitral annular peak E_a, A_a, S_a velocities, and ratio of peak E to peak E_a ($E:E_a$). The Index of Myocardial Performance (IMP), a global measurement of left ventricular systolic and diastolic performance, was calculated.

Mean age in years (CVD 8.9; NL 8.6), weight in kg (CVD 8.1; NL 9.3) and heart rate in bpm (CVD 83; NL 95.1) were not significantly different between groups. CVD dogs varied in degree of severity as evidenced by the range in atrial size based on left atrial to aorta M-mode (CVD 1.20, 0.8-1.7; NL 1.05, 0.7-1.5) and two dimensional (CVD 1.59, 1.1-2.5; NL 1.29, 0.7-1.8) measurements and elevated vertebral heart scores (CVD 11.3, 10-13; NL 10.0, 8.5-11.3) reported as mean and range. Differences were identified by Student's *t* test and results are recorded in the table as mean +/- SE, * = significant differences (P<0.05).

	E (m/s)	A (m/s)	E:A	VTI_E (cm)	VTI_A (cm)	DT_E (ms)	E_a (m/s)	A_a (m/s)	S_a (m/s)	$E:E_a$	IMP_{LV}
CVD	0.79* ± .08	0.61* ± .06	1.21* ± .12	5.79* ± .59	3.53* ± .36	98.18* ± 9.12	0.09* ± .01	0.08* ±.01	0.09* ±.004	8.66 ±.77	0.54* ± .07
NL	0.49 ± .02	0.35 ± .02	1.54* ± .07	4.01 ± .18	2.07 ± .09	125.22 ± 5.66	0.07 ±.003	0.06 ±.003	0.07 ±.004	7.84 ±.49	0.37 ± .04

Peak E velocity in normal dogs was significantly correlated with age (r=0.5, P<0.05). The elevation in E, A, E_a, A_a and abbreviation of DT_E is suggestive of enhanced preload in CVD. Increase in the IMP_{LV} suggests alterations in systolic and/or diastolic function in CVD. Echo may offer insight into diastolic dysfunction associated with increasing age and CVD.

ABSTRACT #66

MAST CELL BLOCKADE ATTENUATES THE COLLAGEN LOSS, MYOCARDIAL HYPERTROPHY AND VENTRICULAR DILATION ASSOCIATED WITH EXPERIMENTAL MITRAL REGURGITATION IN DOGS. A. Ray Dillon[a], Pat Rynders[a], Michael Tillson[a], Lou Dell'Italia[b], Debra Beard[a], Jim Redmon[a] College of Veterinary Medicine[a], Auburn University, AL. University of Alabama at Birmingham Medical School[b], Birmingham, AL.

In a model of mitral regurgitation (MR) induced by chordal rupture, we have demonstrated that MR is associated with infiltration of LV by mast cells, increased activity of MMPs, decreased TIMPs, and increase chymase activity, which results in a rapid loss of extracellular matrix collagen, increased LV compliance, increased myocyte integren adhesion, myofiber slippage, myocyte hypertrophy, and decreased total LV contraction. In experimental and spontaneous MR in dogs, the LV collagen loss allows LV dilation and dysfunctional contractility. The myocardial remodeling in volume overload is distinctly different than that observed in pressure overloads and ischemic cardiomyopathy. Blockage of ACE activity and Ang II receptors failed to attenuate the collagen loss and myocardial dilation and hypertrophy.

This study evaluated the effect of a mast cell blocker (ketotofen, K) (n=6) in dogs with MR compared to non-treated dogs with MR (n=6) and normal surgical controls (n=6). Dogs had mitral regurgitation induced by transvenous chordal rupture to a defined end-point of LV regurgitation, a decrease in forward stroke volume, and increased pulmonary arterial wedge pressure. Treatments in the ketotofen (K) dogs was initiated on day three and all dogs were observed for four weeks.

Compared to untreated MR dogs, mean pulmonary artery pressures (13±1 vs. 20±3 mmHg, p<0.05) and SVR (1684±132 vs. 2151±147, p<0.05) were significantly decreased in the K-treated MR dogs. In addition, collagen volume of LV was significantly higher in K-MR dogs compared to untreated MR dogs (0.50±0.033 vs. 0.34±0..017, p<0.05), but collagen was significantly lower than in normal dogs (0.79±.031, p<0.05). The improvement in filling pressures and collagen matrix in K-treated dogs was also reflected in significantly smaller increases in LV end-diastolic dimensions, left atrium/aortic root ratio, and wall thinning (LVfw), suggesting improvement of LV and left atrial remodeling in response to the volume overload of MR. At sacrifice, K-treated dogs had significantly less LV hypertrophy (LVwt/bw) compared to non-treated MR dogs. Mast cells numbers were significantly increased (p<0.05) in K-treated (0.360±.039) and non-treated dogs (0.311±.034) with MR compared to normal controls (0.17±.026), but morphology of the mast cells were distinctly different in ketotofen treated dogs. Mast cell blockade did attenuate the collagen loss and destructive remodeling associated with MR volume overload in dogs.

ABSTRACT #67

UTILITY OF AN ELISA B-TYPE NATRIURETIC PEPTIDE ASSAY IN THE DIAGNOSIS OF CONGESTIVE HEART FAILURE IN DOGS PRESENTING WITH COUGH OR DYSPNEA. TC DeFrancesco, JE Rush*, EA Rozanski*, BW Keene,

BD Hansen, CE Atkins. College of Veterinary Medicine, North Carolina State University, Raleigh, NC and Tufts University School of Veterinary Medicine, N. Grafton, MA*.

B-type Natriuretic Peptide concentrations [BNP] are higher in humans with congestive heart failure (CHF) than in patients with dyspnea of other causes. Measurement of [BNP] is helpful in distinguishing cardiac from non cardiac cause of dyspnea in humans. Previous canine studies have shown elevated [BNP] in dogs with CHF. We conducted a prospective study of dogs presenting to the veterinary teaching hospitals at NC State University and Tufts University with an owner complaint of cough or dyspnea from February 2003 to February 2004. One to seven mls of blood was collected from each dog from one of the following groups: 1) dogs presenting with cough and/or dyspnea (n=173) 2) dogs presenting for evaluation of sub-clinical heart disease (n=86) and 3) normal dogs with no physical examination evidence of heart disease (n= 75). The plasma sample was frozen at -70oC within one hour of collection until analysis was performed by BIOSITE Inc. in August 2004. The canine BNP measurement was performed with an ELISA sandwich monoclonal antibody assay. For each dog in the cough/dyspnea group, a boarded cardiologist who was blinded to the results of the [BNP] classified the diagnosis of the dogs with cough/dyspnea as either cardiac or non-cardiac causes based on medical record review.

The diagnosis of cough/dyspnea was cardiac causes in 93 dogs (54%) and non-cardiac causes in 80 dogs (46%). The median [BNP] in dogs with cardiac cause of cough/dyspnea was 24.46 + 48.98 pg/ml while the median [BNP] in dogs with non-cardiac cause of cough/dyspnea was 2.66 + 10.11 pg/ml. This difference was statistically significant ($p<0.05$). The median [BNP] in dogs with sub-clinical heart disease was 3.03 + 7.59 pg/ml. Normal healthy dogs with no evidence of heart disease had a median [BNP] of 0.96 + 1.53 pg/ml. The area under the receiver operating characteristic curve when [BNP] was used to differentiate CHF from other causes of cough/dyspnea was 0.91.

In conclusion, this canine ELISA [BNP] assay was helpful in the diagnosis of CHF in dogs presenting with cough or dyspnea.

ABSTRACT #68

EVALUATION OF A NEW BRAIN NATRIURETIC PEPTIDE ASSAY IN DOGS. William E. Herndon, California Veterinary Specialists. San Marcos, CA; Justine A. Lee, Veterinary Teaching Hospital University of Minnesota, St. Paul, MN; Kenneth J. Drobatz, Matthew J. Ryan, Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA.

Dog brain natriuretic peptide (BNP) is a 32 amino acid peptide liberated by the modification of pro-BNP produced in the heart. BNP has various biologic effects including natriuresis, vasodilation, and neurohormonal modulation. It has been shown to be elevated in dogs and people with cardiac disease, especially those with congestive heart failure.

The purpose of this study is to report dog BNP concentration ([BNP]) obtained from a newly developed assay. Whole blood was anticoagulated with EDTA and plasma samples were frozen for subsequent assay. Samples were prospectively obtained from patients at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia. Data were not normally distributed and were compared using the Wilcoxon ranksum test. P values less than 0.05 were considered significant. [BNP] pg/ml ([BNP]) is reported as median (maximum, minimum). Dogs (n=149) were grouped into the following categories: healthy (n=38) 0.87 (19.3, 0), heart disease without congestive heart failure (CHF) (n=14) 2.15 (15.1, 0.52), CHF (n=11) 13.95 (28.7, 4.2), respiratory (n=5) 6 (7, 0.62), trauma (n=24) 0.71 (58, 0), neurologic (23) 1.51 (26.3, 0), gastrointestinal (n=12) 0.68 (45.1, 0), renal (n=9) 2.2 (18.7, 0.36), neoplasia (n=4) 1.4 (2.2, 0.36), toxicity (n=5) 1.7 (2, 0.24), and metabolic (n=4) 4.1 (6, 1.84). [BNP] was significantly elevated in dogs with CHF compared to all other categories. When compared to normal dogs, those with CHF, renal, metabolic, respiratory, and heart disease without CHF had significantly different [BNP].

Although some conclusions are limited due to the low number of dogs in particular categories, this new Biosite dog BNP assay accurately identifies dogs in CHF. Furthermore, although some dogs had moderate to severely elevated [BNP], most dogs with noncardiac disease have normal to only mildly elevated [BNP]. With further investigation, this new BNP assay may someday provide a widely available noninvasive diagnostic test with rapid turnaround time to help diagnose and/or treat heart disease and congestive heart failure in the dog.

ABSTRACT #69

SHORT-TERM HEMODYNAMIC EFFECTS OF CHRONIC ORAL CARVEDILOL IN CAVALIER KING CHARLES SPANIELS WITH ASYMPTOMATIC CHRONIC DEGENERATIVE VALVE DISEASE. S.G. Gordon[1], A. Bahr[2], M.W. Miller[1], D.M. Boothe[3] & K. Glaze[1]. [1]Department of Small Animal Clinical Sciences & Michael E. DeBakey Institute, & [2]Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, [3]Department of Anatomy, Physiology & Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL.

Chronic degenerative valve disease (CVD) is the leading cause of canine heart failure. No medication has been demonstrated to delay the progression of asymptomatic CVD. Carvedilol is a 3rd generation non-selective beta-blocker with ancillary $alpha_1$-blocking and antioxidant properties that may be useful in the treatment of CVD.

The purpose of this study was to evaluate the safety and tolerability of an up titration protocol and target chronic oral dose of carvedilol (approximately 1mg/kg twice per day) in asymptomatic dogs with cardiomegaly secondary to CVD. A physical examination, complete biochemistry panel, complete blood cell count, indirect systemic blood pressure (BP), thoracic radiographs, electrocardiogram, echocardiogram (ECHO), and gated radionuclide ventriculography (GRV) were performed at baseline (B) and repeated in 4.8+/-0.2 months (R). Plasma carvedilol concentrations (2-hour post dose) were measured during the 2nd evaluation.

The initial dose of carvedilol was approximately 0.3 mg/kg twice daily. Dose increases were carried out based on phone re-evaluations approximately every two weeks. The dose was increased from 0.3 mg/kg to 0.6 mg/kg and then to the target dose of 1.2 +/-0.1 mg/kg. Five Cavalier King Charles Spaniels were available for analysis. No dog developed clinically significant adverse signs while receiving carvedilol. Two-hour plasma carvedilol concentrations were 12.2+/1.5 ng/ml. Mean systolic BP in mmHg (B=122 vs R=112), mean heart rate in bpm (B=95 vs R=80) and mean radiographic vertebral heart scores were not significantly different (B=11.2 vs R=11.1). Left ventricular (LV) internal dimension in systole and diastole and left atrial:aorta ratio by M-mode, peak mitral inflow E, A and E:A and lateral mitral annular tissue Doppler parameters; peak S_a, E_a, A_a were not significantly different. Maximum diastolic left atrial area (cm^2) obtained from the right parasternal 4-chamber view was significantly reduced (B=5.2 vs R=4.4, p=0.03) and E:Ea was significantly reduced (B=9.8 vs R=8.3, p=0.03). GRV derived regurgitation fraction, LV ejection fraction, and Peak Emptying Rate were not significantly different. Peak Filling Rate in end diastolic volume per second was significantly reduced (B=2.0 vs R=1.2, p=0.05) and there was a trend towards reduction in Time to Peak Filling in milliseconds (B=106 vs R=57, p=0.09).

These data suggest that chronic oral carvedilol at approximately 1mg/kg PO BID is safe and well tolerated when gradually up-titrated in this population. There was no evidence of disease progression over the duration of this study (approximately five months). Some ECHO

and GRV parameters suggest a reduction in left atrial size, improvement in LV function and reduced filling pressures with chronic carvedilol in dogs with CVD. Plasma carvedilol concentrations were >10 ng/ml consistent with clinically meaningful beta-blockade but higher doses may be necessary to achieve plasma levels between 50-100 ng/ml consistent with more complete beta-blockade.

ABSTRACT #70

QUANTIFICATION OF CANINE RIGHT VENTRICULAR MOTION USING TISSUE DOPPLER IMAGING IN HEALTHY DOGS: DESCRIPTION, REPEATABILITY AND REPRODUCIBILITY. V. Chetboul[1], C. Carlos[1], A. Nicolle[1], D. Concordet[2], T. Lamour[3], J. Ginesta[3], V. Gouni[1], J.-L. Pouchelon[1], and H.P. Lefebvre.[2] [1]Cardiology Unit of Alfort, National Veterinary School of Alfort, France. [2]UMR 181 Physiopathologie et Toxicologie Experimentales INRA-ENVT, National Veterinary School of Toulouse, France. [3]Base Cinophile de l'Armée de Terre, Suippes, France.

Right ventricular (RV) motion is poorly documented in dogs. Although RV function is probably altered in many heart diseases involving the right and/or left ventricle, its quantitative assessment has not been used for clinical investigations in canine cardiology. It would therefore be relevant to develop an accurate non-invasive method for evaluating canine regional RV function. The aim of the present study was to describe the right ventricular myocardial motion and determine the within-day (repeatability) and the between-day (reproducibility) variability of RV myocardial velocities using 2D color Tissue Doppler Imaging (TDI) in awake healthy dogs.

Six healthy Beagle dogs (four to seven years; 11.9 to 16.8 kg) were used. A total of 36 2D color TDI examinations were performed by the same trained observer on four different days with three dogs examined per day at three non-consecutive times. Longitudinal RV velocities were recorded in two segments (basal and apical) of the RV myocardial wall (RVMW) using the left apical 4-chamber view. Longitudinal left ventricular free wall (LVFW) velocities were also recorded in a basal and an apical segment. A Student paired t test was used to compare the right basal and apical velocities at each phase of the cardiac cycle, and to compare LVFW and RVMW velocities at each phase of the cardiac cycle. A general linear model was used to determine the within-day and between-day coefficients of variation (CV).

As described for the LVFW, right velocity profiles included one positive systolic wave and two negative diastolic waves. The RVMW velocities were significantly higher than the LVFW velocities of the corresponding segment at each phase of the cardiac cycle in the basal segment ($p<0.01$), and in systole and late diastole in the apical segments ($p<0.05$). RVMW velocities were higher in the basal than in the apical segments ($p<0.001$), thus defining right intramyocardial velocity gradients (cm/s) from the base to the apex (6.0 ± 1.6, 6.7 ± 1.2, and 4.7 ± 1.1, in systole, early and late diastole, respectively). Most within- and between-day CV values (10/12) measured in the basal segment of the RVMW and LVFW were < 15%, the lowest being observed in the basal segment of the RVMW (3.5%) in early diastole.

In conclusion, TDI provides a rapid and non-invasive evaluation of the systolic and diastolic RV function in the awake dog with adequate repeatability and reproducibility of the measurements particularly at the base. Further studies in canine patients are however required to determine the clinical relevance, sensitivity and specificity of these new indices of RV function.

ABSTRACT #71

TREATMENT OF CAUDAL OCCIPITAL MALFORMATION SYNDROME IN DOGS BY FORAMEN MAGNUM DECOMPRESSION. CW Dewey[1], JM Berg[2], G Barone[1], DJ Marino[1], JD Stefanacci[1]. [1]Long Island Veterinary Specialists, Plainview, NY; [2]County Animal Specialty Group, Yonkers, NY.

Caudal occipital malformation syndrome (COMS) is the canine analog of human Chiari I malformation. As with human Chiari I malformation, dogs with COMS can display a variety of clinical signs; these include cerebellovestibular dysfunction, myelopathy (usually cervical), and seizure activity. Foramen magnum decompression (FMD) is usually performed for humans with symptomatic Chiari I malformation; results are favorable, with better outcomes being attained with early surgical intervention. The purpose of this study was to describe clinical results of a FMD procedure in 16 dogs with COMS. Cases were restricted to dogs with MRI evidence of COMS and no other neurologic disorders. All dogs underwent a FMD procedure that included either meningeal resection or marsupialization to the surrounding musculature. Fifteen of 16 dogs were receiving medical therapy for COMS prior to surgery. Breeds included Cavalier King Charles spaniel (9), Maltese (2), Yorkshire terrier (1), and Pomeranian (1). Mean age was 3.86 yrs. Neuroanatomic localization included multifocal CNS dysfunction (7), isolated cervical myelopathy (6), isolated cerebellovestibular dysfunction (2), and L4-S1 myelopathy (1). All dogs with multifocal CNS signs had evidence of both cerebellovestibular and cervical spinal cord disease. Other specific abnormalities included cervical hyperesthesia (13), diminished menace responses (7), positional strabismus (7), excessive scratching behavior (6), torticollis (3), abnormal mentation (2), "fly-biting" episodes (2), head tilt (2), chewing at the paws (2), excessive licking (1), eye rubbing (1), and generalized seizures (1). Mean duration of clinical signs prior to surgery was 32.19 wks (1-208 wks). Syringohydromyelia was evident on MRI in 15/16 dogs (93.75%). No intraoperative complications occurred. Postoperative complications occurred in two dogs. One dog had worsening of a head tilt, which resolved in three weeks. Another dog experienced neck pain after the initial FMD (resolved in four wks), and was nonambulatory tetraparetic following repeat FMD. Resolution of clinical signs occurred in seven dogs (43.75%), and improvement occurred in six dogs (37.5%), for an overall positive result of 81.25%. One dog did not improve, one dog worsened and was euthanized, and one dog died nine days following repeat FMD (the tetraparetic dog), due to a suspected ruptured viscus. Repeat surgery was performed in 4 dogs (25%), due to constrictive scar tissue formation at the original FMD site. Drugs were discontinued in all dogs with resolution of signs. Four of the 6 improved dogs remained on medical therapy. Five of 6 dogs with scratching behavior prior to FMD continued to do so postoperatively; two of these five dogs required medical therapy to control scratching. Mean duration of signs prior to FMD was 3.8 wks for the resolved group, and 78.08 wks for the improved group. Results suggest that FMD is often an effective treatment for COMS, especially if performed early in the disease course.

ABSTRACT #72

CSF FLOW ABNORMALITIES IN CAUDAL OCCIPITAL MALFORMATION SYNDROME. PA March[1], CJ Abramson[1], M Smith[2], J Murakami[2]. [1]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH; [2]Department of Neuroradiology, Children's Hospital, Columbus, OH.

The pathogenic factors responsible for the progression of signs in the caudal occipital malformation syndrome (COMS) are not well understood. The development of secondary syringohydromyelia appears to coincide with the onset of clinical neurologic deficits but the CSF flow abnormalities that lead to syrinx formation have not been described in dogs with COMS. The purpose of this study was to characterize and compare CSF flow dynamics in normal dogs and in dogs with COMS using cine phase contrast MRI. Dogs with COMS were grouped according to severity of syrinx formation. Cine flow

MRI imaging was performed in the mid-sagittal and axial planes using a 1.5 Tesla MR scanner (GE Medical Systems®) equipped with a retrospectively graded phase contrast sequence to measure CSF flow velocity throughout the cardiac cycle. Velocity encoding was cranial to caudal. Sixteen image frames per cardiac cycle were evaluated for cranial and caudal flow velocities using a GE Medical Systems® software program. Regions of interest were drawn manually to encompass specific areas of subarachnoid space, syrinx cavities, and cord parenchyma. CSF velocities, ratios of diastolic to systolic flow times, and parenchymal movements were measured.

Cine flow MRI was successfully performed on 30 dogs. Flow determinations in the axial plane were more reproducible and reliable than those in the mid-sagittal plane. Axial and sagittal flow was significantly impaired in the region of the cisterna magna and foramen magnum in dogs with COMS compared to normal dogs. In severely affected dogs, reversal of CSF flow was observed during diastole. Caudal movement of the caudal brainstem and cerebellum was significantly greater and more prolonged in dogs with COMS. CSF flow in the ventral subarachnoid compartment was minimally affected. Increased flow gradients were found between more cranial and caudal dorsal subarachnoid spaces in dogs with COMS. In the areas of syrinx formation, flow of CSF in the dorsal subarachnoid space was less impaired but exhibited a longer duration of caudal versus cranial flow. In dogs with moderate to severe COMS, caudal and cranial CSF flow within the syrinx was marked and was equal to flow velocities in adjacent subarachnoid spaces.

This study demonstrated that non-invasive cine flow MRI measurements can be performed in dogs and that dogs with COMS have abnormalities of CSF flow and neural tissue shifts during systole and diastole. Magnitudes of some of these changes were correlated with the degree of syrinx formation. This imaging tool may be useful in predicting progression of disease in dogs with COMS and further studies are planned to investigate the role of cine flow MRI in monitoring CSF flow dynamics before and after foramen magnum decompression surgery.

ABSTRACT #73

COMPARISON OF CONVENTIONAL SPIN-ECHO AND FAST SPIN-ECHO MAGNETIC RESONANCE IMAGING OF THE CANINE BRAIN. Jaime Sage, Valerie Samii, Eric Green, Carley Abramson, Mark Smith, Cheryl Dingus. The Ohio State University, Columbus, OH.

The purpose of this study was to compare the resolution of fast spin-echo (FSE) and conventional spin-echo (CSE) MRI sequences with maximized resolution parameters and a limited acquisition time to determine if the benefits afforded by FSE are significant enough to warrant replacement of CSE for imaging the canine brain. T2-weighted FSE and CSE are two MRI sequences used to define pathologic brain lesions. Given the same imaging parameters the resolution of FSE images is inherently inferior to that of CSE. However, FSE images can be acquired in a shorter amount of time allowing resolution parameters to be increased. The ability to obtain higher quality images in less time has lead to the replacement of CSE by FSE in human medicine.

MRI of the brain of 46 dogs with clinical neurological signs referable to the brain and eight dogs suspected of having Chiari type-I malformation were performed. Resolution of individual FSE and CSE images from each dog were compared in blinded fashion by three observers (part I) based on distinction of the lateral ventricles, third ventricle, gray and white matter junctions and gyri and sulci definition. The observers also reviewed the complete MRI study of each dog for the presence of pathology and motion artifact (part II).

Acquisition times ranged from 4:24 to 7:16 minutes for FSE scans and from 6:32 to 11:26 minutes for CSE scans. There was a significant difference in the resolution of the CSE and FSE scans when the variables used to evaluate resolution were considered together ($p = 0.000$) and separately ($p = 0.001$). All reviewers consistently rated the resolution of FSE scans higher than the CSE scans ($p = 0.000$).

Overall inter-rater reliability was poor ($\kappa \leq 0.25$) to fair ($\kappa = 0.26 – 0.50$) with weighted Kappa values ranging from 0.082 to 0.589 (average = 0.251) and Pearson correlation ranging from 0.182 to 0.757 (average = 0.369).

Ratings for all variables were higher when the complete MRI study was evaluated as compared to using only the individual images (p-value = 0.000). Positive correlation of all reviewers' ratings between the individual images in part I and the complete MRI study in part II (Pearson correlation 0.141 to 0.475) was shown.

Although inter-observer reliability was poor, all reviewers agreed that fast spin-echo images had significantly higher resolution than conventional spin-echo images for all parameters. This study supports the replacement of T2-weighted CSE with FSE when imaging the canine brain due to higher resolution capabilities in a shorter acquisition time.

ABSTRACT #74

LONG-TERM EFFECT OF CERVICAL FUSION ON NEUROLOGICAL STATUS AND VERTEBRAL CANAL DIAMETER IN GIANT BREED DOGS WITH CERVICAL STENOTIC MYELOPATHY. H. Galano, N. Olby, N. Sharp, T. Skeen, K. Muñana, P. Early, S. Sullivan. North Carolina State University Veterinary Teaching Hospital, Raleigh, North Carolina.

Cervical stenotic myelopathy (CSM) is a complex disorder of the cervical spine in large and giant breed dogs. Spinal cord compression occurs as a result of proliferation of the dorsal portion of the annulus fibrosus, the dorsal longitudinal ligament (large breed dogs), the articular facets and the ligamentum flavum (giant breed dogs). It is theorized that underlying instability between congenitally malformed vertebrae causes proliferation of these aforementioned structures, ultimately compressing the spinal cord. We hypothesized that progression of signs in giant breed dogs is due to both repeated concussive injury to the spinal cord and progressive spinal cord compression. We proposed that fusion of the cervical vertebrae would address underlying instability, thus preventing concussive spinal cord injury, and causing regression of excess bone and soft tissues alleviating compression of the spinal cord. The goals of this study were to determine whether cervical fusion of giant breed dogs with CSM results in regression of excess bone and an improvement in neurological status.

Four giant breed dogs diagnosed with CSM and treated with fusion of the affected cervical vertebrae using PMMA plugs were included in the study. All dogs had dorsolateral compression of the cervical spinal cord by abnormal articular facets visible on CT images and were at least one-year post surgery. All dogs underwent a neurological examination and a CT scan of their cervical spine under sedation. The severity of neurological signs was graded as follows: 0=normal, 1=neck pain, 2=ataxia, 3=ambulatory tetraparesis, 4=non-ambulatory tetraparesis, 5=tetraplegia. The diameter of the vertebral canal at the points of greatest compression in the pre and post-operative CT images were compared.

All dogs were graded as 3 on their initial neurological examination and showed an almost immediate improvement postoperatively. Three dogs returned to normal and one dog had a mild ataxia but was significantly improved since the surgery. Long term complications included a domino lesion in one dog, and superficial dermatitis secondary to splinting in two dogs. Repeat CT images revealed vertebral fusion and remodeling of the articular facets in all dogs, causing an apparent increase in the diameter of the vertebral canal and decompression of the spinal cord.

We conclude that cervical fusion does result in regression of excess bone with subsequent widening of the vertebral canal and improvement in neurological status. Vertebral fusion prevents

repeated concussive injuries to the spinal cord, thus improving the neurological status in the short-term as well as in the long-term benefit of articular facet remodeling.

ABSTRACT #75

COMPARISON OF MAGNETIC RESONANCE IMAGING AND MYELOGRAPHY IN THE DIAGNOSIS OF CERVICAL SPONDYLOMYELOPATHY IN DOBERMAN PINSCHER DOGS: 18 CASES. Ronaldo C. da Costa, Joane Parent, Gary Partlow, Howard Dobson, David Holmberg, Jonathan LaMarre. Ontario Veterinary College, Univ. of Guelph, Guelph, ON, Canada.

Despite a high incidence of cervical spondylomyelopathy (CSM) and the widespread use of myelography and MRI, there has been no study comparing the diagnostic efficacy of both methods in dogs with CSM. The authors present a comparison of both techniques in 18 Doberman dogs.

Eighteen Dobermans affected with CSM were prospectively studied. Neurological examination was performed in all dogs. Cervical myelography was performed using 0.3 ml/kg of iohexol, and lateral (static and traction) and ventro-dorsal views were obtained. Linear traction was obtained using a neck harness designed to exert 9 kg traction. MRI was performed with a 1.5 Tesla magnet. On the sagittal plane, T1, T2, proton density (PD) and STIR weighted images (WI) were acquired. On the transverse plane, T1, T2, and gradient echo (GE) combined with magnetization transfer (MT), with and without intravenous gadolinium injection were obtained. After acquisition of all sequences with the cervical spine in neutral position, traction studies were acquired as described above with T2 WI. The imaged area extended from C1 to T2, imaging all intervertebral discs. On the myelogram and the MRI T2 weighted mid-sagittal images, each disc space – pre and post-traction - was classified based on a compression scale as normal, partial or complete subarachnoid space compression, or spinal cord compression. On the MRI, parenchymal spinal cord changes were also recorded. Foraminal stenosis was evaluated on the GE transverse images at each intervertebral disc level and classified as absent, mild, moderate or severe.

The clinical signs were caused by a disc-associated problem in 16 dogs (88%). In one dog the signs were caused by bilateral foraminal stenosis and in another by bilateral dorsolateral cord compression caused by impingement of articular facets. Based on MRI 10 dogs had a lesion at C6-7 (55%) and eight at C5-6 (45%). Fourteen dogs (77%) had foraminal stenosis, which was severe in three (21%), moderate in five (36%) and mild in six (43%). Ten dogs (55%) had parenchymal spinal cord changes observed as increased signal intensity on T2 WI. On the myelogram the lesion was classified as dynamic in 10 dogs (55%) and static in eight dogs (45%), while on the MRI it was classified as dynamic in seven dogs (39%) and static in 11 dogs (61%). Eight dogs had disagreement between MRI and myelogram results. The most common divergence (four cases) was a lesion being classified as dynamic on myelogram but static on MRI. In two dogs the myelogram markedly underscored the severity of spinal cord compression, and in another it failed to identify the cause of the signs (foraminal stenosis). The agreement on weighted Kappa test between the myelogram and MRI pre-traction scores was 0.66 (substantial) and post-traction 0.59 (moderate).

The results of this study indicate that although the myelogram can identify the location of the lesion in most patients, MRI is more accurate in predicting the site and nature of the spinal cord compression.

ABSTRACT #76

MORPHOMETRIC AND ANATOMO-PATHOLOGIC MAGNETIC RESONANCE IMAGING FEATURES OF DOBERMAN PINSCHER DOGS WITH AND WITHOUT CLINICAL SIGNS OF CERVICAL SPONDYLOMYELOPATHY – 32 DOGS. Ronaldo C. da Costa, Joane Parent, Gary Partlow, Howard Dobson, David Holmberg, Jonathan LaMarre. Ontario Veterinary College – University of Guelph, Guelph, Ontario, Canada.

An investigation of the cervical spinal anatomy of normal dogs is fundamental to our understanding of the pathophysiology of the cervical spondylomyelopathy (CSM). We present the anatomo-pathological abnormalities and the morphometric analysis of the cervical spine of Doberman dogs with and without clinical signs of CSM studied with magnetic resonance imaging.

Thirty-two dogs were studied, 16 clinically normal mature Dobermans and 16 affected with CSM. The average age was 4.3 years and six years, respectively. The gait abnormalities were graded and scored. MRI was performed with a 1.5 Tesla magnet. On the sagittal plane and transverse planes, T1, T2 and proton density weighted images (WI) were obtained. Gradient echo combined with magnetization transfer were also acquired transversally. After acquisition of all sequences with the spine in neutral position, linear traction studies (T2 weighted) were obtained using a neck harness designed to exert 9 kg traction. On the sagittal T2 WI, the vertebral canal and the spinal cord diameters (height) were measured from C2 to T1. The intervertebral disc space was measured, pre and post-traction, from C2-C3 to C7-T1. On the transverse T2 WI, the vertebral canal and spinal cord area, width and height were measured. On the sagittal T2 WI, each disc space – pre and post-traction - was classified as normal, partial or complete subarachnoid space compression, and spinal cord compression. The intervertebral discs were classified based on signal intensity as normal, partially or completely degenerated. A two-way ANOVA and a Wilcoxon Mann-Whitney test were used to analyse the results.

The results demonstrated that within the group of clinically normal dogs, 3 (19%) had spinal cord compression and another 5 (31%) had complete subarachnoid space compression, caused by disc herniation in all instances. Twelve normal dogs (75%) had disc degeneration, which affected multiple discs in 11 dogs (92%). Foraminal stenosis was observed in 11 normal (69%) and 14 affected dogs (87%); no significant difference was seen between groups. Signal changes within the spinal cord parenchyma were not observed in normal dogs but were seen in 10 affected dogs (62%). Overall the morphometric analysis indicated significant differences ($p<0.05$) only for the measurements at C5-6 and C6-7 between groups. The mean intervertebral disc space of all discs pre and post-traction in normal dogs was 4.3 mm and 5.2 mm, while in the affected dogs was 4.6 mm and 5.7 mm. The pre and post-traction measurements were significantly different between groups ($p<0.05$), but the amount of disc distraction was not statistically different between groups.

This study established the reference measurements of the cervical spine of normal Dobermans and demonstrated that clinically normal Dobermans have anatomo-pathological abnormalities comparable with those seen in dogs affected with CSM.

ABSTRACT #77

T_2 AND DIFFUSION MRI AID IN ASSESSING NEUROPATHOLOGY OF FELINE ALPHA-MANNOSIDOSIS. CH Vite[1], S Magnitsky[2], D Aleman[1], ME Haskins[3], P O'Donnell[3], K Cullen[3], S Pickup[2], JH Wolfe[3], H Poptani[2]. [1]Clinical Studies, School of Veterinary Medicine; [2]Radiology, Medical School; [3]Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

A mutation in the MANB gene causes the lysosomal storage disease alpha-mannosidosis (AMD) which is characterized by a deficiency in lysosomal α-mannosidase activity leading to intra-

lysosomal accumulation of mannose-rich oligosaccharides. AMD cats have neurologic deficits, a generally uniform disease course, and death by six months without treatment. The progression of the CNS disease can be studied in the living animal using clinical evaluation, electrodiagnostic testing, and MRI. The neuropathology has been well described and is characterized by swelling of neurons and glia, neuronal loss, gliosis and demyelination. Since cell swelling would lead to changes in the apparent diffusion coefficient (ADC), the goal of this study was to investigate the utility of diffusion and T_2 imaging in the detection and progression of neuropathology associated with AMD.

Two groups of 16-week-old animals were studied: normal controls (n=4) and AMD affected cats (n=3). Multi-slice axial MR images were acquired on a 4.7T magnet equipped with 12cm 25 G/cm gradients using a 12 cm Litz coil. To generate T_2 maps, 2D spin-echo images were acquired with four echo times (TE = 15, 35, 55 and 75 ms). The trace of tensor (ADCav) diffusion-weighted images were acquired using five b-values (0, 1038.6, 44538.5, 93461.6, 166154). AMD cats exhibited a decrease in T_2 of the gray matter (caudate nucleus, cerebral cortex, thalamus) and a significant increase in T_2 of the white matter (corona radiata, internal capsule, centrum semiovale) compared to normal cats. The percent change in T_2 of these cats from different structures of the brain were similar and homogenous throughout the brain. ADCav of the gray matter was decreased throughout the brain in all the AMD cats compared to normal controls.

Since demyelination has earlier been reported in this model using magnetization transfer contrast, we hypothesize that the observed increase in T_2 of the white matter may reflect demyelination. The reason for the decrease in T_2 of the gray matter is unknown, however, our working hypothesis is that it may represent changes in the extracellular space resulting from cell swelling. A major histopathological hallmark of CNS disease in cats with AMD is swelling of the neurons and glia caused by the large amounts of stored mannose-rich oligosaccharide substrate, which may have led to the observed decrease in the ADCav of the gray and white matter. In conclusion, we have shown in our preliminary studies that AMD can be quantitatively detected using T_2 and ADCav. MRI studies may assist in monitoring the progression of the disease as well as evaluating response to therapeutic interventions in the management of this disease.

ABSTRACT #78

FRAMELESS STEREOTACTIC CT-GUIDED NEEDLE BRAIN BIOPSY IN DOGS. PF Adamo, AS Lang. University of Wisconsin, Madison, WI.

As alternative to conventional stereotactic CT-guided brain biopsy we developed a new technique that does not require the complicated and expensive stereotactic frames used in previous studies. In this new procedure, the stereotactic frame is replaced by a biopsy needle guide inserted into a surgical arm, which in turn is attached to a frame. The technique was first performed in cadavers (five dogs) in which a brain lesion was simulated by injecting 0.2 ml of methylene blue mixed with 0.2 ml of Hypaque-76 in one side of the forebrain. The technique was then successfully performed in four clinical cases.

Technique description: The animals were anesthetized in the CT suite, the hair on the head was clipped over the region of interest, transverse contrast CT images were obtained, the target area was localized on one transverse image, and the table position was noted. The skin was marked with indelible ink corresponding to the laser localizer at this position (transverse plane). Several barium marks were applied at 90° along the ink line. We then scanned the target area with four to five slices of 3mm thickness each. These images displayed the lesion in the brain as well as the barium lines over the skin. The barium line best aligned with the intracranial lesion was chosen as a reference point for the skin incision and the craniotomy. After a sterile scrub a small skin incision was made. The underlying muscles were incised and dissected from the calvarium, and a hand drill was used to make a small hole in the calvarium. The apparatus holding the biopsy needle was then mounted over the patient's head, the table was re-positioned at the same point of the previous chosen target area, and the needle was aligned to the lesion by using serial transverse CT scans. A 2.1mm diameter Sedan Side-Cutting Biopsy Needle was used. The distance from the tip of the needle to the center of the lesion was measured by using CT, and the needle was then advanced. A gentle negative pressure was created with a 10ml syringe applied to the proximal end of the inner needle to introduce the sample to be collected into the needle window, and the inner biopsy needle was rotated 180° to cut the collected sample. The inner needle was then removed (the external needle was left in place), and the sample were extracted by flushing the needle with air and with sterile saline. Three samples were collected and submitted for cytologic and histologic examination, bacterial and fungal culture and antimicrobial sensitivity testing.

No complications were noted and all dogs were discharged 48 hours after the biopsy. Biopsy samples indicated astrocytoma, GME, choroid plexus papilloma respectively in three cases. In one case (Case # 4) the final histopathological diagnosis was not possible. In two dogs the diagnosis was confirmed at necropsy. The technique, which was effective and relatively simple, may be a valid alternative to the conventional stereotactic brain biopsy.

ABSTRACT #79

STEREOTACTIC RADIOSURGERY FOR THE TREATMENT OF PRIMARY INTRACRANIAL NEOPLASMS IN DOGS. Christopher L. Mariani[1], Roger M. Clemmons[1], Michael A. Wong[1], Andrew L. Hopkins[2], Cheryl L. Chrisman[1], Heidi L. Barnes[1], Rowan J. Milner[1], Nola V. Lester[1], Didier A. Rajon[3], William A. Friedman[3], Francis J. Bova[3]. Departments of Small Animal Clinical Sciences[1] and Neurosurgery[3], University of Florida, Gainesville, FL and North Florida Neurology[2], Orange Park, FL.

Stereotactic radiosurgery (SRS) is a technique utilizing advanced diagnostic imaging to deliver a highly conformal dose of radiation to a defined target. Radiation is typically produced with a cobalt source (^{60}Co, gamma rays) or with a linear accelerator (LINAC, x-rays). We retrospectively reviewed dogs with primary intracranial neoplasms treated with LINAC SRS at our institution to determine tumor type and location, survival time and side effects of therapy. Neoplasia was not confirmed histologically in all animals, as dogs with computed tomographic or magnetic resonance imaging scans consistent with neoplastic lesions were included in the study. Dogs with tumors invading the brain from extracranial areas and non-neoplastic lesions were excluded. The majority of patients were treated with a frameless targeting system (utilizing a "bite plate" attached to the targeting array), although three animals were irradiated with the aid of a modified Brown-Roberts-Wells headframe rigidly fixed to the skull.

Twenty-two dogs met the inclusion criteria (12 males, 10 females). The median age at treatment was 9.2 years (range 3.7-13.5 years) and a variety of breeds were represented. Tumors treated included 16 meningiomas (nine histologically confirmed, one cytologically confirmed, six presumptive based on diagnostic imaging), three pituitary tumors (one histologically confirmed, two presumptive), two gliomas (one histologically confirmed, one presumptive), and one choroid plexus papilloma (presumptive). There were 20 supratentorial and two infratentorial lesions. The median radiation dose delivered was 1500 cGy (range 1000-2500 cGy). Five dogs had their tumors surgically debulked prior to SRS, four dogs had ventriculoperitoneal shunts placed for obstructive hydrocephalus before SRS and four dogs received chemotherapy (hydroxyurea) in addition to SRS.

The median survival time for the entire cohort at the time of writing is 426 days (range 1-1595 days; six dogs still alive). There was no

significant difference in survival between dogs with meningiomas (322 days) and dogs with other tumors (426 days). If animals dying due to causes other than tumor progression were censored from the analysis (three dogs), median survival times were 584 days for dogs with meningiomas and 426 days for dogs with other tumors. Side effects attributable to the SRS procedure were rare, but included obtundation and status epilepticus.

This technique compares favorably to other treatment options available for intracranial neoplasia in dogs. A distinct advantage of SRS over fractionated radiotherapy is the requirement of a solitary anesthetic episode for treatment, and its noninvasive nature is appealing to owners reluctant to pursue definitive surgical therapy.

ABSTRACT #80

RESULTS OF A KETOGENIC FOOD TRIAL FOR DOGS WITH IDIOPATHIC EPILEPSY. Edward (Ned) E. Patterson[1], Karen R. Munana[2], Claudia A. Kirk[3], Steve R. Lowry[4], P. Jane Armstrong[1]. 1. University of Minnesota, St. Paul, MN. 2. North Carolina State University, Raleigh, NC. 3. University of Tennessee, Knoxville TN. 4. Hill's Pet Nutrition Center, Topeka, KS.

The objective of this study was to determine if a high fat, low carbohydrate food (ketogenic food; KF) had a significant effect on seizure frequency in dogs with idiopathic epilepsy compared to a control food (CF). A multi-institutional, prospective, double masked, placebo controlled study was performed from 1999 to 2002. Dogs were enrolled if they had a diagnosis of idiopathic epilepsy, were receiving phenobarbital and/or potassium bromide at steady state blood concentrations, and had at least three seizures in the previous three months. Over a 3- to 6-month baseline monitoring period, during which all dogs were fed CF to ensure consistency of dietary chloride content, an initial seizure frequency was established.

After the baseline monitoring, dogs with five or more seizures were randomized to begin receiving CF (16% crude fat, 54% NFE, 25% crude protein; as dry matter) or KF (57% fat, 5.8% NFE, 28% protein; as dry matter) after a 36-hour fast. Seizure frequency and laboratory results were evaluated at 0, 0.5, 3 and 6 months into the test period. Thirty-one dogs entered the baseline monitoring period, 17 dogs underwent randomization for the test period with 12 completing the study (6 CF, 6 KF).

Dogs in the KF group had significantly higher serum concentrations of beta-hydroxybutyrate (BHB) at 3 and 6 months during the test period (2.10, 1.99 mg/dl) than the CF group (0.87, 0.62 mg/dl) ($p = 0.012$, $p = 0.0072$). One third of dogs in each group had a 50% or greater reduction in seizure frequency. There was no difference in seizure frequency between KF group dogs (2.02, 2.41/month) and CF group dogs (2.35, 1.36/month) at 0 and 6 months respectively ($p = 0.71$, 0.17) despite the differences in BHB concentrations. There were no significant differences in serum concentrations of glucose, phenobarbital, or bromide, blood pH, or body weight between the groups. Three of nine dogs fed KF developed pancreatitis, and 2 of 31 dogs fed CF developed pancreatitis. There was no statistical difference in the incidence of pancreatitis between the two groups (Chi-square 4.61, $p = 0.203$). These data suggest that this population of dogs appear at risk for developing pancreatitis regardless of food fed. High owner satisfaction with the results of the diet to which their dog was assigned may have been due to close monitoring, anti-epileptic drug dose modifications, placebo-type effects and/or stable dietary chloride helping stabilize bromide levels.

Power calculations performed following completion of the test period indicated that 22 dogs in each group would be required to show significant differences between food groups using seizure frequency as the major outcome variable. Therefore there was insufficient power in this study to determine effect of food treatment.

ABSTRACT #81

CLINICAL PHARMACOKINETICS AND SAFETY OF THE ANTICONVULSANT ZONISAMIDE IN HEALTHY DOGS FOLLOWING SINGLE AND MULTIPLE DOSING. Boothe DM[1], Perkins J[1], Dewey C, Auburn University, Auburn AL[1], and Long Island Veterinary Specialists, NY[2].

The purpose of this study was to design a dosing regimen and evaluate the safety of zonisamide (ZNS). Eight adult (four male and four female) dogs were studied following single (6.9 mg/kg) and multiple dosing (10.25) using a randomized crossover design.Blood samples were collected intermittently for 48 hours and then dogs were dosed orally (10 mg/kg) twice daily for eight weeks. Blood samples were collected weekly and at discontinuation of the drug. Additionally, urine was collected to determine 24 hour urine ZNS clearance following IV administration. Safety was based on clinical pathology, thyroid and urine testing during both studies. ZNS was measured using HPLC validated in canine serum, plasma, erythrocytes (RBC) and whole blood. Data were subjected to standard non-compartmental pharmacokinetic analysis (WinNonLin®) and compared using one-way ANOVA. Safety parameters at study beginning and end were compared using a Student t-test. ZNS (mcg/ml) oral maximum concentration (Cmax) was greatest in RBC (28.73µg/ml) and least (14.36µg/ml) in plasma. The unbound portion was 60$\pm$13%. Volume of distribution also was greater (1L/kg) in plasma and least (0.4 L/kg) in RBC. Clearance was 7.55 ml/hr/kg from plasma and 5.06 ml/hr/kg from RBC. Elimination half- life in plasma was 16.4 hr in serum and 57.4 hr in RBC. Bioavailability was 126.8% for RBC and 189.6% for plasma. Following multiple dosing, at steady-state (by 7 days), Cmax averaged 65.8µg/ml with fluctuations of 17.2% between Cmax and Cmin dosings. Concentrations did not differ among blood compartments at the end of multiple dosing. Although differences did occur across time in clinical pathology tests, all were within normal limits at study end except for T4 (at 8 weeks 1$\pm$0.4 ng/dl total; 1$\pm$0.3 ng/dl free and 0.18 ng/dl TSH). Urine citrate and calcium were greater at study end but not when corrected by urine creatine. In conclusion, ZNS dosed at 10 mg/kg twice daily for dogs should maintain therapeutic levels (10 to 70µg/ml) recommended in human epileptic patients. Therapeutic monitoring should be based on serum or plasma; the impact on potential stone formation should be further assessed.

ABSTRACT #82

PHARMACOKINETICS OF ZONISAMIDE ADMINISTERED ALONE AND IN COMBINATION WITH PHENOBARBITAL IN DOGS. M Saito, K Orito, S Takikawa, T Kageyama, M Muto. Azabu University, Sagamihara, Kanagawa, Japan.

Zonisamide (ZNS) is an antiepileptic drug (AED) recently approved for human use in the United States. The drug has been used in Japan to treat humans with epilepsy since 1984, and has been utilized by veterinarians in this country for approximately 10 years. Limited clinical studies have shown the efficacy of the drug for canine epilepsy in the absence of significant adverse effects. Despite these promising results, only minimal pharmacokinetic data is available and a standardized dosage regimen has not been established. Moreover, potential drug interactions with other AEDs have not been evaluated. This may be of significance when phenobarbital (PB) and ZNS are used in combination, as PB is known to induce hepatic enzymes, one of which is responsible for the metabolism of ZNS. The objectives of this study were to determine the pharmacokinetics of ZNS before and after chronic administration of PB and to evaluate the pharmacokinetic properties of ZNS when used to treat naturally occurring epilepsy in dogs.

ZNS was administered to 4 healthy adult beagles as a single oral dose of 5 mg/kg. Serum ZNS concentrations were determined using HPLC. After a 7-day washout, PB was administered orally *q* 12 h at 5

mg/kg for 35 days, followed by a single oral ZNS dose of 5 mg/kg, and serum ZNS concentrations were again measured. Initial mean peak serum concentration (C_{max}) and time (T_{max}), and elimination half-life ($T_{1/2}$) of ZNS were 3.75µg/ml, 4.9 h and 14.6 h, respectively, with steady state levels achieved after three to four days of ZNS administration. After PB dosing, mean C_{max} T_{max} and $T_{1/2}$ were 3.1 µg/ml, 5.4 h, and 8.4 h respectively with steady state concentrations being achieved after 2 to 2.5 days. Simulation analysis using these pharmacokinetic parameters suggested that about twice the dose of ZNS is needed in dogs receiving PB compared with those on ZNS alone in order to achieve the same serum ZNS concentration with long-term therapy.

Trough serum ZNS concentrations were determined using HPLC after a minimum of seven days of dosing in 14 epileptic dogs. All dogs were receiving oral ZNS *q* 12 h, had no evidence of hepatic or renal dysfunction, and were not receiving any concurrent drugs known to induce hepatic enzymes. The mean dose was 5.6 mg/kg (range, 2.5 to 8) *q* 12 h and the mean trough concentration was 14.4µg/ml (range, 5.3 to 27.9). No adverse effects were attributable to ZNS. Serum levels were relatively proportional to dose ($r=0.78$, $p<0.01$). The minimum dose required to attain a reported minimum therapeutic concentration (12.5 µg/ml) was 6 mg/kg *q* 12 h. Based on the pharmacokinetic properties demonstrated in this study, ZNS appears to be a suitable AED in dogs although special attention may be needed if PB is concurrently used. The pharmacokinetic variability seen in this study suggests that dosage should be tailored in each dog based on serum ZNS concentrations.

ABSTRACT #83

MUSCLE AND NERVE BIOPSIES IN 138 CATS: DIAGNOSIS AND OUTCOME. R. Pettigrew[1], M. Kent[2], W.L. Berry[3], G.D. Shelton[1]. [1]Department of Pathology, University of California, San Diego, La Jolla, CA; [2]Department of Small Animal Medicine and Surgery, University of Georgia, Athens, GA; [3]Southern California Veterinary Referral Group, Irvine, CA.

With the exception of diabetic neuropathy, descriptions of feline neuropathies in the veterinary literature are few and limited to single case reports. This retrospective study describes the histological diagnosis, clinicopathological data, and long term outcome of 138 cats for which muscle and nerve biopsies were submitted to the Comparative Neuromuscular Laboratory between 2000-2004. Cases lacking a peripheral nerve biopsy were included in the study if intramuscular nerve branches were observed in the muscle. In the muscle biopsies, neuropathic changes were present in 85(61.6%), myopathic changes in 20(14.5%) and no changes present in 33(23.9%). Variable depletion of nerve fibers within intramuscular nerve branches was present in 27(19.6%) of the muscle specimens. In the nerve biopsies (n=131), axonal degeneration (AD) was present in 44(33.6%), demyelination (D) in 13(9.9%), mixed axonal degeneration and demyelination (AD/D) in 16(12.2%), and no abnormalities observed in 58(44.3%).

The majority of cats were domestic short hair without a sex predilection (55; 39.9 %). Several purebred cats were represented, there were 12 Bengal cats (8.7% of the cases) including both sexes. The average age of presentation was 6 years for cases of AD or AD/D, and 4 years for cats with D or depletion of intramuscular nerve branches. Of note, the average age at presentation in Bengal cats was 1.2 years. Approximately 50% of the 138 cases were 5 years of age or younger. Weakness was the primary presenting clinical complaint. Lower motor neuron signs were present in 120 (87.9%), upper motor neuron signs in 2 (1.5%) and a normal neurological examination in 16 (11.6%) of cats. Other presenting signs included stridor with suspected laryngeal paralysis (n=2), megacolon (n=1) and chronic non-healing wounds (n=2). Routine laboratory work was unremarkable. Of the cats tested two were positive for FeLV, one was FIV positive, and none had antibody titers indicative of active toxoplasmosis. There was a strong association between electrophysiology and histopathologic changes for the majority of cases. Of 108 cats undergoing electrodiagnostic testing, 98 had abnormal EMG findings, 40 had slowed motor nerve conduction velocities (MNCV), and 16 had decreased compound muscle action potential amplitudes (CMAP). Six cases revealed histopathologic evidence of axonal degeneration with a normal MNCV. Of the 79 cases with long-term follow-up, 45 (57%) improved or had a complete resolution of clinical signs. The average age of cats that recovered or had complete resolution of signs was 3.2 years. Relapse of clinical signs occurred in five of the 79 cats.

In summary, early age of onset appears to be associated with recovery from peripheral neuropathy in cats. Bengal cats have an early onset neuropathy with average age of 1.2 years. Axonal degeneration despite a normal MNCV, in a percentage of cats, indicates the necessity of nerve and muscle biopsies when neuromuscular disease is suspected.

ABSTRACT #84

HAPLOTYPE ANALYSIS OF MULTIPLE SYSTEM DEGENERATION IN KERRY BLUE TERRIERS AND CHINESE CRESTED DOGS. Dennis P. O'Brien, Jeremy F. Taylor, Joan R. Coates, Shahnawaz Khan, Robert D. Schnabel, and Gary S. Johnson. University of Missouri, Columbia, MO.

The purpose of this study was to refine the mapping of the locus for canine multiple system degeneration (CMSD). CMSD is a fatal, hereditary movement disorder of Kerry Blue Terriers and Chinese Crested dogs characterized by degeneration of cerebellar Purkinje cells, substantia nigra, and caudate nucleus (deLahunta & Averill 1976; O'Brien et al 1996). Breeders refer to the condition as PNA (progressive neuronal abiotrophy). The mode of inheritance is autosomal recessive necessitating breeding trials to identify carriers. We previously used a 51-dog three-generation family of both breeds and their crosses, including 18 dogs diagnosed with CMSD based on characteristic signs and confirmed at necropsy, to demonstrate that the disease is allelic in the two breeds and to map CMSD to a 30 Mb region of canine chromosome 1. We now report that haplotype analysis has enabled us to further narrow the target region for CMSD.

Haplotypes were determined using a panel of seven microsatellite markers within the target region. These microsatellites were identified from the first build of the canine whole genome sequence. Haplotypes were determined by genotyping the above-mentioned family members and an additional CMSD-affected Kerry Blue Terrier of unknown relationship.

Breed-specific CMSD-associated haplotypes identified an 11 Mb region of CFA1 as harboring the causal gene. The orthologous region of human chromosome six contains 41 known genes. We are currently examining candidate genes within the target region and evaluating additional microsatellite markers to identify a marker panel for use in genetic counseling to distinguish normal dogs from potential carriers.

ABSTRACT #85

COMPARISON OF AN IN-HOUSE CORTISOL TEST KIT WITH A REFERENCE LABORATORY CHEMILUMINESCENT ASSAY. PP Kintzer and L Turgeon, Boston Road Animal Hospital, Springfield, MA.

Endocrine testing of dogs for adrenal disorders is commonplace in clinical practice. Measurement of serum cortisol concentrations is necessary in the screening tests (ACTH stimulation test, dexamethasone suppression tests) for adrenal disorders as well as for monitoring therapy in many cases. The current study was designed to compare the serum cortisol concentrations measured using a validated in-clinic assay (SNAP Cortisol, IDEXX Laboratories, Inc.) with the cortisol concentrations measured using a validated reference

laboratory chemiluminescent assay (Immulite, Diagnostic Products Corp.).

Eighty-one serum samples were collected from 39 patient events (dogs being tested for suspected adrenal disease and dogs being monitored during therapy for hyperadrenocorticism). Each sample was divided into two aliquots. One aliquot was tested in-clinic using the SNAP Cortisol assay. The other aliquot was sent out to a reference laboratory (ILS, North Grafton, MA) and tested using a chemiluminescent assay. The results were analyzed for correlation and bias using standard statistical methods.

The results obtained from the SNAP Cortisol test correlated very well with the Immulite results (r=0.92). Bias analysis showed that the SNAP Cortisol to Immulite bias was 0.8 μg/dL. Using current veterinary medical diagnostic and therapeutic recommendations, as well as the patient's historical and clinical findings and response to therapy, only 5 of the 39 (12.8%) test procedures gave results that could have led to a different clinical decision depending on the assay methodology used. In three situations (7.7%) the Immulite results better corresponded to the patient's clinical status, whereas in two cases (5.1%) the SNAP Cortisol results gave a more accurate assessment of the clinical picture.

The SNAP Cortisol results correlated very well with the Immulite results. It appears that either methodology can be used to accurately measure serum cortisol concentrations.

ABSTRACT #86

USE OF COMPOUNDED ACTH FOR ADRENAL FUNCTION TESTING IN DOGS. RJ Kemppainen, EN Behrend, KA Busch. Auburn University College of Veterinary Medicine, Auburn, AL.

To date, the only commercially available form of ACTH proven to be effective for stimulation testing in dogs is cosyntropin (Cortrosyn). However, manufacturer issues have made cosyntropin difficult to obtain at times, and the cost has recently increased dramatically. Compounded formulations are available from veterinary pharmacies, but their efficacy has not been examined for the most part. The purpose of this study was to compare adrenal stimulation in dogs in response to four compounded ACTH formulations to that achieved with cosyntropin administration.

Five mixed-breed, healthy dogs were used. ACTH stimulation tests were performed on five occasions. Each dog was given a different ACTH on each test date, so all individual dogs received each product in random order. Cosyntropin (ACTH A) was administered IV (5 mcg/kg) and the compounded forms IM (ACTH B-E, 2.2 U/kg, per manufacturers' instructions). ACTH B = Corticotropin LA injectable gel, Pet Health Pharmacy; C = Corticotrophin LA gel, Wedgewood Pharmacy; D = Corticotrophin LA gel, Red Oak Drug; E = ACTH solution, Meds for Vets. Blood was drawn before (t=0) and 30, 60, 90 and 120 min post-injection. Samples were centrifuged after clotting, and the serum separated and stored at –20C until analysis. Data were analyzed using a two-way ANOVA for repeated measures. Serum cortisol concentrations in response to cosyntropin were treated as the controls. Comparisons between control values and others were made using Dunnett's test, while the Tukey test was used to compare values at different times within a group. Significance was set at $p \leq 0.05$. Immunoreactive ACTH in each of the compounded forms of ACTH was measured.

Serum cortisol concentrations were not different between the cosyntropin-treated dogs and the other groups at t=0, 30 and 60. However, at t=90 and 120, cortisol concentrations in dogs given ACTH B and E were significantly lower than those in dog given cosyntropin. Serum cortisol concentrations at 120 minutes in some dogs that received ACTH B or E were near or below their baseline values (t=0). At 120 minutes, cortisol concentrations in dogs given ACTH D were significantly greater than those measured in dogs given cosyntropin as well as higher than the concentrations at t=60 in dogs given ACTH D. Immunoreactive ACTH concentrations varied considerably between forms (11, 52, 925 and 813 pg/ml in ACTH B, C, D and E, respectively), and did not correlate with the duration or magnitude of the serum cortisol response.

In conclusion, administration of all four forms of compounded ACTH increased serum cortisol concentrations to a similar degree as a maximally stimulating dose of cosyntropin, when values were compared at 60 minutes following injection. Due to variability in duration of response, we recommend that veterinarians using compounded ACTH products collect several post-ACTH samples, at a minimum, one and two hours following injection.

ABSTRACT #87

STEROID PRECURSOR CONCENTRATIONS IN DOGS WITH PITUITARY-DEPENDANT HYPERADRENOCORTICISM DURING TRILOSTANE TREATMENT. N. S. Sieber-Ruckstuhl[1], F. S. Boretti[1], M. Wenger[1], Ch. Maser-Gluth[2], C. E. Reusch[1]. [1]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty University of Zurich, Zurich, Switzerland [2]Steroid laboratory, Institute of Pharmacology, Ruprecht-Karls-University, Heidelberg, Germany.

Trilostane is thought to be a competitive inhibitor of the 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme system. In research animals it has been shown to inhibit the conversion of pregnenolone to progesterone and of 17α-OH-pregnenolone to 17α-OH-progesterone. This leads to changes in steroid precursor and steroid concentrations, especially an increase in pregnenolone and 17α-OH-pregnenolone and a decrease of progesterone, 17α-OH-progesterone, cortisol, and aldosterone. In dogs it is known so far, that trilostane therapy leads to a decrease of cortisol and aldosterone.

The objective of our study was to investigate the effect of trilostane on steroid precursor concentrations in dogs with pituitary-dependant hyperadrenocorticism (PDH).

Steroid precursor and cortisol concentrations were evaluated before (time 0) and 1 hour (time 1) after injection of synthetic ACTH on day 0, weeks 1-2 and weeks 3-7 of trilostane treatment. 17α-OH-pregnenolone, 17α-OH-progesterone, dehydroepiandrostenedione, androstenedione, 11-deoxycortisol, and cortisol concentrations were measured in 15 dogs with PDH.

At each re-evaluation, a significant decrease of cortisol (time 0 and 1) and a significant increase of 17α-OH-pregnenolone (time 0 and 1) and dehydroepiandrostenedione (time 0 and 1) were seen. 17α-OH-progesterone and androstenedione concentrations did not change significantly. The 11-deoxycortisol concentration at time 0 showed a significant increase at both re-evaluations. No change of the 11-deoxycortisol concentration at time 1 was seen.

We conclude that Trilostane effectively inhibits the steroid production in the adrenal glands in dogs. The significant increases of 17α-OH-pregnenolone and dehydroepiandrostenedione confirm an inhibitory effect of the 3β-HSD enzyme system. Unexpected was the failure of 17α-OH-progesterone and androstenedione concentrations to decrease. This could indicate an incomplete inhibition of the 3β-HSD with an additional inhibition of the 21-hydrolase or the 11β-hydroxylase. However, an up-regulation of the conversion of cortisol to cortisone by 11-β-hydroxysteroid-dehydrogenase could also lead to the same steroid precursor profile. To completely understand the activity profile of trilostane further studies on a larger group of dogs with analysis of further precursors (especially cortisone) and evaluation in cell culture are needed.

ABSTRACT #88

URINARY CATECHOLAMINE-CREATININE-RATIOS (UCaC) IN HEALTHY DOGS AT HOME AND IN A HOSPITAL ENVIRONMENT. P.H. Kook[1], F.S. Boretti[1], T. Wiederkehr[2], M. Hersberger[3], T.M. Glaus[1], C.E. Reusch[1], [1]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, [2]Private

Practice, Buch, [3]Center of Medical Research, University Hospital, Zurich, Switzerland.

In humans, urine catecholamine concentrations may be useful to diagnose pheochromo-cytoma (pheo). The aims of this study were to establish preliminary reference values for concentrations of canine urine catecholamines (norepinephrine, epinephrine, dopamine) and their metabolites (normetanephrine and metanephrine) expressed as a ratio to urine creatinine (UCaC), and to investigate the influence of the acute stress associated with examinations at a veterinary hospital on these ratios.

Urine samples of 10 healthy dogs were caught by the owners seven days before taking the dog to our clinic (t_1), collected in our clinic after physical examination, blood sampling and ultrasonographic examination of the adrenal glands (t_2), and again caught by the owners 24 hours (t_3) and seven days (t_4) after the visit to the clinic. Ten ml of urine were brought into tubes containing 280 µl 26% HCl. Urine-pH was immediately measured by owners or DVMs, the urine further acidified to reach a urine pH <2.0, and samples kept at 4ºC protected from light until delivery to the laboratory. Free urinary catecholamines and free and deconjugated metanephrines were measured by HPLC (BIO RAD, Munich, Germany). Differences of results between each catecholamine at each time point were statistically analyzed using a Dunn's multiple comparison test. UCaC were also measured in one dog with pheo.

Results of each UCaC (median and range, [nmol/mmol]) at the different time points are shown in the table.

UCaC	t_1	t_2	t_3	t_4	dog pheo
Norepinephrine	12 (5-67)	9 (4-19)	6 (3-11)	5 (3-12)	132
Epinephrine	3 (1-10)	4 (2-8)	2 (1-4)	1 (1-16)	<3
Dopamine	17 (7-44)	15 (5-58)	16 (5-27)	13 (7-25)	37
Normetanephrine	63 (33-103)	66 (14-98)	55 (32-100)	43 (21-79)	6430
Metanephrine	174 (50-473)	128 (46-255)	118 (40-170)	55 (27-108)	202

There was no statistical difference between dopamine and normetanephrine at t_2 compared to any other time point. Epinephrine and metanephrine were significantly higher at t_2 compared to t_4. Norepinephrine was significantly higher at t_2 compared to t_3 and t_4. In the dog with pheo, norepinephrine and normetanephrine were markedly higher, epinephrine was below the detection limit, and dopamine and metanephrine did not differ from healthy control dogs. Even though differences between hospital and home samples were small, urine samples are recommended to be collected at home to avoid influence of stress. Measurement of UCaC maybe useful to diagnose pheo in dogs, however, results in one dog imply that various UCaC should be measured, as not ratios of all may be elevated.

ABSTRACT #89

EFFECT OF CENTRIFUGATION AND STORAGE TEMPERATURES AND APROTININ ON STABILITY OF CANINE PLASMA ADRENOCORTICOTROPHIC HORMONE PRECURSORS PRIOR TO ANALYSIS. Pauline de Fornel-Thibaud, Nicolas Granger and Dan Rosenberg. Internal Medicine Unit, National Veterinary School of Alfort, Maisons-Alfort, France.

Adrenocorticotrophic hormone (ACTH) is a labile hormone that is highly sensitive to temperature degradation. Sample handling is critical for this hormone. We recently validated in dogs an assay allowing quantitative determination of plasma ACTH precursors (pro-opiomelanocortin, POMC, and pro-ACTH) and obtained data indicating that the use of this assay may be of value in the characterization of tumor size in dogs with Cushing's disease (*J Vet Int Med*, 2005, 19:23-28). These data were obtained with blood collected into pre-cooled EDTA tubes, immediately centrifuged at 4°C and after immediate freezing of plasma. Thus, the question arises as whether to POMC and pro-ACTH have the same properties as ACTH and whether similar sample handling conditions are necessary to preserve canine ACTH precursors. The aim of this study was to evaluate the impact of different temperatures of centrifugation and storage and the use of collection tubes containing aprotinin on the stability of ACTH precursors in canine plasma.

Whole blood was collected from three dogs with a pituitary tumor causing hyperadrenocorticism. One half of each sample was collected into EDTA-tubes and the other half into EDTA-aprotinin tubes with a final concentration for aprotinin of 50 UI/ml. The aprotinin containing samples and equivalent amounts of non aprotinin samples were divided in half for immediate centrifugation at either room temperature (22°C) or 4°C. All the samples were then aliquoted in plastic vials for storage at room temperature, refrigerated (4°C) or frozen (-20°C). At 0, 24 and 48 hours, aliquots were removed from each storage condition and frozen at -80°C until analysis. Samples were assayed for ACTH precursors by the enzymeimmunoassay previously validated in the dog.

ACTH precursors concentrations in plasma samples collected with aprotinin were comparable with those in samples collected without aprotinin whatever the centrifugation temperature. There was no significant degradation of ACTH precursors in samples stored at room temperature, 4°C or -20°C for up to 48 hours. After two days, whatever the storage temperature, aprotinin provided no benefit when compared to samples without aprotinin.

ACTH precursors (POMC and pro-ACTH) appear stable in sample handling conditions associated with ACTH degradation. For canine ACTH precursors assays, collection of samples into EDTA, centrifugation and transport to the laboratory at room temperature within two days is practicable without additional precaution.

ABSTRACT #90

THE ROLE OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN THE DEVELOPMENT OF SYSTEMIC HYPERTENSION IN CATS TREATED FOR HYPERTHYROIDISM. R.E. Jepson, J.Elliott, H.M. Syme. Royal Veterinary College, London, UK.

A proportion of hyperthyroid cats that have normal blood pressure at diagnosis become hypertensive following treatment. The aim of this study was to evaluate the role of RAAS in the pathogenesis of systemic hypertension following treatment for hyperthyroidism by measurement of plasma renin activity (PRA) and aldosterone (ALDO) concentration.

Cats were selected retrospectively for inclusion in the study if they were diagnosed as hyperthyroid (T4 >55nmol/L) and developed systemic hypertension within six months of documented stable euthyroidism (HT group, n=11). A control group of cats that remained normotensive during treatment for hyperthyroidism were selected at random (NT group, n=10). All cats were normotensive and non-azotaemic (plasma creatinine <1.9mg/dl) prior to treatment. Systolic blood pressure (SBP) was measured using the doppler technique and hypertension was defined as SBP>175mmHg on multiple occasions or in association with clinical manifestations of hypertension. Treatment was with carbimazole therapy alone or in combination with surgical thyroidectomy. PRA and ALDO concentrations were measured using commercially available radioimmunoassays (Diasorin GammaCoat, DPC Coat-A-Count Aldosterone) before treatment and after euthyroidism was established in both NT and HT cats. Wilcoxon signed rank tests were used to compare data before and after treatment. Mann-Whitney U tests were used to make comparisons between NT and HT groups. Data are reported as the median [25th, 75th percentiles].

PRA did not decrease significantly with treatment of hyperthyroidism in either group (pre 1.52 [1.09, 2.14], post 0.65 [0.19, 1.97] ng/ml/hr, P=0.18; NT and HT combined). ALDO declined significantly in both groups (pre 202 [125, 268], post 159 [92, 206] pg/ml, P=0.012; NT and HT combined), and plasma creatinine increased significantly (pre 1.3 [0.9, 1.5], post 1.9 [1.6, 2.9] mg/dl, P<0.001) with treatment. There was no difference in

PRA, ALDO or creatinine concentrations between NT and HT groups either before or after treatment.

The cause for the development of hypertension in cats that are being treated for hyperthyroidism remains enigmatic. Activation of the RAAS tends to decrease with treatment both in cats that remain normotensive and those that develop hypertension. Although renal function declined significantly with treatment, cats in both NT (3/10) and HT (6/11) groups developed azotaemia, and creatinine concentrations were not significantly different between the groups. The role of declining renal function in development of hypertension is worthy of further study.

ABSTRACT #91

USE OF RECOMBINANT HUMAN TSH IN TSH-STIMULATION TESTS IN SUSPECTED HYPOTHYROID DOGS. F.S. Boretti[1], N.S. Sieber-Ruckstuhl[1], R. Hofmann-Lehmann[2], B. Willi[2], H. Lutz[2], C.E. Reusch[1], [1]Clinic for Small Animal Internal Medicine and [2]Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Switzerland.

Recombinant human TSH (rhTSH) has been proposed to replace bovine TSH for TSH-stimulation tests in dogs. Aims of the present study were in a first part to establish criteria for TSH-test interpretation using rhTSH in healthy dogs and in a second part to evaluate rhTSH in suspected hypothyroid dogs to confirm its clinical usefulness.

Part 1 consisted of 38 clinically healthy dogs: 18 beagle dogs with a median age of 2 years (range 1-2) and a median body-weight of 14.3 kg (range 10.4-16.5) and 20 non-beagle dogs with a median age of 4 years (range 1-11) and a median body-weight of 28.5 kg (range 20.3-49.7). All dogs were T4-, T3- and Thyroglobulin-Auto-Antibody negative and had a normal CBC, serum biochemistry and cTSH. Part 2 included 48 dogs presented to the Clinic for Small Animals at the University of Zurich with clinical signs consistent with hypothyroidism. Their median age was six years (range 1-14) and median body-weight 32 kg (range 4.2-76.5). In all dogs TSH-stimulation tests were performed using 75µg rhTSH (Thyrogen®) IV. Blood samples for determination of T4 were taken before and 6 hours after stimulation.

None of the dogs showed adverse reactions after rhTSH administration. There was a significant increase in T4 in all dogs of part 1. Post-TSH T4 was at least 1.5 times basal T4 concentration. Although there was a significant difference in age and body-weight between the healthy beagle and non-beagle dogs, there was no difference in post-TSH T4 between the two groups. Median, 5- and 95- percentile of post-TSH T4 of all 38 healthy dogs were 3.8, 2.5 and 5.5 ug/dl, respectively. According to criteria established in part 1, hypothyroidism could be clearly confirmed in 12 (hypothyroid group) and excluded in 27 (euthyroid group) dogs of part 2. Nine dogs showed a reduced stimulation (intermediate group). In the hypothyroid group T4 and post-TSH T4 were significantly lower and cTSH was significantly higher compared to the euthyroid and intermediate group. T4 and cTSH were not significantly different between the euthyroid and intermediate group, although post-TSH T4 values were significantly higher in the euthyroid dogs. There was no difference in sex, age and body weight between the groups. One of the nine intermediate stimulators had severe non-thyroidal disease and 4 of them had received medication (NSAIDS, steroids) before the test had been performed. Using T4 and cTSH as sole criteria for diagnostic assessment, one of the euthyroid (normal stimulation, low T4, elevated cTSH) and one of the hypothyroid dogs (no stimulation, low-normal T4 and cTSH) would have been misinterpreted as hypothyroid and euthyroid, respectively.

The results of our study show that TSH-stimulation test using 75ug rhTSH is a safe and valuable diagnostic tool to assess thyroid function in suspected hypothyroid dogs. It can be recommended as additional test in selected cases. Dogs can be considered euthyroid if post-TSH T4 is $\geq$2.5ug/dl and at least 1.5 times basal T4 concentration. However, stimulation can be reduced in dogs having concurrent disease or receiving medication.

ABSTRACT #92

USE OF RHEOLYTIC™ THROMBECTOMY FOR THE TREATMENT OF FELINE DISTAL AORTIC THROMBOEMBOLISM. SB Reimer, MD Kittleson, AE Kyles. School of Veterinary Medicine, University of California, Davis, CA.

Feline thromboembolic disease is a well-recognized and often devastating complication associated with feline cardiomyopathy. Conventional therapies centered upon the use of supportive care or the administration of thrombolytic agents have had mixed results.

The purpose of this prospective clinical study was to evaluate the efficacy of a commercially available Rheolytic™ Thrombectomy system (AngioJet® catheter, Possis Medical Inc, Minneapolis, MN) in the treatment of feline distal aortic thromboembolism. Six cats with naturally occurring distal aortic thromboembolism were included in this preliminary study. All six cats presented with bilateral clinical signs which included pelvic limb paralysis and a lack of a palpable pulse in either femoral artery. The range of time between the initial thromboembolic event and presentation for assessment was two hours to eight days. Following a clinical diagnosis of distal aortic thromboembolism, cats were anesthetized and arterial access was achieved via a surgical approach to the carotid artery. An arteriotomy was performed, and a diagnostic catheter was advanced over a guide-wire to the level of the thrombus. Selective arterial angiography was performed to confirm the presence of the thrombus in the distal aorta. The procedural catheter was then advanced to the region of arterial obstruction and thrombectomy was performed. Repeat angiography was intermittently performed through the procedural catheter to assess progress of the thrombectomy. Upon relief of the obstruction, the catheter and guide-wire were removed and the arteriotomy was closed via ligation of the carotid artery. Cats successfully discharged from the hospital were placed on low-molecular-weight heparin therapy.

Rheolytic™ Thrombectomy was successful in relieving the arterial obstruction in 83% (5/6) of the cases. Of the five cases in which the thrombus was removed, three survived to discharge resulting in a survival to discharge rate of 50%. The median survival time of the three cats that were discharged was 4 months (mean: 10.7 months; range: 4 to 24 months). There was no obvious relationship between the time presented after thromboembolic event and outcome. The cat that presented the longest period of time after the initial event experienced the longest survival time. One cat experienced cardiopulmonary arrest under general anesthesia after the procedure was instituted, and another cat exhibited signs attributable to progressive central nervous system dysfunction upon recovery from anesthesia and was eventually euthanized.

The rate of relief of arterial obstruction as well as the survival to discharge rate using the Rheolytic™ Thrombectomy system compare favorably with rates achieved using conventional therapies. Feline distal aortic thromboembolism is a frustrating disease which warrants a guarded to poor prognosis. These preliminary results indicate that Rheolytic™ Thrombectomy may be a viable alternative therapy in the treatment of thromboembolic diseases, including feline distal aortic thromboembolism.

ABSTRACT #93

INSULIN SENSITIVITY IS HALVED AND FASTING INSULIN CONCENTRATION INCREASED FOUR TIMES IN SPONTANEOUSLY OBESE DOGS. Kurt R Verkest, Linda M Fleeman, Jacquie S Rand, and John M Morton. Centre for Companion Animal Health, School of Veterinary Science, University of Queensland, Australia.

Type 2 diabetes mellitus occurs in human beings and cats when insulin secretion fails to compensate for obesity-induced insulin resistance. Although obese dogs develop insulin resistance, they are not known to progress to type 2 diabetes, and little is known about the long-term effects of obesity on insulin secretion in dogs. Previous studies of insulin secretion in obese dogs involved dogs with induced, short-term obesity, or were confounded by the inclusion of intact bitches, or used glucose tolerance tests that had insufficient early samples to assess first-phase insulin secretion. The aim of this study was to evaluate glucose tolerance and insulin secretion in dogs with spontaneous obesity.

Frequently-sampled, intravenous glucose tolerance tests were performed on six spontaneously obese (body condition score (BCS) ≥8 on a 9-point scale) and six lean (BCS 4-5 /9) dogs, matched for age and gender. Female dogs were neutered >2 years prior to the study. Blood was collected prior to and over 180 min after IV glucose (1g/kg) to include both first and second phase responses. Outcomes measured included the MINMOD-derived insulin sensitivity index (S_I), and the disposition index (DI), which is the product of insulin sensitivity and insulin secretion between 0 and 10 min. Additional outcomes included absolute plasma glucose concentrations at 45, 60, 90, 120, and 180 min, maximum insulin conc, and mean insulin concentrations. Differences between obese and lean dogs were assessed using ANOVA or Wilcoxon matched pairs rank sum tests.

Mean glucose concentrations were similar in obese and lean dogs and differences were not statistically significant (p=0.46-0.66), after first adjusting for glucose concentration at three minutes. One obese dog had substantially higher plasma glucose concentrations than all other dogs at 45, 60, 90, 120, and 180 minutes, indicating glucose intolerance. The insulin sensitivity index in obese dogs was approximately half the value for lean dogs (mean ±SEM 2.99±0.77 vs 6.47±1.54 $x10^{-4}min^{-1}$/(uU/mL), respectively; p=0.04). Obese dogs had approximately four times higher fasting insulin concentrations than lean dogs (mean ±SEM 24.09±2.35 vs 5.51±1.27 μU/mL, respectively; p=0.001). Mean insulin concentrations were significantly higher for obese dogs at all time intervals, but the greatest difference was in the first phase (0 to 5 min). Mean DI was similar in obese and lean dogs and did not differ significantly (p=0.91), suggesting an appropriate compensatory increase in first-phase insulin secretion in response to insulin resistance. The glucose-intolerant, obese dog had a substantially lower DI than all other dogs, suggesting beta cell failure. Further investigation is required to determine the cause of the beta cell failure.

We conclude that (1) insulin sensitivity is decreased by 50% and fasting insulin concentration increased four times in dogs with spontaneous obesity, and (2) dogs compensate for obesity-induced insulin resistance by increasing insulin secretion.

ABSTRACT #94

COMPARISON OF GLARGINE AND LENTE INSULINS IN CATS WITH DIABETES MELLITUS. KE Weaver, EA Rozanski, O Mahony, DL Chan, LM Freeman. Tufts University School of Veterinary Medicine, North Grafton, MA.

Most cats with diabetes mellitus require insulin therapy to prevent symptomatic hyperglycemia and the development of ketosis. Recently, the use of insulin Glargine (Lantus®) has been described in cats. Glargine is a genetically modified human recombinant insulin designed to function as a basal insulin with relatively peak-less activity and sustained duration of action. Glargine has been proposed as a once-daily insulin for cats, which would simplify treatment for clients. The two goals of this study were to 1) compare the efficacy of once-daily administered Glargine insulin to twice-daily administered Lente insulin in cats with diabetes mellitus and 2) to describe the effects of a commercial high protein, low carbohydrate diet designed for the management of feline diabetes mellitus (Purina DM®).

The study was a prospective randomized trial. Cats with naturally-occurring diabetes mellitus which was either newly diagnosed or poorly responsive to current therapy were eligible for study inclusion. Baseline testing included a physical examination, biochemistry profile, urinalysis and urine culture, T4 and fructosamine concentrations. Body weight and body condition scores (BCS, 1-9 scale) were recorded and a thorough dietary history was obtained. All owners were instructed to feed the cats the high protein, low carbohydrate diet exclusively once the study began. Cats were randomized to receive either Lente or Glargine insulin. The initial starting doses were 0.5 U/kg SQ Lente q 12 hours or 0.5 U/kg Glargine q 24 hours. Re-evaluations were performed on all cats at weeks 1, 2, 4, 8, and 12 and included an assessment of clinical signs (eg, polyuria, polydipsia and appetite), physical examination including BCS and weight, a 16-hour blood glucose curve and fructosamine concentrations. Insulin dosage was increased, left the same, or decreased based upon the findings at each re-examination. Differences in the number of cats with effective control between the two insulin groups were compared using Chi Square analysis and glucose and fructosamine concentrations were compared using analysis of variance with repeated measures.

Seventeen cats were enrolled in the study. One cat was excluded (aggression, n=1), and three died during the study (diabetic ketoacidosis n=1; anesthetic accident n=1; cancer n=1). Therefore, 13 cats completed the 12-week study (Lente n=7; Lantus n=6). There was a significant improvement in the fructosamine and glucose concentrations in all cats but there was no significant difference between the two insulin groups. Four of the thirteen cats achieved complete remission, and two cats had a marked decrease in their insulin needs. There was no difference between insulin types in regard to treatment response or remission rates. The results of the study suggest that Glargine in combination with a high protein, low carbohydrate diet represents a viable option for treatment of feline diabetes mellitus.

ABSTRACT #95

TREATMENT WITH GLARGINE RESULTS IN HIGHER REMISSION RATES THAN LENTE OR PROTAMINE ZINC INSULINS IN NEWLY DIAGNOSED DIABETIC CATS. RD Marshall[1,2], JS Rand[1] [1]Centre for Companion Animal Health, University of Queensland, [2]Creek Rd Cat Clinic, Brisbane, Australia.

Insulin glargine is a synthetic insulin analogue that is very long-acting in human patients. The aim of this study was to compare the effectiveness of glargine, PZI and lente insulins in newly diagnosed diabetic cats.

Twenty-four newly-diagnosed diabetic cats (17m,7f) were treated either with glargine, PZI or lente (n=8 for each group) and fed a very low carbohydrate-high protein diet (Purina DM canned). Insulin was initially given at 0.5U/kg BID S/C if blood glucose was >360mg/dl, and 0.25U/kg BID S/C if blood glucose was <360mg/dl. Insulin dose was then adjusted based on serial blood glucose curves and water intake. Cats were defined as achieving diabetic remission if normoglycemia was maintained without insulin therapy for more than two weeks.

At diagnosis, there was no statistical difference between treatment groups for age, body weight, body condition score, or concentrations of fructosamine, blood glucose, B-hydroxybutyrate or bicarbonate. Four of eight cats in each group were Burmese.

There was a non-significant trend for glargine treated cats to have lower 12-hour glucose concentrations after 10 and 17 days, than those treated with PZI or lente. Mean 12-hour blood glucose at four weeks was significantly lower for glargine (239±61mg/dl) than PZI (389±20mg/dl) and lente (410±38mg/dl) treated cats. Fructosamine concentration after four weeks of treatment was significantly lower than at diagnosis for glargine treated cats (343±38 and 553±21umol/l

respectively) but not for PZI (469±42 and 570±22umol/l) or lente (465±49 and 574±34umol/l).

All eight cats treated with glargine went into diabetic remission within four months of beginning treatment (mean=5.2±1.6 weeks, median=3 weeks), while three cats treated with PZI (mean=3.3±0.7 weeks) and two cats treated with lente (mean=2±0 weeks) achieved diabetic remission. Of the seven glargine treated cats alive, six cats remain in remission at the time of publication (mean remission time=13±3.5 months, range=3-27 months). One of the remaining two cats alive treated with PZI (mean remission time=8.3+3.3 months, range=6-10months) and both cats treated with lente (mean remission time=8±2 months, range=6-10 months) remain in remission.

Only one cat treated with glargine required an increase in insulin dose above 0.5U/kg BID, and seven of eight cats had their insulin dose reduced in the first three days of treatment. The mean dose of glargine at day 3 was 0.3±0.04U/kg BID. Clinical hypoglycemia occurred in two cats treated with lente and one cat treated with PZI, but in none of the cats treated with glargine.

In conclusion, glargine was safe to use and resulted in higher remission rates than lente or PZI insulins in newly diagnosed diabetic cats. It is postulated that improved glycemic control with glargine resulted in better reversal of B-cell glucose toxicity and higher diabetic remission rates.

ABSTRACT #96

EVALUATION OF DOG LEUKOCYTE ANTIGEN (DLA) GENE POLYMORPHISMS IN CANINE DIABETES MELLITUS AND THE DEVELOPMENT OF ANTI-INSULIN ANTIBODIES FOLLOWING INSULIN THERAPY. L.J.Davison[1], L.J.Kennedy[2], A.Barnes[3], D.Isherwood[3], M.E.Herrtage[1], W.E.R.Ollier[2] and B.Catchpole[4]. [1]Department of Clinical Veterinary Medicine, University of Cambridge, UK. [2]Centre for Integrated Genomic Medical Research, University of Manchester, UK. [3]Faculty of Veterinary Science, University of Liverpool, UK. [4]Royal Veterinary College, University of London, UK.

Certain dog breeds such as Samoyeds and Tibetan terriers are predisposed to developing diabetes mellitus (DM), suggesting that the disease has a genetic component. The Human Leukocyte Antigen (HLA) genes, coding for Major Histocompatibility Complex (MHC) class II proteins, are the major susceptibility genes for Type 1 DM in man. It has also been reported that human diabetics expressing certain HLA alleles are more likely to produce anti-insulin antibodies (AIAb) following insulin therapy. The aim of this study was to determine whether particular Dog Leukocyte Antigen (DLA) haplotypes are associated with an increased risk of canine DM and to evaluate whether development of anti-insulin antibodies in insulin-treated diabetic dogs is associated with specific DLA alleles.

DNA was isolated from EDTA blood samples from 393 canine diabetic dogs and genotyped at the DLA-DRB, -DQB and -DQA loci using a sequence-based approach. Non-diabetic dogs (n = 830), breed-matched where possible, were used as controls. Serum reactivity to bovine insulin was measured by enzyme-linked immunosorbant assay (ELISA) in 94 of the diabetic dogs who had received treatment exclusively with bovine insulin (Insuvet lente, Schering Plough Animal Health) for greater than 1 month. Serum samples from 30 non-diabetic dogs were used as controls, and dogs with ELISA O.D. reactivity >mean+2SD of this group were considered positive for AIAb.

One DLA haplotype (DLA-DRB1*009/DQA1*001/DQB1*008; Odds Ratio = 2.26, $p<0.0002$) was found to have an increased frequency in the diabetic dog population compared to controls. Fifty-nine of 94 diabetic patients demonstrated serological reactivity to insulin, however no DLA haplotype was consistently associated with the presence or absence of AIAb.

These results suggest that there is an association between DLA haplotype and susceptibility to DM in some dogs, implicating the immune response in the pathogenesis of their disease. Larger studies are required to clarify the role of the MHC in the development of anti-insulin antibodies. Future work will focus on breed-specific immunogenetic and autoantibody analysis to investigate DLA polymorphisms in different phenotypic subgroups of canine diabetic patients.

ABSTRACT #97

TISSUE-SPECIFIC EXPRESSION OF LIPOPROTEIN LIPASE FAVORS PARTITIONING OF FREE FATTY ACIDS TO MUSCLES IN OBESE, INSULIN-RESISTANT CATS. M. Hoenig, D.C. Ferguson, J.B. Mcgoldrick, M. Debeer, *P.N. Demacker; Department Of Physiology And Pharmacology, College Of Veterinary Medicine, University Of Georgia, Athens, Ga 30602, And *Division Of General Internal Medicine, University Medical Center Sint Radboud, Nijmegen, The Netherlands.

Obese cats show alterations in lipoprotein metabolism. They also have increased lipid deposits in muscle. These changes suggest that lipase activity, in particular that of LPL, is altered with obesity because modulation of tissue-specific LPL expression determines the partitioning of triglycerides between tissues. Increased lipid deposition in muscle has been associated with insulin resistance in cats and many other species. The increased partitioning of fatty acids into muscle tissue is thought to cause insulin resistance because uptake of free fatty acids negatively influences glucose transport.

The goal of this study was to examine if obese cats show alterations in plasma and tissue lipase activity and mRNA expression which might account for the changes in lipoprotein metabolism and tissue lipid deposition. Twelve lean and 12 obese adult cats (six neutered males and six neutered females each) were used. Postheparin plasma activity of lipoprotein lipase (LPL) and hepatic lipase (HL), and fat and muscle activity of LPL were measured using glyceroltri[9,10(n)-^{3}H] oleate (triolein) as triglyceride substrate. LPL and hormone-sensitive lipase (HSL) mRNA expression were measured in muscle and fat tissue with real-time PCR using primers for feline LPL and HSL. Feline beta actin was used as control. LPL plasma and fat activity and fat mRNA expression were significantly lower (50%, 80%, and 50%, respectively) in obese cats, whereas muscle LPL activity and mRNA tended to be higher leading to a higher fat/muscle ratio in obese compared to lean cats ($p < 0.001$). HL was not different between the groups. HSL mRNA expression was twofold higher in muscle in obese cats compared to lean ($p = 0.011$). The decrease in LPL activity and mRNA expression in fat and the increase in LPL and HSL mRNA expression in muscle favors a redistribution of fatty acids from fat to muscle tissue in obese cats where they can either be deposited or used for energy in times of caloric need because glucose uptake is diminished.

ABSTRACT #98

DIABETIC KETACIDOSIS IN 127 DOGS. Daniel Z. Hume, Kenneth J. Drobatz, and Rebecka S. Hess. Matthew J. Ryan Veterinary Hospital, University of Pennsylvania, Philadelphia, PA.

The purpose of this study was to define the clinical signs, clinicopathologic abnormalities, concurrent disorders, treatment, and outcome associated with canine diabetic ketoacidosis (DKA). Large studies of spontaneous canine DKA have not been reported in peer review literature. To this end, a retrospective study of all dogs with DKA examined at the MJR Veterinary Hospital between 1993 and 2003 was performed.

Median age of 127 dogs with DKA was eight years (four months to 16 years). Eighty-two (65%) dogs were diagnosed with DKA at the time of initial diagnosis of diabetes mellitus (DM). Fifty-two (41%) were neutered male dogs, 44 (35%) were neutered female dogs, 16 (12%) were intact female dogs, and 15 (12%) were intact male dogs. Mixed breed dogs were of the most common breed category

represented in the study. The most common clinical signs were polyuria and polydipsia (90%), lethargy (87%), inappetence (87%), vomiting (83%), and weight loss (54%). Most dogs (90%) were dehydrated, 51% had cranial organomegaly, and 36% had abdominal pain. Hypokalemia (102 dogs, 85%), hypophosphatemia (53, 44%), hypomagnesemia (40, 60%), and low ionized calcium (60, 52%) at presentation or during treatment were common. The mean venous pH and serum bicarbonate were 7.24±0.1 and 12.6±4.6 mmol/L, respectively. Forty-one (37%) dogs were anemic and 74 (63%) had a leukocytosis. Forty-four dogs (38%) had elevations of both BUN and creatinine. ALT, AST, and ALP were elevated in 82%, 86%, and 97% of dogs, respectively. Eighty-seven dogs (69%) had one or more concurrent disorder diagnosed at the time of hospitalization. The most common identified concurrent conditions included acute pancreatitis (41%), bacterial urinary tract infection (20%), hyperadrenocorticism (15%), and bacterial pneumonia (6%). Oral corticosteroid therapy had been administered to 10% of dogs within a week prior to hospitalization. Most dogs (121, 95%) received IV fluid supplementation, IV CRI of insulin (111, 92%), and IV CRI of potassium (96, 79%).

Eighty-nine dogs (70%) survived to be discharged from the hospital. The median duration of hospitalization was 6 days. Survival was correlated with initial hematocrit (p=0.03), venous pH (p=0.006), and base deficit (p=0.006). Time from admission to initiation of SQ insulin therapy was correlated with potassium concentration (p=0.006), phosphorus concentration (p=0.004), white blood cell count (p=0.006), base deficit (p=0.002), and venous pH (p=0.0006). Length of hospitalization was correlated with IV continuous rate infusion (CRI) of magnesium (p=0.0001), phosphorous (p=0.0001), bicarbonate (p=0.0015), and potassium (p=0.0185).

In conclusion, most dogs with DKA were not previously diagnosed with DM. Electrolyte abnormalities are common in DKA and are associated with length of hospitalization. Survival was correlated to degree of anemia and acidosis. Concurrent conditions are present in about 70% of dogs with DKA.

ABSTRACT #99

PATHOGENESIS OF ACID INJURY IN THE NON-GLANDULAR EQUINE STOMACH: THE DOSE AFFECT OF VOLATILE FATTY ACIDS. F. Andrews[1], B. Buchanan[1], S. Smith[1], S. Elliott[1], N. Clariday[1], L. Edwards[1], and A. Saxton[2]. [1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, and [2]Department of Animal Science, The University of Tennessee, Knoxville, TN.

Equine Gastric Ulcer Syndrome (EGUS) is common in performance horses and these horses are commonly fed diets that are high in soluble carbohydrates. Volatile fatty acids (VFAs), byproducts of carbohydrate fermentation, at high concentrations (60 mmol/l) have been shown to cause acid injury to the equine non-glandular stomach, however lower concentrations of VFAs have not been evaluated. The purpose of this study was to determine the effect of lower concentrations of VFAs (0, 5, 10, 20, and 40 mmol/l), pH, and addition of calcium carbonate ($CaCO_3$) on the nonglandular squamous mucosa of horses using an *in vitro* Ussing Chamber System.

The stomachs of 48 horses of various breeds were removed after humane euthanasia and placed in Ussing chamber systems and exposed to normal Ringers solution (NRS) at either pH 1.5, 4.0 or 7.0 or NRS containing either 5, 10, 20, or 40 mmol concentration of various VFAs (acetic, propionic, butyric, and valeric acids). Short-circuit current (Isc), an indicator of sodium transport across the tissue, and potential difference (PD), an indicator of tissue resistance, were measured for 3.5 hours. After the 3.5 hours, $CaCO_3$ (20 mg/l) was added to the mucosal side of the chamber and readings taken for an additional one hour to determine tissue recovery.

The 48 horses ranged in age from three to 33 years (mean = 13.9 years). There were 27 geldings (56%), one stallion (2%), 19 mares (40%) and one hermaphrodite (2%). Twenty of 45 horses (44%) had gastric ulcers in the non-glandular squamous mucosa. Mean Gastric ulcer score was 1.5/3.0. Tissues exposed to NRS at pH $\leq$ 4.0 showed a significant ($P<0.05$) decrease in Isc and PD when compared to tissues exposed to pH 7.0. At pH $\leq$ 4.0, acetic, butyric, propionic, and valeric acids caused a concentration-dependent decrease ($P<0.05$) in Isc and PD in the exposed tissues when compared to control tissues at pH 4.0 and 7.0. The most dramatic decrease in Isc and PD were seen at the 20 and 40 mmol/l concentration of the VFAs, except for acetic acid, which showed a significant decrease in these values at a lower concentration (5 mmol/l). When $CaCO_3$ was added during the last hour of exposure, Isc and PD increased dramatically to near pretreatment values in all VFA exposed tissues except those tissues exposed to the higher concentrations (20 and 40 mmol) valeric acid at pH $\leq$ 4.0.

VFAs (acetic, butyric, propionic and valeric acids) act synergistically and in a concentration-dependent manner with stomach HCl to cause injury to the non-glandular stomach mucosa. VFAs, especially lower concentrations of acetic acid (5 mmol), in the presence of stomach acid, may contribute to the pathogenesis of EGUS. Also, the addition of $CaCO_3$ to the diet of horses fed high grain rations may help reverse the potentially harmful affects of VFAs.

ABSTRACT #100

LAMINAR LEUKOCYTE EMIGRATION IN THE DEVELOPMENTAL STAGE OF LAMINITIS. S. H. Black, Univ. of Massachusetts, Amherst, MA; D. P. Lunn, Colorado State University, Ft. Collins, CO; M. Hwang, Auburn University, Auburn, AL; J. K. Belknap, The Ohio State University, Columbus, OH.

Due to similarities in clinical presentation of horses at risk of laminitis with human sepsis patients classified as suffering from Systemic Inflammatory Response Syndrome (SIRS), we hypothesized that the laminar inflammation recently reported to occur in the developmental stages of laminitis was due to leukocyte emigration from the vasculature similar to that observed in sepsis-related organ damage.

In this study, we employed immunohistochemical techniques for identifying leukocytes in archived frozen and paraffin-embedded laminar samples from a well-described equine black walnut extract (BWE) model of laminitis in which laminar tissue was harvested after nasogastric (NG) administration of water (control, n=5 horses), at the onset of leucopenia after NG BWE administration (developmental time point, n=5 horses), and at the onset of clinical lameness after NG BWE administration (n=5 horses). A monoclonal anti-CD13 antibody (specific for an aminopeptidase in equine PMNs and monocyte/macrophages) was used as a leukocyte marker. Immunohistochemistry was then performed on sections of skin samples from control horses in order to compare laminar results with those from another dermal structure. In the normal skin, a marginal pool of CD13-positive leukocytes was present around dermal venules. Interestingly, no CD-13 positive cells were present in normal laminar tissue. At the developmental time point post BWE, mild to marked CD13-positive cell infiltration into the laminar perivascular tissue was found. A marked infiltration of perivenular leukocytes was present in laminar sections from animals at the onset of acute lameness post BWE. Upon staining of serial laminar sections at the developmental time point for cell identification, the CD13-positive cells were found to be mainly PMNs; some CD13-positive cells in the affected laminae were monocytes/macrophages. The distinct lack of CD13-positive cells in the normal laminae when compared to skin samples suggests that the laminar vasculature does not have a marginal perivascular pool of leukocytes, possibly due to the protective barrier that the hoof wall provides when compared to

normal skin. The marked infiltration of leukocytes noted (particularly at the developmental stage) suggests that, similar to organ damage in sepsis, an initiating factor in laminar injury may be the emigration of circulating leukocytes into the laminar tissue in the early stage of laminitis.

This leukocyte infiltration may be particularly damaging in the laminar tissue due to a possible lack of protective mechanisms normally present to protect the tissue from leukocyte products such as reactive oxygen species (i.e. we have recently reported a lack of SOD activity in laminar tissue when compared to the skin). The discovery of leukocyte emigration early in the disease process may lead to novel treatment options currently being investigated in human sepsis which address leukocyte/endothelial activation and interaction.

ABSTRACT #101

LYMPHOPROLIFERATIVE RESPONSES AND CYTOKINE MRNA EXPRESSION IN FOALS AND ADULT HORSES INFECTED WITH VIRULENT *RHODOCOCCUS EQUI*. Stephanie Jacks and Steeve Giguère. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Rhodococcus equi, a Gram-positive facultative intracellular pathogen replicating in macrophages, is an important cause of pyogranulomatous pneumonia in foals. As opposed to foals, adult horses are generally resistant to infection. Studies in mice have shown that a Th1 cell-mediated response is sufficient to effect pulmonary clearance of *R. equi*, while a Th2 response is detrimental. Recent studies using adult horses have shown that clearance of virulent *R. equi* is associated with antigen-specific lymphoproliferative responses and IFN-γ induction. We hypothesized that a protective immune response to virulent *R. equi* in adult horses is of the "Th1-like" phenotype whereas foals develop a detrimental "Th2-like" response.

Ten foals between seven and 10 days of age and ten adult horses between two and 12 years of age were used in this study. Five foals and five adult horses were inoculated intrabronchially with virulent *R. equi* ($2x\ 10^4$ CFU/kg of body weight) in PBS. Five foals and five adults were used as controls and were given only PBS intrabronchially. At 15 days post-infection, the animals were euthanized and subjected to a complete post-mortem examination. Bronchial lymph node lymphocytes from each experimental animal were stimulated with a soluble *R. equi* antigen (recall response), *Corynebacterium pseudotuberculosis* antigen (negative control), and ConA (positive control). Proliferative responses were assessed using a non-radioactive colorimetric assay. Expression of mRNA for IL-4 and IFN-γ was measured by real time RT-PCR.

All foals infected with *R. equi* had mild to moderate bilateral and multifocal pulmonary granulomas and abscesses. *R. equi* was recovered from the lungs of each foal at concentrations ranging from $2.3\ x\ 10^4$ to $1.4\ x10^7$ CFU/gram of lung tissue. In contrast, no pulmonary lesions developed in the lungs of adult horses and control foals, and lung cultures were negative for *R. equi*. Lymphoproliferative responses to ConA were significantly higher in foals than in adult horses. In contrast, infected and control adult horses had significantly higher proliferative responses to the soluble *R. equi* antigen than control or infected foals. Control foals had significantly lower IFN-γ mRNA expression than all other groups. Expression of IL-4 and IFN-γ/IL-4 ratios were not significantly different between groups.

The inability of foals to mount lymphoproliferative responses to *R. equi* may contribute to their susceptibility. Poor lymphocyte proliferation in response to *R. equi* in foals is most likely the result of a naive immune system rather than immunodeficiency as evidenced by their significantly higher proliferation to ConA. The IFN-γ and IL-4 profiles of infected foals are similar to that of adult horses.

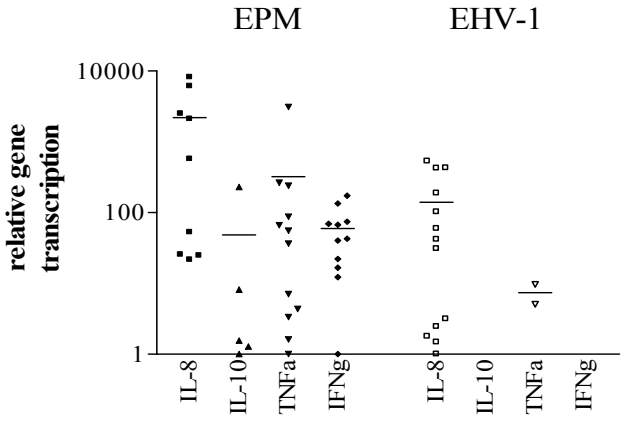
ABSTRACT #102

CYTOKINE GENE SIGNATURES IN NEURAL TISSUE OF HORSES DIAGNOSED WITH EQUINE PROTOZOAL MYELOENCEPHALITIS OR EQUINE HERPESVIRUS-1 MYELOENCEPHALOPATHY. N. Pusterla[1], C.M. Leutenegger[1], P.A. Conrad[2], B.C. Barr[3], G.L. Ferraro[4], B.M. Daft[3], W.D. Wilson[1]. [1]Department of Medicine and Epidemiology, [2]Department of Pathology, Microbiology and Immunology, [3]California Animal Healthy and Food Safety Laboratory System, [4]Center for Equine Health, School of Veterinary Medicine, University of California, Davis, California.

The goal of this study was to determine the antigen load and the gene transcription of selected cytokines in the brain and spinal cord of horses with equine protozoal myeloencephalitis (EPM), equine herpesvirus (EHV)-1 myeloencephalopathy and control horses using TaqMan PCR. The study material consisted of formalin-fixed and paraffin-embedded neural tissue blocks from 35 horses (12 confirmed EPM horses, 11 horses with confirmed EHV-1 myeloencephalopathy and 12 control horses with no neuropathy). Total RNA was extracted from each tissue block, transcribed to complementary DNA and assayed for *Sarcocystis neurona*, *Neospora hughesi*, EHV-1, equine GAPDH, TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, Il-8, IL-10 and IL-12 p40 by TaqMan PCR. Quantitation of pathogen and cytokine transcription was done using the comparative C_T method and was reported as relative transcription or the n-fold difference relative to the weakest signal.

Sarcocystis neurona cDNA was detected in neural tissue from all 12 horses. Two of these also had amplifiable cDNA of *N. hughesi*. The protozoal load ranged from 1 to 461 times baseline in these horses (mean=123). All horses with EHV-1 myeloencephalopathy had positive viral signals on PCR ranging from one to 1,618 times baseline (mean=275). All control horses tested negative for *S. neurona*, *N. hughesi* and EHV-1 cDNA. Cytokine profiles for each disease reflected a balance between pro- and anti-inflammatory markers.

The expression of pro-inflammatory and Th1 cytokines (IL-8, TNF-α, IFN-γ) and the absence or rarity of Th2 and anti-inflammatory cytokines (IL-4, IL-6, IL-10) was commonly observed for the neural tissues from horses with EPM. EHV-1 positive neural tissues expressed mainly pro-inflammatory cytokine IL-8 with no IL-10 or IFN-γ and rare TNF-α expression. Tissue from control horses only expressed the housekeeping gene GAPDH. These findings provide new insight into the immunopathogenesis of EPM and EHV-1 myeloencephalopathy.



ABSTRACT #103

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) SURVEILLANCE IN HORSES AT A VETERINARY TEACHING HOSPITAL. JS Weese[a], M Archambault[b], J Rousseau[a], BM Willey[c], A McGeer[c], DE Low[c]. [a]Dept of Clinical Studies, University of Guelph, Guelph, Ontario, [b]Animal Health Laboratory, University of Guelph, [c]Dept of Microbiology, Mount Sinai Hospital, Toronto, Ontario, Canada.

MRSA appears to be an emerging pathogen in horses, and an emerging zoonosis. Following recognition of nosocomial and community-associated MRSA infection and colonization in horses presented to the Ontario Veterinary College, and colonization of veterinary personnel following contact with horses, equine MRSA screening was initiated. Nasal swabs were collected from horses at the time of admission, weekly during hospitalization and at the time of discharge. Selective culture for MRSA was performed. Isolates were typed via standard *SmaI* PFGE. Colonization or infection was deemed nosocomial if MRSA was not detected on the admission swab but was detected on swabs collected at least three days later.

One or more nasal swabs were collected from 2283/3056 (74.7%) horses admitted between October 4, 2002 and June 15, 2004. MRSA was isolated from 120 (5.3%) horses; 61 (50.8%) were community-associated and 53 (44.2%) were nosocomial while the origin of six cases was unclear. 104/105 (99%) tested isolates were subtypes of Canadian epidemic MRSA-5 (CMRSA-5). The remaining isolate was identified as CMRSA-2. Clinical infections, including septic arthritis (n=4), intravenous catheter site infections (n=3), pneumonia (n=2), incision infection (n=1), wound infection (n=1), mastitis (n=1), rhinitis (n=1) and body wall abscess (n=1), were present or developed in 14/120 (11.7%) horses.

Community-associated MRSA colonization was identified in 4/241 (1.7%) admissions in 2002, 22/1427 (1.5%) in 2003 and 35/615 (5.7%) in 2004 (P<0.0001). The overall rate of community-associated colonization was 27 per 1,000 admissions. Thoroughbreds and horses less than 1 year of age were over-represented (P<0.0001 each). Clinical infection was present or developed in 10 (16%) horses that were colonized at admission but was evident at admission in only 60% of horses. Horses colonized at admission were more likely to develop clinical MRSA infection during hospitalization than those not colonized (OR 38.9, 95% C.I. 9.49-159.5, P<0.0001).

The overall nosocomial MRSA colonization incidence rate was 23 per 1000 admissions, with no difference between years (P=0.0323). Nosocomial clinical infections developed in four (7.5%) of these horses; an incidence rate of 1.8 per 1,000 admissions and an incidence density of 0.88 per 100 patient days. There was no increase in nosocomial infection incidence rate during the study period (P=0.576).

This program has been useful for early identification and isolation of potentially infectious horses to decrease transmission of MRSA to other horses and veterinary personnel, has enabled differentiation of community-associated from nosocomial infection and helped identify farms with endemic MRSA colonization.

ABSTRACT #104

THE EFFECTS OF FENOLDOPAM ON SYSTEMIC HAEMODYNAMCIS AND INDICES OF RENAL FUNCTION IN THE NORMOTENSIVE NEONATAL THOROUGHBRED FOAL. Hollis AR[1], Ousey JC[2], Palmer L[3], Stoneham SJ[3], Allen WR[2] and Corley KTT[1]; [1] Royal Veterinary College, London; [2] Equine Fertility Unit, Newmarket; [3] Rossdale and Partners, Newmarket, UK.

Fenoldopam mesylate, a dopamine-1 receptor agonist, is licensed in the USA as an anti-hypertensive drug for humans. It decreases both systolic and diastolic arterial pressure in hypertensive humans at doses of 0.04mcg/kg/min to 0.8mcg/kg/min. However, no changes in haemodynamics at a dose of 0.03mcg/kg/min were seen in normotensive humans, and a dose of 0.3mcg/kg/min caused a moderate decrease in diastolic pressure, but no change in systolic pressure. Both doses caused increased renal blood flow and urine output, but not creatinine clearance, in normotensive humans.

Six Thoroughbred foals, aged 87-122 hours, were studied. All were born at term and considered to be healthy on the basis of clinical examination, haematology and baseline measurements of cardiac output (lithium dilution) and arterial pressure. The foals were sedated with 5-10mg intravenous diazepam, and instrumented with jugular venous, dorsal metatarsal arterial and urinary catheters for the study. The foals were then allowed to stand and nurse from the dam, and given a recovery period for 1 hour from the administration of diazepam. The foals were then restrained in lateral recumbency on a foal mat and given two doses of fenoldopam ('low dose': 0.04mcg/kg/min and 'high dose': 0.4mcg/kg/min) and a control dose of saline, in a double blind, randomized study. Each infusion was maintained for 30 minutes, and measurements were performed during the last 20 minutes of infusion. The washout period was at least 40 minutes between infusions. Heart rate, arterial blood pressure and cardiac output (lithium dilution) were measured, and systemic vascular resistance, stroke volume, cardiac index and stroke volume index calculated. Renal function was estimated by urine output and endogenous creatinine clearance. Repeated measures and one-way ANOVA tests were used to compare these parameters between each drug and placebo.

Compared to saline, high dose fenoldopam resulted in a significantly increased heart rate, and decreased mean, systolic and diastolic arterial blood pressure. There were no changes in renal indices. Low dose fenoldopam had no significant effect on systemic haemodynamics, but increased urine output. There was a trend to increased creatinine clearance with low dose fenoldopam, which was significant when compared to high dose fenoldopam.

These data suggest that, unlike in the normotensive human, high dose fenoldopam results in a significant decrease in arterial blood pressure in the neonatal foal. Low dose fenoldopam results in no significant changes in haemodynamics, with increased urine output. Low dose fenoldopam therefore has a potential clinical application in neonatal foals with acute renal failure, and this warrants further investigation.

ABSTRACT #105

RETROSPECTIVE STUDY OF DYSTOCIA IN HORSES AT A REFERRAL HOSPITAL. Norton JL, Johnston JK, Palmer JE, Sertich PL, Dallap BL, Boston RC, Wilkins PA. University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square PA.

The duration of Stage II labor impacts fetal survival in cases of equine dystocia. Institution of an organized, protocol driven approach to equine dystocia has been reported from a large private equine referral hospital located in central Kentucky, where the hospital and the patient population are proximate. We instituted a coordinated dystocia management protocol (CDMP) at our hospital in 1997 in an effort to improve time from presentation to dystocia resolution. The purpose of this study was to evaluate the effects of instituting this CDMP on time to resolution and outcome in mare and foal, for both emergent cases (EM) and dystocia occurring in mares enrolled in a high-risk pregnancy (HRP) program, at a referral hospital where location of the hospital and the EM patient population generally results in longer transport time.

Case records of mares with a complaint of dystocia, defined as Stage II labor of >30 minutes, between the years 1991 and 2004 were retrieved and data recorded. Mares were assigned to either EM (N=57) or HRP (N=14) and to prior to 1997 or from 1997 forward, when CDMP was instituted. A total of 71 cases met criteria for inclusion. For EM mares, time to resolution decreased by ~55 minutes (>44%) after CDMP institution, HRP mares experienced an ~ 9 minute (>15%) improvement. However, time from onset of Stage

II to hospital presentation in EM mares was significantly prolonged after CDMP institution. Survival rate of all mares at discharge was 86%. Of the fetuses thought to be alive at the time of presentation, 82% were alive at delivery prior to 1997. This improved to 88% following CDMP implementation, although the change was not statistically significant. Survival of foals delivered alive remained low overall, with 9% of foals delivered from EM mares prior to 1997, and 13% of foals delivered from EM mares post-CDMP, surviving to discharge; again the difference was not statistically significant. Survival of foals delivered from HRP dystocia remained good, with an overall survival to discharge of 71%.

Institution of a coordinated dystocia management protocol significantly reduced the time from presentation at the hospital to resolution for emergency presentations. However, no significant difference in outcome for EM foals was observed, primarily related to longer duration of Stage 2 labor prior to referral after 1996. For HRP mares, the small change in time to resolution was not unexpected as most of these mares had a clear plan developed for approaching dystocia prior to the onset of Stage II labor The large and significant difference in foal survival between EM and HRP groups underscores the importance of minimizing time to resolution in cases of dystocia in mares. Where the hospital and patient are not proximate, referral should be instituted as soon as it become clear that dystocia will not be rapidly corrected, if fetal survival is important.

ABSTRACT #106

A RETROSPECTIVE STUDY OF MYOPATHIES AND ASSOCIATED GAIT ABNORMALITIES IN 65 WARMBLOOD HORSES. LM Hunt, SJ Valberg, K Steffenhagen and JB Bender. College of Veterinary Medicine, University of Minnesota, St.Paul, MN.

The purpose of this study was to evaluate the types of myopathies that occur in Warmblood (WB) horses and determine their response to written diet and exercise recommendations provided after a diagnosis was established. Records of 1,400 muscle biopsies submitted to the Neuromuscular Diagnostic Laboratory were searched to identify 65 horses that were WB or WB crosses. The signalment, history, clinical abnormalities and muscle biopsy diagnoses were tabulated for each case. A questionnaire was administered to owners regarding their horse and an additional control horse to identify management factors prior to and after biopsy diagnosis, compliance with recommendations and response to treatment. Fifty % of owners responded. The data set contained 32% mares, 60% geldings and 8% stallions with mean age of 8.3 ± 0.7 yrs. The primary presenting complaint was muscle soreness of the hind quarters and back, as well as stiffness, with 18% of horses showing overt signs of tying-up and 18% showing signs of Shivers. 60 % of horses were diagnosed with polysaccharide storage myopathy (PSSM), 6% with recurrent exertional rhabdomyolysis (RER), 5% with myofiber atrophy, 5% had nonspecific myopathic signs and 24% had no abnormalities. 44% of horses with reported Shivers were diagnosed with PSSM. Owners of all horses received similar written recommendations that included feeding grass hay and replacement of dietary grain with a high fat supplement combined with a gradually increasing daily exercise program and maximal turnout. Prior to diagnosis, 87% of WB horses received a concentrate without additional fat and 13% received a fat supplement. 8 % of control horses were fed a concentrate without additional fat and 20 % received a fat supplement. Prior to diagnosis, 54% of WB horses were getting no or light exercise only (30% in controls). After receiving our recommendations, 46% of owners followed the exercise, turnout and diet recommendations, 19% changed the diet, increased turnout without an increase in exercise, 31% changed the diet only and 4% of owners did not comply. Significant improvement in original clinical signs was noted in 54% of horses (46% changed diet, exercise, turn-out, 8% changed diet and turnout). Within the remaining horses that did not show appreciable improvement, 67% only changed the diet and 8% made no changes. Signs of Shivers did not resolve with the recommendations.

Results from this study suggest that in WB horses, PSSM frequently presents as muscle soreness without overt signs of tying-up and that improvement in clinical signs may be achieved by reducing the level of dietary starch and increasing fat in conjunction with daily exercise and turnout. Partial compliance with these recommendations was met with less success. Clinical signs of Shivers did not respond favorably to diet, turnout and/or exercise changes despite excellent owner compliance.

ABSTRACT #107

IMMUNE-MEDIATED MYOSITIS IN 31 HORSES. SS Lewis and SJ Valberg, University of Minnesota, St Paul MN.

Immune-mediated myositis (IMM) is commonly reported in human beings and dogs, however, there are few detailed reports of IMM in horses. The purpose of this study was to characterize the signalment, history, clinical signs, muscle biopsy findings and response to treatment of 31 horses with potential IMM. A retrospective study was done by reviewing records of 797 biopsies submitted to the Neuromuscular Diagnostic laboratory at the University of Minnesota. Biopsies from 31 horses were selected for inclusion based on the presence of lymphocytic vasculitis or myositis. Age and signalment were compared between horses with putative IMM and the 765 horses in the Neuromuscular Laboratory database with other diagnoses. Information was obtained from biopsy submission sheets and augmented by a standardized questionnaire administered to horse owners regarding the horse's health prior to muscle disease, course of the muscle disease, treatment given and response to treatment. Immunohistochemical staining was done on 9 formalin fixed epaxial biopsies with marked mononuclear infiltrates to evaluate the contribution of macrophages, B and T lymphocytes as well as IgG to the inflammatory infiltrates.

A bimodal distribution of age for horses with putative IMM was identified with 25 horses ≤ 8 yrs of age and 4 horses ≥ 17 yrs of age. Quarter Horse related breeds were over-represented amongst horses with IMM (27/31), although 4 other breeds were also affected. No gender predilection was detected. A respiratory disease prior to the onset of clinical signs was not consistently reported. Clinical signs most frequently reported were depression, stiffness and muscle atrophy (often severe and rapidly progressive), particularly involving epaxial and gluteal muscles. CK was elevated in 20/22 cases where measured (mean 19,677; range 314-190,000 U/L) and AST activity was elevated in all cases. Muscle biopsies of the epaxial and gluteal muscles showed greater inflammatory changes surrounding blood vessels and myofibers than semimemr/tendinosus biopsies. Macrophages were prominent in the cellular infiltrate. Immunohistochemical stains showed a few focal B-cells (CD20,79) near necrotic fibers and/or blood vessels in 6/9 biopsies. CD3 cell staining for T cells showed marked cross-reaction with equine muscle fibers in all specimens. Monoclonal CD4 and CD8 staining is in progress. Myofibers did not stain for equine IgG.. Treatment with corticosteroids resolved signs of depression and halted the progression of atrophy. Muscle mass redeveloped over several months. Muscle atrophy recurred in several horses that again was responsive to corticosteroid treatment.

IMM appears to be a distinct cell mediated myopathy, particularly affecting young Quarter Horses, that results in malaise, elevated CK and rapid onset of epaxial and gluteal muscle atrophy. Diagnosis is best achieved with biopsy of affected muscles. Corticosteroids treatment appears to result in resolution of clinical signs; however, some affected horses are susceptible to reoccurrence.

ABSTRACT #108

PREVALENCE OF POLYSACCHARIDE STORAGE MYOPATHY AND Shivers IN BELGIAN DRAFT HORSES. AM Firshman[1], JD Baird[2], M Estes[3], SJ Valberg[1]. [1]College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA, Ontario Veterinary College, [2]University of Guelph, Guelph, ON, Canada, [3]College of Veterinary Medicine, The Ohio State University, OH.

Shivers represents a common confounding disorder in Belgian Draft horses (BDHs) characterized by muscle tremors and hyperflexion of the hindlimbs, which may progress to weakness, atrophy and recumbency. Some horses with Shivers have been diagnosed with polysaccharide storage myopathy (PSSM) based on accumulation of abnormal polysaccharide in muscle biopsies. However, a high prevalence of PSSM has been noted in many draft horses at post-mortem (>40%), which may confound the suggested causal relationship between Shivers and PSSM. We performed a large-scale study of muscle biopsies from a well-defined population of BDHs that included healthy horses, horses with Shivers and horses with muscle weakness. Our specific aims were 1) to determine the prevalence and clinical signs, of horses with PSSM and horses with Shivers in a large population of BDHs, 2) to determine if clinical signs of Shivers were consistently associated with a histological diagnosis of PSSM.

All available BDHs were examined on four farms (>100 adult horses) located in New York (2), Ohio (1) and Ontario (1). A history, gait evaluation including backing of horses from a stocks or tie stall and neurologic examination including scoring of muscle mass was performed. Serum [Se],[Cu],[Fe],[Zn], [vitamin E] and creatine kinase (CK) activity were determined. Histochemical and biochemical analyses were performed on gluteal muscle biopsies. Muscle biopsies were evaluated blindly and a diagnosis of PSSM was based on the presence of amylase resistant PAS positive inclusions and muscle [glycogen] >150 mmol/kg ww.

Thirty percent of BDHs were diagnosed with PSSM and 20% with Shivers. Seven percent of BDHs had both PSSM and Shivers, 24% had PSSM alone and 13% had Shivers alone. Chi-squared analysis indicated no significant association between PSSM and Shivers. Muscle wasting and weakness were found in 25% of BDHs. Of these, 50% had PSSM and 39% had Shivers. Sixty percent of BDHs with PSSM and 50% of BDHs with Shivers had no signs of muscle wasting and weakness. Neither PSSM nor Shivers were associated with abnormal serum CK activity, nor abnormal trace mineral or vitamin E concentrations.

BDHs have a high prevalence of PSSM and Shivers within the general population. Both disorders may present with weakness, muscle wasting and an abnormal gait, however, our results suggest that Shivers is a separate disorder from PSSM and the high incidence of PSSM in BDHs may have resulted in a mistaken assumption of a causal relationship.

ABSTRACT #109

PLATELET ACTIVATION IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION. Hammond, AN; Marr, CM; Bailey, SR and Cunningham FM. Royal Veterinary College, London University, London, UK.

The aim of this study was to look for evidence of platelet activation in horses with recurrent airway obstruction (RAO) in response to a controlled antigen challenge. Five RAO and five healthy control horses were maintained at pasture and received no medication for a minimum period of two months prior to the study. The investigation was carried out under a UK Home Office licence. The antigen challenge comprised placing each horse in a closed loose box for five hours with poor quality hay and straw, shaken hourly. Pleural pressure measurements were obtained immediately before, and at five and 24 hours post challenge. Venous blood samples were also collected at these times for measurement of platelet numbers (Coulter counter), PAF-induced platelet aggregation and thromboxane (Tx) production (Radioimmunoassay [RIA]), protein tyrosine phosphorylation (Western blotting) and plasma Tx (RIA) and 5-hydroxytryptamine (5-HT) concentrations (High Performance Liquid Chromatography). Data were analysed using one or two way ANOVA.

Four of the five RAO horses, but none of the controls, responded to the challenge with an increase in pleural pressure and the one non-responder was excluded from the analysis. PAF caused concentration dependent aggregation and Tx production by platelets from both RAO and healthy horses and, although antigen challenge did not alter these responses, the EC_{50} for PAF-induced aggregation was significantly greater in the RAO horses at all the time points studied. Similarly, plasma Tx and 5-HT were unaffected by antigen challenge but the 5-HT concentration was significantly higher ($p<0.05$) in the RAO horses before and at five hours post challenge. Western blot analysis revealed a number of tyrosine phosphorylated proteins in unstimulated platelets obtained from control and RAO horses prior to challenge. Addition of PAF (10^{-7}M) or thrombin (2iu/ml) increased the intensity of several different molecular weight bands and, following challenge, there was an increase in expression of an, as yet unidentified, protein of >250 kD in three of the RAO horses.

These results, although providing no conclusive evidence for platelet activation following antigen challenge, suggest there are some differences between platelets from RAO and healthy horses. The increased 5-HT concentrations in the plasma of RAO horses may be due to impaired amine uptake by platelets or endothelial cells and warrants further investigation.

ABSTRACT #110

ARTERIAL HYPOXEMIA IN MAXIMALLY EXERCISING THOROUGHBRED HORSES IS NOT CAUSED BY INTRAPULMONARY ARTERIOVENOUS SHUNTING. T. E. Goetz and M. Manohar. College of Veterinary Medicine, University of Illinois, Urbana.

Diffusion limitation, inadequate alveolar hyperventilation, rightward shift of the hemoglobin-0_2 dissociation curve, and ventilation:perfusion mismatching are considered to be the principal factors contributing to the development of exercise-induced arterial hypoxemia. Recently, in human subjects (Eldridge and coworkers. *J Appl Physiol* 97:797-805, 2004), it was demonstrated that increasing exercise intensity caused recruitment of dormant intrapulmonary arteriovenous shunts thereby contributing to the magnitude of exercise-induced arterial hypoxemia. To determine whether, similar to exercising human subjects, strenuously exercising horses exhibit intrapulmonary arteriovenous shunting which may contribute to arterial hypoxemia, experiments were carried out on seven healthy Thoroughbred horses at rest, and during submaximal (8 m/s on a 3.5% uphill slope) and maximal (14 m/s on a 3.5% uphill slope) exercise.

Intrapulmonary arteriovenous shunting was studied with neutron activation analysis using 15 μm diameter microspheres, labeled with various stable isotopes (gold, samarium, lanthanum, ytterbium or lutitium), which were injected into the right atrium while blood was being withdrawn at a constant rate (25 ml/minute) from the pulmonary artery and aorta. The sequence of microsphere injections was randomized for every horse.

Although significant arterial hypoxemia (96 ± 2 mm Hg at rest versus 75 ± 2 mm Hg during maximal exertion) and pulmonary hypertension (29 ± 2 mm Hg at rest versus 96 ± 2 mm Hg during maximal exertion) occurred during maximal exercise, 15 μm diameter microspheres did not traverse the pulmonary microcirculation to appear in the aortic blood. Thus, our findings did not support a role for intrapulmonary arteriovenous shunts > 15 μm in diameter in the development of exercise-induced arterial hypoxemia in Thoroughbred racehorses. Our observation in going

from 30 to 120 s of maximal exertion that arterial 0_2 tension remained unchanged despite significant reduction in the mixed-venous blood 0_2 tension, 0_2 saturation and 0_2 content, also discounts the importance of intrapulmonary arteriovenous shunts in causing arterial hypoxemia. The importance of intrapulmonary shunts is discounted because, assuming that a constant fraction of total pulmonary blood flow bypasses the gas-exchange areas of the lungs via intrapulmonary arteriovenous shunts during 30 to 120 s of maximal exertion, the observed significant reductions in mixed-venous blood oxygenation should have caused further significant reductions in the arterial 0_2 tension, which was not the case in our horses.

The results of the present study did not support a role for functional intrapulmonary arteriovenous shunts > 15 μm in diameter in the development of exercise-induced arterial hypoxemia in Thoroughbred racehorses. These findings suggest that species differences may exist between humans and Thoroughbred horses regarding exercise-induced intrapulmonary arteriovenous shunting.

ABSTRACT #111

FLUID MOVEMENT FROM PULMONARY VASCULATURE AT REST AND DURING EXERCISE IN HORSES. M. Vengust[1], H. Stämpfli[2], F. Teixeira-Neto[3], G. Monteith[2], L. Viel[2], G. Heigenhauser[4]. [1]University of Ljubljana, Veterinary Faculty, Slovenia; [2]University of Guelph, Ontario Veterinary College, Guelph, Ontario, Canada; [3]FMVZ-UNESP, Botucatu, Sao Paulo State, Brasil.[4]McMaster University Medical Centre, Hamilton, Ontario, Canada.

Fluid exchange across the capillary endothelium is believed to obey Starling's law. Exercise at near peak VO_2 causes a marked increase in cardiac output with a less prominent increase in pulmonary vascular pressures. These adaptations coexist with redistribution of blood flow across the lung through the capillary recruitment and increase in the pulmonary surface area. Hence, changes in pulmonary hemodynamics during exercise contribute to increase in fluid movement across the alveolar-capillary barrier. The purpose of the study was to determine fluid movement across the alveolar-capillary barrier at rest and during exercise in horses.

Six Standardbred horses (5-6 years) were exercised on a high-speed treadmill (Säto Sweden) at 80% VO_2 peak until fatigue. Resting arterial and mixed venous blood, as well as CO_2 elimination and O_2 uptake, were sampled simultaneously 5 minutes apart. During exercise, the sampling was performed in 60 sec intervals until fatigue. Blood volume changes (ΔBV %) across the lung were calculated from changes in plasma protein, hemoglobin and hematocrit (Strauss et al. 1951; Dill and Costill 1974; Harrison, 1985). Cardiac output (Qp L/min) was calculated using Fick principle using VO_2 and blood O_2 content from central venous and arterial blood. Fluid movement (FM L/min) across the lung was then quantified based on Qp and ΔBV (FM = (Qp x ΔBV). Variables were analyzed using two-way repeated-measures ANOVA ($P<0.05$), and data expressed as mean ± SE.

No fluid moved from or into the pulmonary vasculature was present at rest (0.3 ±0.8 L/min). During exercise 11.6 ±2.1 L/min at 1 min ($P = 0.0004$), 5.5 ±2.6 L/min at 2 min ($P = 0.02$), 9.2 ±1.9 L/min at 3 min ($P = 0.006$), 11.2 ±2.5 L/min at 4 ($P = 0.001$), and 8.3 ±2.1 L/min at fatigue ($P = 0.006$) of fluid moved from the pulmonary circulation. Mean exercise Qp was 290L ±19.3 L/min. Fluid loss from the pulmonary circulation decreased to the resting value at 2 min of recovery (1st min of recovery 6.3±0.8 L/min, $P = 0.03$; 2nd min of recovery 4.5 ±0.7 L/min, $P = 0.17$).

In horses, exercise at 80% VO_2 peak resulted in a marked increase in Qp associated with a significant movement of fluid from the pulmonary circulation into the pulmonary interstitium. Increased capillary recruitment and increased pulmonary capillary surface area are a possible cause for the initial (1 min of exercise) calculated fluid movement from the pulmonary circulation. With the continuation of the exercise fluid continued to leave the pulmonary vasculature; results indicate that around 3% of Qp is moved from the pulmonary vasculature into the pulmonary interstitium. Findings presented herein should help in future research on specific airway conditions.

ABSTRACT #112

QUANTIFICATION OF EIPH: COMPARISON OF RBC ENUMERATION IN TW AND BALF WITH VISUAL ENDOSCOPIC SCORING. MM Durando and EK Birks. University of Pennsylvania, Kennett Square, PA.

Quantitifying pulmonary hemorrhage associated with exercise (EIPH) is important for determining the efficacy of various EIPH therapeutic modalities. Despite numerous studies, such quantification has proven difficult and controversial, with no consensus as to the most accurate/reproducible method. In addition, a commonly utilized method, bronchoalveolar lavage (BAL) is not feasible in many settings. Most commonly, EIPH is detected by visual endoscopic evaluation of the upper airway and trachea. This study was performed to compare right and left-sided quantification of erythrocytes (RBC) in BAL fluid with RBC counts from tracheal wash fluid (TW) and to determine how well the common methods of detecting EIPH correlate with each other and with visual endoscopic scoring of hemorrhage in the trachea and/or bronchioles.

Six Thoroughbred horses had a videoendoscopic examination (3 m long, 13 mm dia) of their upper airway, trachea, and bronchioles along with TW and BAL of the right and left lung 45-60 minutes following high speed treadmill exercise. All exams were recorded for later scoring. 120 mls of saline were instilled for the right and the left-sided BAL, and 30 mls of saline instilled for the TW. Each horse exercised a total of 10 times, with each run separated by a minimum of two weeks, for a total of 60 post-exercise examinations. Four veterinary clinicians familiar with endoscopic airway evaluation, and blinded as to the identity of the horse, reviewed the videotaped endoscopies and provided scores (0-4) based on a previously published system. BAL and TW fluid were collected in tubes containing EDTA and submitted to the clinical laboratory for RBC enumeration. All samples were counted manually, in duplicate, and RBC numbers reported as cells/ul.

RBC counts from the right-sided BAL were not strongly correlated with left-sided BAL RBC counts ($R^2=0.46$). When TW RBC counts were compared with right-sided BAL RBC counts, there was a poor correlation ($R^2=0.18$), however, when TW RBC counts were compared with left-sided BAL RBC counts, the correlation was strong ($R^2=0.82$). Visual scoring of the right vs left-sided BAL had a moderate correlation ($R^2=0.56$), while TW visual scoring was weakly correlated with left-sided BAL visual scores ($R^2=0.38$), and right-sided BAL visual scores ($R^2=0.38$). When visual scoring was compared with RBC counts, the correlations were weak to moderate: right-sided BAL visual score vs right-sided BAL RBC counts $R^2=0.39$; left-sided BAL visual score vs left-sided BAL RBC counts $R^2=0.47$; TW visual score vs TW RBC counts $R^2=0.54$. Overall mean RBC counts (n=60) were 8218±1056, 5816±836, and 1431±218 cells/μl for the left BAL, right BAL and TW, respectively. Left and right BAL RBC counts were significantly different ($p=0.004$). In 43/60 examinations, there were more RBCs in the left-side BAL than the right-side.

These data suggest that the accuracy of RBC quantification would be improved by lavaging and averaging RBC counts from both sides of the lung. On average, more RBCs were recovered from the left than the right lung, however, this observation did not occur in every examination.

ABSTRACT #113

TRACKING INFLAMMATORY AND RENAL PARAMETERS IN DOGS PRE- AND POST-TREATMENT FOR PERIODONTAL DISEASE. JE Rawlinson[1], RE Goldstein[2], HN Erb[2], and CE Harvey[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]College of Veterinary Medicine, Cornell University, Ithaca, NY.

Periodontal disease (PD) is one of the most common medical conditions found in the dog. Few studies have been conducted in the dog to evaluate the systemic effects of PD; one revealed an association between PD and morphologic changes in the heart, liver, and kidney of dogs. Our goals were to explore associations between the severity of PD and concentrations of inflammatory and renal parameters and to track changes in these parameters after appropriate treatment for PD.

Twenty-two client-owned dogs of varying age with clinical signs of PD (gingivitis +/- recessive alteration of the periodontium) were identified. A full physical exam, chemistry panel, complete blood count, urinalysis, urine culture, urine protein:creatinine ratio (UPC), blood pressure, serum C-reactive protein (CRP), and microalbuminuria (MA) test were performed on each dog the day before dental therapy for PD. Dogs with concurrent disease and/or other apparent causes of oral inflammation were excluded. Under anesthesia, PD was scored for degree of gingival inflammation (0-3 scale) and attachment loss (ATL) (measured in millimeters). After scoring, the PD was treated clinically as indicated. Approximately 4 weeks following dental treatment, all dogs were re-examined and all tests were repeated except dental scoring.

Non-parametric statistical analyses were performed; significance set at $p \leq 0.05$. A numerical PD score was tabulated using the Total Mouth Scoring System (ATL weighted for teeth and size of patient). A positive correlation between pre-treatment CRP and PD was found using Spearman's rank correlation ($r_{sp}=0.64$ and $p=0.0016$). Pre and post PD treatment results were compared using Wilcoxon signed rank test. Median and range CRP (μg/ml) (pre 5.65 (0.8-33), post 3.5 (0.9-11.7), $p=0.026$) and serum globulins (g/dl) (pre 3.45 (2.4-4.5), post 3.3 (2.8-3.8), $p=0.043$) decreased post-treatment. BUN concentrations (mg/dl) (pre 13.5 (6-26), post 17 (11-68), $p=0.0001$) increased. No change was identified in WBC, MA, urine specific gravity (USG) or serum creatinine (CR) (mg/dl) (pre 1.1 (0.6-1.9), post 1.15 (0.6-2.1)) concentrations. When samples of the 11 most severe PD dogs were analyzed separately, post-treatment CRP decreased ($p=0.007$) whereas BUN ($p=0.0015$) and CR ($p=0.0059$) increased when compared to pre-treatment values. There was no post-treatment change in WBC, MA, UPC or USG in these 11 dogs.

We conclude that systemic signs of inflammation are present in dogs with PD and that the degree of inflammation correlates with the severity of the PD. This inflammation is reduced one month following appropriate dental therapy. Proteinuria and MA did not appear to be associated with the severity of PD and did not change with dental therapy. The mild increase in BUN and CR (in severe PD) following treatment is of unknown significance and requires further investigation. As all renal values remained well within normal limits and no changes were seen in USG, UPC, and MA, true renal damage post-dental treatment appears to be unlikely.

ABSTRACT #114

COMPARISON OF THE VITROS 250 AND THE IDEXX VETTEST CHEMISTRY ANALYZER FOR URINE PROTEIN:CREATININE RATIOS IN DOGS AND CATS. Michelle Kahn, Pete Fernandes, Mona Jensen, Gina Panagakos, David Dieffenbach. IDEXX Laboratories, Westbrook, ME.

The importance of rapid laboratory results has been well established in veterinary medicine. An in-clinic diagnostic test gives the practitioner the ability to make an immediate diagnosis and provide timely treatment. The new IDEXX Urine P:C Ratio is an in-house, fully quantitative assessment of urinary protein loss. The objective of this study was to compare the UPC results between a reference instrument (Vitros 250) and the new IDEXX Urine P:C Ratio for the VetTest.

Paired feline and canine urine samples were collected from 150 patients (100 canine samples, 50 feline samples). Testing was performed on each analyzer with in twp hours of one another and according to manufacturer's instructions with appropriate dilutions. Each analyzer was maintained to manufacturers' specifications, including handling of the test materials and performance of quality control procedures.

The linear regression scatter plot for the paired UPC ratios shows a high level of association between the two instruments ($R^2=0.97$). The Urine P:C Ratio is comprised of two components, a urine protein test and a urine creatinine test. Each test was independently analyzed to ensure that component results were comparable between the instruments. The linear regression scatter plot for the paired urine protein tests shows a high level of agreement between the instruments ($R^2=0.97$). Samples above the dynamic range were diluted according to manufacturer's instructions with dynamic range maximums of 200 mg/dL for Vitros 250 400 mg/dl for the IDEXX VetTest. The linear regression scatter plot for the paired urine creatinine tests also shows a high level of association between the two instruments ($R^2=0.96$). As per manufacture instructions, all urine samples were diluted 1:21 with samples greater than 350 mg/dl requiring a second dilution of 1:2.

There was strong association between the Vitros 250 and the IDEXX VetTest analyzers when comparing UPC results. Moreover, the individual analytes for urine protein and urine creatinine also showed a high level of association. This result correlates well with the fact that both the Vitros 250 and the IDEXX VetTest use dry slide technology. The urine protein slide (UPRO) uses a dye-binding assay and has dynamic ranges of 5 to 200 mg/dL (Vitros 250) and 5 to 400 mg/dL (IDEXX VetTest). The urine creatinine slide uses an enzymatic assay and has a dynamic range of 1.05 to 346.5 mg/dL (Vitros 250) and 5 to 350 mg/dL (IDEXX VetTest). The UPC Ratios and both urine protein and urine creatinine results compare favorably between the Vitros 250 and the IDEXX VetTest analyzers.

ABSTRACT #115

URINE PROTEIN EXCRETION OF HEALTHY BERNESE MOUNTAIN DOGS AND OTHER DOGS WITH AND WITHOUT ANTIBODIES AGAINST BORRELIA BURGDORFERI. B Gerber[1], S Eichenberger[1], MM Wittenbrink[2], CE Reusch[1]; [1]Clinic for Small Animal Internal Medicine and [2]Institute of Veterinary Bacteriology, Vetsuisse Faculty University of Zurich, Switzerland.

The breed Bernese Mountain Dog (BMD) is associated with familial glomerulonephritis. Furthermore *Borrelia burgdorferi (B.b.)* infection was suspected to be a possible cause of glomerulonephritis in BMD. We found that BMD have more often serum antibodies against *B.b.* compared to dogs of other breeds. The aim of this study was to compare the amount and pattern of proteins in the urine of healthy BMD and healthy dogs of other breeds both with and without antibodies against *B.b.*, to investigate if healthy BMD show changes in urine proteins as early signs of glomerular disease and if this is influenced by antibodies against *B.b.*

Urine and blood samples from the dogs were taken within one year. As controls, large breed dogs with long hair were chosen. Health status of all dogs was assessed by questionnaire filled out by the owner, complete blood count, chemistry panel, urinalysis and urine culture. Antibodies against *B.b.* were determined using an ELISA with a whole cell sonicate as antigen. The results were confirmed using Western blot. Urine protein excretion was measured by the urine protein-to-urine creatinine ratio (UPC), urine albumin concentrations were assessed by a commercial in-clinic test for canine microalbuminuria and to further characterise the urine protein

pattern proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Ninety-five BMD and 27 control dogs were included. All dogs were considered healthy. BMD were 1-11 years of age (median four years) and control dogs 1-8 years (median five years). There was no significant difference in age between the two groups. The gender distribution was the same in both groups (34% males). In the control group eight different breeds and a mixed breed dog were represented. Seroprevalence of *B.b.* in BMD was 62% compared to 19% in control dogs. This difference was significant. Although the dogs were randomly chosen, the geographical area where the dogs lived were similar in both groups. The UPC was not significantly different between BMD and control dogs and between dogs with and without antibodies against *B.b.* In 11 BMD and in one control dog, the UPC was >0.3. There was no significant difference in the occurrence of microalbuminuria between BMD and control dogs and dogs with and without antibodies against *B.b.* In 23 BMD and in five control dogs the test for microalbuminuria was positive. 18 of them had an UPC <0.3. 17 of the 23 BMD with microalbuminuria but none of the five control dogs had antibodies against *B.b.* Preliminary interpretation of the SDS-PAGE did not reveal differences between dogs with and without antibodies against *B.b.*

These results indicate that antibodies against *B.b.* are not associated with changes in amount and pattern of urine proteins and that healthy BMD do not show early changes in urine proteins.

ABSTRACT #116

COMPARISON OF METHODS USED FOR DETERMINING URINE PROTEIN-TO-CREATININE RATIO IN DOGS AND CATS. Peter Fernandes[1], Michelle Kahn[2], Vera Yang[3], Aimee Weilbacher[4]. Idexx Laboratories, Elmhurst, IL[1], Westbrook, ME[2], Sacramento, CA[3], North Grafton, MA[4].

The urine protein-to-creatinine ratio (UPC) is a cornerstone for quantitative assessment of persistent proteinuria in dogs and cats. Since there are no collectively accepted reference methods, inter-laboratory comparison of UPC are complicated by the variety of assay methodologies used for measuring urine protein and urine creatinine concentrations.

The purpose of this study was to evaluate common methodologies used for the measurement of urine protein and urine creatinine concentrations. Urine samples were collected from 21 dogs and 48 cats; and for each sample, urine protein and urine creatinine concentrations were measured using the Vitros® 250 chemistry system and Hitachi® 717 automated analyzer. Urine protein methods compared include a colorimetric pyrocatechol violet dye-binding assay (UPRO dry slide, Ortho-Clinical Diagnostics, Rochester NY) and a turbidimetric benzethonium chloride assay (U/CSF protein, Roche Diagnostics, Indianapolis, IN). Urine creatinine methods compared include an enzymatic technique (CREA dry slide, Ortho-Clinical Diagnostics, Rochester, NY) and a Jaffé reaction (Randox® creatinine, Equal Diagnostics, Exton, PA). Data were analyzed by simple linear regression scatter plots, Pearson's correlation, and NCCLS EP9-A difference and bias plot tests. Data were split and analyzed separately for dogs and cats.

Linear regression analysis, correlation, bias data analysis for Canine urine samples						
	Vitros (X)	Hitachi (Y)	slope	intercept (mg/dL)	correlation (r)	Mean bias
Protein	Pyrocatechol	Benzethonium	0.68	33.83	0.60	25.26
Creatinine	Enzymatic	Jaffé	1.03	-7.78	0.98	-2.21
Ratio	Vitros UPC	Hitachi UPC	0.26	0.35	0.47	0.05
Linear regression analysis, correlation, bias data analysis for Feline urine samples						
	Vitros (X)	Hitachi (Y)	slope	Intercept (mg/dL)	correlation (r)	mean bias
Protein	Pyrocatechol	Benzethonium	0.48	50.70	0.85	-3.70
Creatinine	Enzymatic	Jaffé	1.02	2.04	0.97	6.99
Ratio	Vitros UPC	Hitachi UPC	0.64	0.19	0.99	-0.25

This study demonstrates that urine protein and urine creatinine concentrations can vary depending on methodology, therefore making inter-laboratory UPC comparisons problematic. When monitoring renal disease, serial UPC determinations require using the same methodology as the baseline. If a new methodology is used then a new baseline must be established.

ABSTRACT #117

SEMI-QUANTITATIVE EVALUATION OF PROTEIN IN FELINE URINE. HM Syme and J Elliott, Royal Veterinary College, London, UK.

The importance of protein in feline urine as evidence of significant kidney and other systemic diseases has been highlighted recently with survival of both azotaemic and non-azotaemic cats being inversely related to urine protein to creatinine ratio (UPC) at initial presentation. The aim of this study was to evaluate a semiquantitative highly sensitive assay of urine albumin concentration (E.R.D.-HealthScreen™ Feline Urine Test, Heska Corporation.) and assess its performance against more quantitative measures of urine protein.

Urine samples were collected from 69 cats by cystocentesis during routine clinical evaluation. A complete routine urinalysis was undertaken. Samples were then diluted to a urine specific gravity of 1.010 with distilled water and urine albumin concentration was measured semiquantitatively by the E.R.D.-HealthScreen test. After screening by this method, 10 urine samples in the negative, low, moderate and high/very high positive categories were selected and aliquots were submitted to a clinical laboratory for UPC measurement. The same samples were also assayed for urinary albumin using a polyclonal ELISA previously validated for feline urine. A further 38 urine samples, where the UPC had been measured previously and the samples stored at -80ºC, were selected to give samples with a wide range of protein concentrations. Aliquots of these samples were thawed and subjected to the E.R.D.-HealthScreen test. The median (25^{th} and 75^{th} percentile) UPC and urine albumin to creatinine ratios (UACs) for the four categories of proteinuria in the E.R.D.-HealthScreen test were calculated and statistical comparisons were made between the four categories using Kruskal Wallis with Dunn's Multiple comparison test.

The UPC and UAC values respectively found for each category of the E.R.D.-HealthScreen test were: 0.14 (0.10 to 0.21) and 19 (11-30) mg/g for negative tests (n=19); 0.23 (0.17 to 0.32) and 56 (33-81) mg/g for the low category (n=16); 0.23 (0.21 to 0.40) and 88 (70-180) mg/g for the moderate category (n=19) and 0.66 (0.44 to 1.11) and 260 (138-415) mg/g for the high category (n=22). Statistically, the negative category had significantly lower UPC and UAC results when compared to the moderate and high categories.

This study has defined the range of UPC and UAC values encompassed by the different categories on the E.R.D.-HealthScreen test. A negative result using the E.R.D.-HealthScreen test consistently gave UPCs below 0.25 and UACs below 58 mg/g. High positive results using the E.R.D.-HealthScreen test usually yielded UPC and UAC results outside of our laboratory normal reference ranges (>0.4 and >82 mg/g respectively).

ABSTRACT #118

DEVELOPMENT OF ALBUMINURIA AND OVERT PROTEINURIA IN HETEROZYGOUS (CARRIER) FEMALE DOGS WITH X-LINKED HEREDITARY NEPHROPATHY (XLHN). Olivia F. Hsieh,[1] George E. Lees,[1] Sean E. Clark[2], Wayne A. Jensen,[2] and Clifford E. Kashtan.[3] [1]Texas A&M U., Coll. Sta., TX, [2]Heska Corp., Ft. Collins, CO, and [3]U. of Minn., Mlps, MN.

Dogs with XLHN have juvenile-onset glomerular disease due a *COL4A5* mutation that causes type IV collagen in their glomerular basement membranes (GBM) to be defective. Affected males have complete absence of normal GBM collagen and develop severe,

rapidly progressive disease. However, carrier females have mosaic expression of normal and defective collagen in their GBM and develop relatively mild disease that usually does not progress clinically while they are young adults. Previous studies have characterized the onset of albuminuria in relation to that of overt proteinuria in male dogs with XLHN. The purpose of this study was to similarly characterize the onset and magnitude of albuminuria and proteinuria in carrier females with this disease.

Twenty-one (seven normal, 14 carrier) females from six litters were studied after their genotypes were determined by an allele-specific DNA test. Beginning at eight weeks of age and continuing until the dogs were 26-32 weeks of age, urine was obtained weekly by antepubic cystocentesis for complete urinalysis and determination of protein:creatinine ratio (UPC). Aliquots of urine stored at -80°C were later assayed for albumin concentration using a canine-specific immunoassay. Urine albumin concentration was normalized to a 1.010 urine specific gravity (nUAlb), as well as expressed in a ratio to urine creatinine concentration (UAlb/Cr). A total of 483 urine specimens were evaluated.

Albuminuria (nUAlb ≥ 1.0 mg/dL or UAlb/Cr ≥ 30 mg/gm) and/or proteinuria (UPC ≥ 0.5) was found in 28 of 169 specimens from seven normal dogs, but not in consecutive weeks in any one dog. All 14 carriers developed persistent proteinuria and/or albuminuria. Onset of persistent proteinuria was at 13-20 weeks of age. Onset of persistent albuminuria usually preceded that of proteinuria by 1-4 (median, 2) weeks. Using either nUAlb or UAlb/Cr to define onset of persistent albuminuria gave identical results. Striking differences in magnitudes of proteinuria were observed among the dogs. Magnitude of proteinuria typically increased for 2-11 (median, 6) weeks after its onset, and then partly subsided before it stabilized in each dog by the end of the study. Magnitudes of stable proteinuria exhibited by 6- to 7-month-old carriers ranged from microalbuminuria alone (1 dog) to UPC > 10 (1 dog); six dogs stabilized with UPCs < 2.5, 4 dogs with UPCs = 2.5-6.0, and four dogs with UPCs > 6.0. Rate of UPC increase (average weekly increment) for the first 4-8 weeks after onset of proteinuria was a better indicator of eventual magnitude of proteinuria classification than was the duration of persistent albuminuria before the onset of overt proteinuria in these 14 dogs.

We conclude that carrier female dogs with XLHN have diverse severity of their renal disease manifested in part by different magnitudes of proteinuria during adolescence. Different ratios of random inactivation of the normal or the mutated *COL4A5* allele in cells that synthesize GBM collagen in these dogs probably account for much of this diversity of renal disease severity.

ABSTRACT #119

DAY-TO-DAY VARIATION OF URINE PROTEIN:CREATININE RATIO IN FEMALE DOGS WITH STABLE GLOMERULAR PROTEINURIA CAUSED BY X-LINKED HEREDITARY NEPHROPATHY (XLHN). Mary B. Nabity,[1] George E. Lees,[1] May M. Boggess,[1] and Clifford E. Kashtan.[2] [1]Texas A&M Univ. College Station, TX, and [2]Univ. of Minn., Minneapolis, MN.

Urine protein:creatinine ratio (UPC) is an index of magnitude of proteinuria that is widely used to assess dogs with proteinuria due to renal disease. Greater magnitude of proteinuria (ie, higher UPC) is associated with worse clinical outcomes, and serial UPC evaluations are used to monitor changes in disease severity and responses to treatment. Especially when serial UPCs are used for monitoring, clinical decisions hinge on distinguishing differences that reflect real changes in the prevailing magnitude of proteinuria from differences that may occur due to day-to-day biological variation of UPC in dogs with unchanged magnitudes of proteinuria. Such decisions currently are hindered by a paucity of data regarding day-to-day variation of UPC in proteinuric dogs.

Dogs with XLHN develop glomerular disease because a *COL4A5* mutation causes the type IV collagen in their glomerular basement membranes (GBM) to be abnormal. Heterozygous (carrier) females have mosaic GBM expression of normal and abnormal collagen and develop proteinuria during adolescence. They typically remain otherwise healthy with good renal function that is clinically stable for extended periods, especially while they are young adults. During 1999-2002, 49 different 1- to 3-year-old XLHN-carrier females were used in studies that included daily UPC determinations for 3-5 consecutive days while their magnitude of proteinuria was ostensibly stable. All studies used the same protocol for morning urine collections by cystocentesis, and all urine protein and creatinine assays were performed using one dry-film chemistry auto-analyzer. Each dog was evaluated one or more times while not receiving diets or drugs that might modify the magnitude of proteinuria. Many dogs were also evaluated at the ends of 2- to 16-week intervals during or after administration of such diets or drugs. In this analysis, we used a total of 233 such evaluations to assess day-to-day UPC variation by calculating the variance components and intraclass correlation coefficient (ICC) from a random effect ANOVA model for each 3- to 5-day evaluation period.

In evaluations of untreated dogs, the ICC was 74% (95% confidence interval, 64-84%). Thus, variance attributable to day-to-day variation was such that an average of 2-4 measurements provides a reliable estimate of UPC, and 3-day evaluation periods were used in all further studies. No difference was detected in the day-to-day variability in untreated dogs with low UPC (< median value, 3.4) as compared to dogs receiving a UPC-lowering treatment, and in untreated dogs with a high UPC (≥ 3.4) as compared to dogs receiving UPC-raising treatments. Thus, all 233 evaluations (treated and untreated) were used to examine the effect of mean UPC value on variance, which was greater when UPC was >5.

These data suggest that serial UPC values in individual dogs may differ by up to 50% before one can confidently conclude that the prevailing magnitude of proteinuria has actually changed.

ABSTRACT #120

TRANSURETHRAL COLLAGEN IMPLANTATION FOR TREATMENT OF CANINE URINARY INCONTINENCE. J. K. Byron, D. J. Chew, M. A. McLoughlin, S. P. DiBartola, S. Arnold, J. Q. Jaeger. College of Veterinary Medicine, The Ohio State University, Columbus, OH.

A retrospective study was performed to assess the success of and client satisfaction with transurethral bovine cross-linked collagen implantation for treatment of canine urinary incontinence secondary to urethral sphincter mechanism incompetence or post-operative ectopic ureters (EU). Fifty-eigth procedures were performed on 44 dogs. Fourteen dogs had two procedures performed. Each procedure was treated as a separate event. Nine of these had previously undergone surgery to correct EU. Clients were contacted and the degree and duration of incontinence prior to and after the procedure was assessed as well as the effect of any estrogen or alpha-adrenergic medications. Incontinence score (CS) was assessed on a scale of 1 to 5 as noted below.

CS	Description
1	Patient is never continent. Dribbles urine when awake as well as when sleeping. Constantly leaves urine on surfaces when getting up from a sitting or recumbent position.
2	Poorly continent. Patient urine soils where it has been sleeping more than 50% of the time. Dribbles urine or has a wet perineum when awake 25 to 75% of the time.
3	Patient urine soils where it has been sleeping more than 50% of the time. Dribbles urine or has a wet perineum when awake up to 25% of the time.
4	Patient urine soils where it has been sleeping up to 50% of the time, but does not dribble or have a wet perineum when awake.
5	Patient is always continent.

Follow-up information was available for 34 of the procedures. Of these cases, seven were dogs with EU. All dogs had a mean age of 58.4 months at the time of the procedure. Dogs without EU had a mean age of 62.6 months and EU dogs had a mean age of 36.8 months. All dogs had a mean duration of incontinence of 26.1 months. Five dogs without EU underwent 2 separate procedures. Mean CS in all dogs prior to collagen implantation, without medical therapy, was 1.9. Mean CS with medical therapy was 2.7. Mean CS in all dogs after the procedure was 4.5. 16 dogs had estrogen or alpha agonist therapy after the procedure to improve continence. Mean CS in these dogs was 4.4. Duration of continence without medical therapy was 5.7 months in those dogs that needed additional medical therapy. Sixteen dogs did not receive medical therapy and had a CS of 4.9 a mean of 17.8 months after the procedure (range one – 56 months). These dogs had stable CS at the time of follow-up. EU dogs had a mean CS of 4.4 after the procedure. Three EU dogs had a mean CS of 4.6 after the addition of medications. Mean CS was 4.8 in 3 dogs not needing additional medication and two of these dogs had stable CS after 24 months at the time of follow-up. Overall client satisfaction with the procedure was 88% with 26 cases of 100% satisfaction. Transurethral collagen implantation appears to be effective in improving urinary continence, particularly in those patients with poor response to estrogen and alpha agonist therapy.

ABSTRACT #121

CLINICAL EVALUATION OF EFFECTS OF DIETARY MODIFICATION IN CATS WITH SPONTANEOUS CHRONIC RENAL FAILURE. S Ross[1], C Osborne[1], D Polzin[1], S Lowry[2], C Kirk[3], L Koehler[1]. [1]College of Veterinary Medicine, University of Minnesota, St. Paul, MN. [2]Hill's Science and Technology Center, Topeka, KS. [3] College of Veterinary Medicine, The University of Tennessee, Knoxville, TN.

A double-masked, controlled, randomized, clinical trial was designed to determine if a renal diet (modified in protein, phosphorous, sodium, and lipid composition) was superior to an adult maintenance diet in minimizing uremic episodes and mortality rate in cats with mild to moderate chronic renal failure.

Forty-five client owned cats were randomly assigned to a maintenance diet or a renal diet and evaluated tri-monthly for up to 24 months. Kaplan-Meier survival analyses were used to evaluate efficacy of the renal diet compared to the maintenance diet in minimizing uremia, renal-related mortality, and all causes of mortality.

Events	Renal diet (%)	Maintenance diet (%)	P value
Uremic crises	0/22 (0)	5/23 (22)	0.02
Renal cause mortality	0/22 (0)	4/23 (17)	0.03
All causes of mortality	3/22 (14)	9/23 (39)	0.06

Serum urea nitrogen concentrations were significantly lower and blood bicarbonate concentrations were significantly higher in the group fed the renal diet at baseline and during the 12- and 24-month intervals. Cats fed the maintenance diet had a significantly greater number of uremic episodes (22%) compared to cats fed the renal diet (0%). A significant reduction in renal-related mortality was observed in cats fed the renal diet.

The renal diet evaluated in this study was superior to an adult maintenance diet in minimizing uremic episodes and mortality rate in cats with mild to moderate spontaneous chronic renal failure.

ABSTRACT #122

CLINICAL BENEFIT OF CALCITRIOL IN CANINE CHRONIC KIDNEY DISEASE. D Polzin, S Ross, C Osborne, J Lulich, and L Swanson. University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

Parathyroid hormone (PTH) has been identified as a uremic toxin. It has been implicated in promoting progression of chronic kidney disease (CKD). Oral administration of low doses of calcitriol has been shown to reduce PTH in dogs with CKD. Uncontrolled clinical observations suggest that calcitriol therapy may prolong survival in dogs with CKD.

We performed a double-masked, randomized, controlled clinical trial to test the hypothesis that calcitriol reduces mortality in dogs with CKD. Thirty-seven client owned dogs with serum creatinine concentrations ranging from 2.0 to 6.3 mg/dl were enrolled between May 2000 and January 2003. All dogs were >1 year old and had stable creatinine values. They were randomly assigned to calcitriol (n=18) or placebo (n=19) and followed for 1 year. Initial mean serum creatinine values did not differ between groups (placebo = 4.00±0.74; calcitriol = 4.08±1.62). Calcitriol dosage, initially 2.5 ng/kg, was adjusted within the range of 0.75 to 5.0 ng/kg/day according to serial determinations of ionized calcium and PTH. Except for calcitriol, patient management was similar for both groups. We compared the effectiveness of calcitriol versus placebo on limiting mortality using Kaplan-Meier survival curves with Mantel-Cox analyses.

Calcitriol therapy was associated with a significant reduction in all-cause mortality (p=0.036). All cause mortality rate was 63% in the placebo group and 28% in the calcitriol group. Median survival time was 365 days for dogs receiving calcitriol and 250 days for dogs receiving placebo.

Calcitriol appears to be effective in prolonging survival in dogs with stages 3 and 4 CKD.

ABSTRACT #123

RELATIONSHIP BETWEEN SERUM IOHEXOL CLEARANCE AND RECIPROCAL OF SERUM CREATININE IN DOGS WITH NATURALLY OCCURING CHRONIC RENAL FAILURE. Sherry L. Sanderson*, Mark Tetrick[†], Scott A. Brown*, Larry G. Adams[‡], John M. Kruger[§], Shelly L. Vaden[‖], Lisa E. Moore[¶]. *The Univ. of Georgia-CVM, [†]Iams Company, [‡]Purdue Univ-SVM, [§]Michigan State Univ-CVM, [‖]North Carolina State Univ-CVM, [¶]Kansas State Univ-CVM.

Blood urea nitrogen and serum creatinine (SCr) concentrations are commonly used to evaluate renal function in clinical patients. However, it is well recognized that both tests are insensitive indices of glomerular filtration rate (GFR) and may be influenced by nonrenal factors. Urinary clearance of inulin and exogenous creatinine clearance are valid tests for estimating GFR, however, they are seldom used clinically because they are time-consuming and require carefully conducted urine collections. An alternative method used for evaluating the rate of decline in GFR entails plotting 1/SCr versus time, however, the relationship between 1/SCr and GFR is not a perfect linear relationship. Recently, serum iohexol clearance has been shown to be a reliable method of estimating GFR in dogs. The purpose of this study was to evaluate 1/SCr as a measure of GFR in dogs with naturally-occurring chronic renal failure (CRF).

Ninety-seven paired 1/SCr and iohexol clearances were collected from 41 dogs with various stages of naturally-occurring CRF. Serum Cr concentrations ranged from 1.5 to 9.0 mg/dl; mean = 3.6 mg/dl, and iohexol clearances ranged from 0.242 to 2.611 ml/min/kg; mean = 0.931 ml/min/kg (ref range = 2.89 to 8.07 ml/min/kg). All dogs were clinically hydrated at the time of evaluation. Results from both tests were compared by use of Pearson correlation coefficient. When all 97 paired samples were compared simultaneously, there was modest correlation ($r = 0.629$; $P \leq 0.05$) between the two methods of assessing renal function.

The paired samples were then divided into two groups based on SCr concentration, and Pearson correlation coefficient was repeated for both groups. Group 1 consisted of 66 samples where SCr < 4.0 mg/dl (SCr ranged from 1.5 to 3.9 mg/dl; mean = 2.77 mg/dl; and iohexol clearances ranged from 0.242 to 2.611 ml/min/kg; mean = 1.082 ml/min/kg). Group 2 consisted of 31 sample where SCr ≥4.0 mg/dl (SCr ranged from 4.0 to 9.0 mg/dl; mean = 5.39 mg/dl, and

iohexol clearances ranged from 0.248 to 2.350 ml/min/kg; mean = 0.609 ml/min/kg). Results for Group 1 showed modest correlation ($r = 0.619$; $P \leq 0.05$) between the two methods, however results from Group 2 showed very poor correlation ($r = 0.060$; $P \leq 0.05$) between the two methods.

Results from this study demonstrate that the rate of decline of 1/SCr more closely reflects the rate of decline in GFR in dogs in early stages of CRF. As CRF progresses, small decreases in GFR may be associated with relatively large increases in SCr. In dogs with advanced CRF, changes in 1/SCr do not accurately reflect changes in GFR, and other methods of accurately assessing changes in renal function should be done.

ABSTRACT #124

ANTICOAGULATION WITH UNFRACTIONATED HEPARIN DURING HEMODIALYSIS IN DOGS. ME Kerl[1], CE Langston[2], LA Cohn[1]. [1]Department of Veterinary Med and Surg, Univ of Missouri, Columbia, MO; [2]The Animal Medical Center (AMC) New York, NY.

Blood must pass through an extracorporeal circuit during hemodialysis treatments, thus requiring anticoagulation. Ideal anticoagulation prevents coagulation within the circuit while minimizing hemorrhagic complications. Protocols for human hemodialysis anticoagulation include use of unfractionated heparin (UH), low molecular weight heparin, or citrate. By convention, hemodialysis anticoagulation in dogs is performed with UH. The purpose of this retrospective study was to evaluate the proportion of time the activated clotting time (ACT) was within a predetermined target range in dogs anticoagulated for hemodialysis, and to identify the frequency of bleeding or clotting events during hemodialysis.

Study enrollment included all dogs that received hemodialysis at AMC from 1997 to 2003 with UH as the sole method of anticoagulation. In all cases, UH was used to prime the extracorporeal circuit and was administered via IV bolus to each dog with an ACT below 150 seconds(s) at 50U/kg. For the remainder of the dialysis treatment UH was administered via constant rate infusion. The actual rate was adjusted subjectively with the goal of maintaining measured ACT at 160-200s. ACT was measured ~15, 30, and 60 minutes after initial anticoagulation and q60 min for the duration of the treatment. Information collected from medical records included number of hemodialysis treatments, pre-and post-treatment parameters (ie, body weight, BUN, creatinine, PCV, total protein, and ACT), and ACT as determined at multiple time points during each dialysis treatment. Incidents of bleeding were noted, as was degree of coagulation in the extracorporeal circuit at the completion of dialysis. Two methods were used to determine when ACT was within, above, or below the target range of 160-200s for initial dialysis treatments, for individual dogs, or for all treatments. Correlations were calculated between the proportions of time inside and outside of target range with baseline parameters described above.

Fifty-six dogs each received from one to 37 dialysis treatments (median, 5) for a total of 459 treatments. ACT prior to anticoagulation ranged from 73s to >999s (mean 134.16 +/-88.08s). During dialysis, ACT ranged from 83s to >999 s. The 50th percentile for all ACT after UH administration was 191s, while the 75th percentile was 220s. Using linear interpolation for all treatments, dogs spent roughly 45% of dialysis treatment time in target range, exceeded target range 28% of treatment time and were below target range 17% of the time. There were no meaningful correlations between achieving ACT target and baseline parameters. Bleeding complications were noted in 26 of 459 treatments (10 dogs); mean ACT for those 26 treatments was 130.87s (+/- 32.06). Clots in the extracorporeal circuit were absent after 144 treatments, and graded as mild (n=103), moderate (n=80), or severe (n=108) in the remaining treatments. We conclude that bleeding complications were rare despite frequently exceeding the target range for ACT, while clotting within the extracorporeal circuit was relatively common despite achieving goal or higher ACT.

ABSTRACT #125

ANTICOAGULATION WITH UNFRACTIONATED HEPARIN DURING HEMODIALYSIS IN CATS. CE Langston[1], LA Cohn[2], ME Kerl[2]. [1]The Animal Medical Center (AMC), New York, NY; [2]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO.

Blood must pass through an extracorporeal circuit during hemodialysis treatments, thus requiring anticoagulation. Ideal anticoagulation prevents coagulation within the circuit while minimizing hemorrhagic complications. Protocols for human hemodialysis anticoagulation include use of unfractionated heparin (UH), low molecular weight heparin, or citrate. By convention, hemodialysis anticoagulation in cats is performed with UH. The purpose of this retrospective study was to evaluate the proportion of time the activated clotting time (ACT) was within a predetermined target range in cats anticoagulated for hemodialysis, and to identify the frequency of bleeding or clotting events during hemodialysis.

Study enrollment included all cats that received hemodialysis at AMC from 1997 to 2003 with UH as the sole method of anticoagulation. In all cases, UH was used to prime the extracorporeal circuit and was administered via IV bolus to each cat with an ACT below 150 seconds(s) at 50U/kg. For the remainder of the dialysis treatment UH was administered via constant rate infusion. The actual rate was adjusted subjectively with the goal of maintaining measured ACT at 160-200s. ACT was measured ~15, 30, and 60 minutes after initial anticoagulation and q60 min for the duration of the treatment. Information collected from records included number of hemodialysis treatments, pre-and post-treatment parameters (ie, body weight, BUN, creatinine, PCV, total protein, and ACT), and ACT as determined at multiple time points during each dialysis treatment. Incidents of bleeding were noted, as was degree of coagulation in the extracorporeal circuit at the completion of dialysis. Two methods were used to determine when ACT was within, above, or below the target range of 160-200s for initial dialysis treatments, for individual cats, or for all treatments. Correlations were calculated between the proportions of time inside and outside of target range with baseline parameters described above.

Thirty-four cats were included in the study, each receiving from one to 28 dialysis treatments (median 3) for a total of 167 dialysis treatment sessions. ACT prior to anticoagulation ranged from 94s to >999s (mean 181.31 +/-106.95). During dialysis, ACT ranged from 63s to >999 s. The 50th percentile for all ACT after UF administration was 234s, while the 75th percentile was 298s. Using linear interpolation for all treatments, cats spent roughly 23% of the dialysis treatment in the target range, exceeded the target range 74% of the dialysis treatment time, and were below target range 3% of the time. No meaningful correlations were detected between achieving the target goal for ACT and the baseline parameters. Bleeding complications were noted in 3 of 167 treatments. The mean ACT during those three treatments was 260s (+/- 258s) Coagulation in the extracorporeal circuit was absent during 74 sessions, and graded as mild (n=58), moderate (n=11), or severe (n=4) in the remaining sessions. We conclude that bleeding complications were rare despite frequently exceeding the target range for ACT. Clotting within the circuit was minimal.

ABSTRACT #126

ANALYSIS OF HYPERKALEMIA IN DOGS ON CHRONIC HEMODIALYSIS. V Pantaleo, T Francey, LD Cowgill. School of Veterinary Medicine, University of California, Davis.

Hyperkalemia (HK) is recognized with increased frequency in dogs undergoing chronic hemodialysis (HD) and poses a risk of death. In this retrospective study, records of all dogs with renal failure that received chronic HD between Jan. 1999 and Dec. 2004 were reviewed to identify the prevalence and factors associated with HK. Chronic HD was defined as HD therapy performed for longer than 2 weeks, and only data collected after this time were analyzed. Dogs and treatments (tx) were stratified as non-HK ($K \leq 5.3$ mmol/L), mild HK (5.4-7.0) and severe HK (>7.0), according to preHD K^+; the highest preHD K^+ for the whole period analyzed was used for stratification of individual dogs. Data are presented as median [interquartile range].

A total of 460 chronic HD tx were performed in 27 dogs (11 tx/dog [6-23]). Relevant associations between dogs or tx characteristics and potassium stratification are presented in the following table:

Dogs Stratification†	**Non-HK**	**Mild HK**	**Severe HK**	***P****
# dogs	7 (26%)	6 (22%)	14 (52%)	-
Time on HD (d)	19 [18-25]	40 [32-83]	69 [41-122]	0.03
# tx/dog	2 [2-5]	11 [7-11]	23 [12-36]	<0.01
% HK tx mild (severe)	0	62%	35% (13%)	-
Tx Stratification‡§	**Non-HK**	**Mild HK**	**Severe HK**	***P****
# tx	233 (51%)	190 (41%)	37 (8%)	-
K^+ (mEq/L)	4.6 [4.1-5.0]	6.0 [5.7-6.3]	7.8 [7.3-8.3]	n/a
Na^+ (mEq/L)	141 [136-145]	140 [137-145]	139 [133-142]	0.02
TCO_2 (mEq/L)	17 [15-20]	16 [14-19]	15 [14-17]	<0.01
Total Ca^{++} (mg/dl)	13.3 [12.3-14.4]	13.3 [12.7-14.6]	12.9 [11.7-14.3]	0.06
BUN (mg/dl)	57 [44-72]	58 [46-75]	72 [61-84]	<0.01
Creatinine (mg/dl)	7.7 [6.1-9.6]	8.2 [6.8-10.3]	8.6 [7.4-9.7]	0.01
Ultrafiltration# (ml/kg)	27 [0-51]	25 [3-44]	42 [26-83]	0.03
Blood processed# (L/kg)	2.8 [2.1-3.7]	2.7 [2.2-3.7]	3.3 [2.4-4.2]	0.049

*, ANOVA for difference between groups; †, based on highest preHD K^+; ‡, based on preHD K^+; §, chemistries are preHD values; #, data from previous HD tx.

Mild and severe HK are seen commonly in dogs undergoing chronic HD. HK is associated with differences in sodium, bicarbonate, azotemia, ultrafiltration, volume of bood dialyzed, and duration of dialytic support. The cause is not determined from this study, but could involve dialysis induced events, alterations in potassium load, or altered potassium regulation associated with chronic uremia.

ABSTRACT #127

PREVALENCE OF *MYCOPLASMA HAEMOFELIS*, '*CANDIDATUS* MYCOPLASMA HAEMOMINUTUM', *BARTONELLA* SPP., *EHRLICHIA* SPP., AND *ANAPLASMA PHAGOCYTOPHILUM* DNA IN THE BLOOD OF CATS WITH ANEMIA. AM Ishak and MR Lappin, Colorado State University, Fort Collins, CO.

There are multiple infectious causes of anemia in cats and the anemia can be regenerative or non-regenerative depending on the agent and the timing of laboratory testing. FeLV, FIV, feline infectious peritonitis, *Mycoplasma haemofelis* (Mhf), and '*Candidatus* M. haemominutum' (Mhm) historically are common causes of anemia in cats. Recently, an *Ehrlichia canis*-like organism and *A. phagocytophilum* infections of cats have been documented but only small numbers of cases have been reported. Anemia has been detected in humans infected with *Bartonella henselae* and dogs infected with *Bartonella vinsonii*, but to our knowledge the association of *Bartonella* spp. infection with anemia in cats have not been assessed. While Mhf and Mhm are thought to be the most common causes of feline infectious anemia, many cats submitted for testing are negative. The purpose of this study is to report the prevalence of select infectious agents in cats submitted for Mhf and Mhm PCR assay.

The records of the Infectious Disease Laboratory were reviewed from January 2001 through November 2004 for feline cases from which blood samples had been submitted for Mhf and Mhm PCR assay. The medical records of cats with a hematocrit of < 30% were further reviewed to determine the nature of the anemia (regenerative versus non-regenerative) and to assess for historical, physical, or laboratory evidence of known precipitating causes of anemia in cats including FeLV and FIV infections. Samples from anemic cats for which an obvious cause of anemia could not be detected were thawed and assayed in previously validated PCR assays that amplify the DNA of Mhf, Mhm, *Ehrlichia spp., A. phagocytophilum*, and *Bartonella spp*.. To date, a total of 58 cases have been analyzed. Of the cats, 24 had a non-regenerative anemia and 34 had a regenerative anemia. DNA of *Ehrlichia* spp. or *A. phagocytophilum* were not amplified. Of the cats with regenerative anemia, DNA of Mhf alone (4 cats), Mhm alone (four cats), or both Mhf and Mhm (one cat) was amplified. One cat positive for DNA of Mhm was also positive for DNA of *B. henselae*. DNA of *B. henselae* or *B. clarridgeaie* were each amplified from a Mhf and Mhm negative cat. Of the cats with non-regenerative anemia, DNA of Mhf alone (one cat), Mhm alone (one cat), or both Mhf and Mhm (one cat) was amplified. *Bartonella* spp. DNA was not amplified from any cat with non-regenerative anemia.

In this group of cats with regenerative anemia and non-regenerative anemia of undetermined origin, DNA of infectious agents potentially associated with anemia were amplified from 11 of 34 (32.4%) and three of 24 (12.5%), respectively. While the *Bartonella* spp. prevalence rates were lower than historical controls, further studies will be required to determine whether *Bartonella* spp. infections of cats are associated with anemia.

ABSTRACT #128

SURVIVAL OF *MYCOPLASMA HAEMOFELIS* AND '*CANDIDATUS* MYCOPLASMA HAEMOMINUTUM' IN BLOOD OF CATS USED FOR TRANSFUSIONS. AT Gary, HL Richmond, TB Hackett, MR Lappin. Colorado State University, Ft. Collins, CO.

Blood transfusions are commonly administered to cats; associated risks include the transmission of various infectious diseases including *M. haemofelis* (Mhf) and '*Candidatus* M. haemominutum' (Mhm). In previous experimental studies, both Mhf and Mhm were reliably transmitted by IV inoculation of as little as 1ml of heparinized blood. In a recent study, DNA of Mhf or Mhm were amplified from blood of 14 of 146 (9.6%) active feline blood donors. Blood transfusions in citrate-phosphate-dextrose-adenine (CPDA-1) solution are commonly administered immediately or stored for up to one month at 4ºC prior to administration. It is unknown whether Mhf or Mhm survive in this solution or temperature. The purpose of this study is to determine if *Mycoplasma spp*. remain viable after storage in CPDA-1 for varying periods of time.

Because both organisms are directly associated with feline red blood cells and cannot be cultured, cats must be inoculated to document organism viability. A chronic carrier of Mhf, a chronic carrier of Mhm, and six SPF cats were used in this study. A CBC and a PCR assay capable of amplifying DNA of both organisms were performed twice in all eight cats prior to initiating the study. Blood (60ml) was then collected from each of the carrier cats, placed into a CPDA-1 solution containing bag (Teruflex®, Terumo Co., Tokyo, Japan), and the bags were stored at 4°C. At one hour, one week, and one month of storage, 2.2ml of blood was aseptically collected from

each of the two bags. At each time, the *Mycoplasma* spp. PCR assay was performed on 200 µl of blood and the remaining 2ml were inoculated IV into a *Mycoplasma*-negative cat. After inoculation, 2ml of blood were collected from each cat for CBC and PCR assay weekly for four weeks.

Both chronic carrier cats were positive for Mhf or Mhm DNA throughout the study. All six SPF cats were negative for DNA of Mhf and Mhm before inoculation. DNA of Mhf or Mhm, respectively, was amplified from the CPDA-1 bags after one hour, one week, and one month of storage. The SPF cat administered Mhf containing blood after one hour of storage was PCR positive weeks 1-4, the SPF cat administered Mhm containing blood after one hour of storage was PCR positive weeks 1-3, and the SPF cat administered Mhm containing blood after one week of storage was PCR positive on week 1 after inoculation. *Mycoplasma* spp. DNA was never amplified from the SPF cat administered Mhf containing blood after one week of storage or the 2 SPF cats inoculated with blood stored for one month.

The results provide evidence that Mhf and Mhm can be transmitted to *Mycoplasma* spp. negative cats by administration of infected feline blood that has been stored in CPDA-1 solution for variable time periods. These findings support the recommendation that cats used as blood donors be screened for Mhf and Mhm infections by PCR assay prior to use.

ABSTRACT #129

EFFECT OF CHRONIC FIV INFECTION, AND EFFICACY OF MARBOFLOXACIN TREATMENT, ON '*CANDIDATUS* MYCOPLASMA HAEMOMINUTUM' INFECTION. S Tasker, SMA Caney, MJ Day, RS Dean, CR Helps, TG Knowles, PJP Lait, MDG Pinches, TJ Gruffydd-Jones. School of Clinical Veterinary Science, University of Bristol, Bristol, N. Somerset, UK.

The purpose of this study was to investigate the effect of chronic FIV infection, and efficacy of marbofloxacin treatment, on '*Candidatus* M. haemominutum' (CMhm) infection.

Twelve adult cats were used. Six were chronically infected with FIV-Glasgow 8 (Group A) and the other six cats were FIV-free (Group B). Groups A and B were housed separately for the duration of the study. All cats were infected with CMhm on Day 0 of the study by intravenous inoculation of blood collected from a carrier cat. Over the course of the study from Day 0 until Day 105 post-infection (pi), blood samples were collected three times weekly for PCV and CMhm quantitative real-time polymerase chain reaction (PCR), and once weekly for full haematological examination. FIV provirus quantitative real-time PCR was performed once weekly for Group A cats, and on Days –7 and 105 pi for Group B cats. FIV antibody ELISA testing was performed on all cats on Days –7 and 105 pi. On Day 49 pi three of the six cats in each of Groups A and B were randomly selected to receive marbofloxacin treatment (2 mg/kg PO SID) until Day 76 pi, with the remaining six cats acting as untreated controls. The CMhm copy numbers and haematological data were compared between Groups A and B, and between marbofloxacin-treated and untreated control cats, using a Mann-Whitney U Test. Significance was taken as a P value of < 0.05.

CMhm infection was associated with a drop in PCV between Days 0 and 23 pi. Maximum CMhm copy number was reached around Day 30 pi. No overt cycling or marked variation in copy number was demonstrated, as has been observed with *Mycoplasma haemofelis* infection. All cats in Group A were FIV antibody positive whilst those in Group B were negative. No obvious correlation was found between FIV provirus copy number and CMhm copy number or haematological variables. No significant effect of chronic FIV infection on CMhm copy number kinetics or haematological changes due to CMhm infection, other than the basophil count (P=0.04), was found. Although marbofloxacin treatment was associated with a significant decrease in CMhm copy number (P=0.002), the decrease in copy number plateaued during treatment with no negative PCR results obtained. Additionally, after termination of the marbofloxacin treatment, the CMhm copy numbers of the treated cats increased to reach similar levels to those of the untreated cats within seven to 10 days. Marbofloxacin treatment was associated with a significant effect on lymphocyte count only (P=0.04).

Chronic FIV infection had no significant effect on CMhm infection kinetics or pathogenicity. This study provides a valuable insight into the kinetics of CMhm infection. Although marbofloxacin treatment was associated with a significant reduction in CMhm copy number, it did not induce clearance of CMhm infection.

ABSTRACT #130

EFFECT OF CHRONIC FIV INFECTION, AND EFFICACY OF MARBOFLOXACIN TREATMENT, ON *MYCOPLASMA HAEMOFELIS* INFECTION. S Tasker, SMA Caney, MJ Day, RS Dean, CR Helps, TG Knowles, PJP Lait, MDG Pinches, TJ Gruffydd-Jones. School of Clinical Veterinary Science, University of Bristol, Bristol, N. Somerset, UK.

The purpose of this study was to investigate the effect of chronic FIV infection, and efficacy of marbofloxacin treatment, on *Mycoplasma haemofelis* infection.

Twelve adult cats were used. Six were chronically infected with FIV-Glasgow 8 (Group C) and the other six cats were FIV-free (Group D). Groups C and D were housed separately for the duration of the study. All cats were infected with *M. haemofelis* on Day 0 of the study by intravenous inoculation of blood collected from a carrier cat. Over the course of the study from Day 0 until Day 86 post-infection (pi), blood samples were collected three times weekly for PCV and *M. haemofelis* quantitative real-time polymerase chain reaction (PCR), and once weekly for full haematological examination. FIV provirus quantitative real-time PCR was performed once weekly for Group C cats, and on Days –7 and 77 pi for Group D cats. FIV antibody ELISA testing was performed on all cats on Days –7 and 77 pi. On Day 16 pi three of the six cats in each of Groups C and D were randomly selected to receive marbofloxacin treatment (2 mg/kg PO SID) until Day 43 pi, with the remaining six cats acting as controls with no antibiotic treatment.

The *M. haemofelis* copy numbers and haematological data were compared between Groups C and D, and between marbofloxacin-treated and control cats, using a Mann-Whitney U Test. Significance was taken as a P value of < 0.05.

M. haemofelis infection was associated with the development of macrocytic hypochromic anaemia. Marked variation in *M. haemofelis* copy number was seen over time (> 5 log fold difference within 48 hours in some cats). Cycling of *M. haemofelis* copy number was also evident in some cats. All Group C cats were FIV antibody positive whilst those in Group D were negative. No obvious correlation was found between FIV provirus copy number and *M. haemofelis* copy number or haematological variables. No significant effect of chronic FIV infection on *M. haemofelis* copy number kinetics or haematological changes due to *M. haemofelis* infection, other than mean cell haemoglobin concentration (MCHC) (P=0.03), was found. Marbofloxacin treatment was associated with a significant decrease in *M. haemofelis* copy number (P=0.002), and negative PCR results were obtained at various time points in treated cats, although clearance of infection was not thought likely. Marbofloxacin treatment was also associated with a significant effect on MCHC (P=0.04), platelet count (P=0.03) and punctate reticulocyte count (P=0.03).

Chronic FIV infection had no significant effect on *M. haemofelis* infection kinetics or pathogenicity. Further studies are required to elucidate the reasons for the interesting variation in *M. haemofelis* copy number kinetics demonstrated in this study. Although marbofloxacin was associated with a significant reduction in *M. haemofelis* copy number, further studies are needed to determine an

antibiotic treatment regime appropriate for clearance of *M. haemofelis* infection.

ABSTRACT #131

EFFICACY OF RONIDAZOLE *IN VITRO* AND *IN VIVO* FOR TREATMENT OF FELINE *TRITRICHOMONAS FOETUS* INFECTION. Jody Gookin, Christina Copple, Mark Papich, Matthew Poore, and Michael Levy. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

The protozoan *Tritrichomonas foetus* (TF) is a prevalent cause of chronic large bowel diarrhea in cats, for which no effective treatment has been reported. Two nitroimidazole antimicrobials, tinidazole (TDZ) and ronidazole (RDZ) were tested for activity against feline TF *in vitro* at concentrations ranging from 0.01 to 10 µg/mL. TDZ is registered for use in people and RDZ is available outside the U.S. Both TDZ and RDZ killed TF *in vitro*. Ronidazole was selected for further *in vitro* study. Ten 10-week-old neutered female, TF-negative cats were individually housed, acclimated for three weeks and orogastrically infected with 3 x 10^6 TF organisms. Physical exam findings, fecal consistency (formed, semi-formed, cow-pie, or liquid) and fecal examination results (direct smear, culture, & single-tube nested PCR) were recorded weekly by a blinded observer for 35 weeks.

All cats became TF positive by culture and developed loose feces by two weeks post-infection. Cats were randomized to two groups (n=5 each) and treated with placebo (dextrose) or RDZ (10 mg/kg orally, twice daily for two weeks). If cats in the treatment group had a relapsing infection or if they received a placebo, they were re-treated with RDZ at a higher dose of 30 mg/kg (n=3) or 50 mg/kg (n=7) orally twice daily for two weeks. Treatment with RDZ at 10mg/kg caused initial improvement, but in 5/5 cats there was a relapse infection at 2, 3, 3, 17 & 20-wks after treatment was completed. The TF isolated from these cats remained susceptible to RDZ. At 30mg/kg or 50mg/kg twice daily 9/10 cats have been cured after monitoring for 13 weeks and 4-6 weeks post-treatment, respectively. Spontaneous remission, adverse drug events or clinicopathological abnormalities were not noted. We concluded that oral administration of RDZ at 30-50mg/kg twice daily for two weeks is capable of resolving diarrhea and eradicating infection (on the basis of PCR) in cats infected with TF.

ABSTRACT #132

THE ASSOCIATION OF *BARTONELLA HENSELAE* ANTIBODIES AND UVEITIS IN CATS. JP Fontenelle, AE Hill, CC Powell, MR Lappin. From the Department of Clinical Sciences, Colorado State University, Ft.Collins, CO.

We first reported *B. henselae* associated chronic uveitis in a cat with *B. henselae* antibodies in serum, local ocular production of *B. henselae* antibodies, and response to administration of doxycycline. In a follow-up study, we amplified *B. henselae* DNA from aqueous humor and demonstrated ocular production of *B. henselae* IgM and IgG antibodies in some cats with uveitis, but not healthy cats. In one other clinical study, several additional cats with uveitis and *B. henselae* serum antibodies were reported. However, numbers of cats with proven *B. henselae* associated uveitis are small to date because of difficulties associated with making a definitive diagnosis. The objective of this study was to determine the prevalence of *B. henselae* antibodies in cats with and without uveitis.

In a separate study performed between January 1, 2003 and January 1, 2004, veterinary ophthalmologists were asked to submit samples from cats with endogenous uveitis for infectious disease testing. Cases were classified as idiopathic uveitis (n = 75) if serum was negative for *T. gondii* antibodies, FIV antibodies, and FeLV antigen and aqueous humor was negative for *Toxoplasma gondii* DNA and FHV-1 DNA. Cats that were positive in one or more of these tests were classified as non-idiopathic (n = 34). Two groups of control cats were used in the analysis. Control group 1 consisted of serum samples from 109 clinically ill cats that were sent for infectious disease testing during the same time period; samples were excluded if ocular abnormalities or abnormalities consistent with feline bartonellosis (fever, lympadenopathy, stomatitis, or seizures) were mentioned. Control group 2 consisted of serum from 64 healthy cats. Age was recorded for each cat and the cat categorized as high or low risk for flea exposure by state based on previously published work. IgG antibodies against *B. henselae* were measured in all sera by a previously validated ELISA. Samples were categorized as being positive for *B. henselae* using cutoff points of ≥1:64 and ≥1:128. The association between *Bartonella* status and uveitis was analyzed using logistic regression in two separate analyses accounting for age and risk of flea exposure in the model. While serological evidence of exposure to *B. henselae* was common in cats with uveitis (53.2% at the 1:64 cutoff), results were not significantly different between any of the groups regardless of which serological cutoff (1:64 or 1:128) was utilized.

These results indicate that as with other causative agents of uveitis (e.g. *T. gondii*), the presence of serum antibodies to *B. henselae* may not correlate with the precise cause of the intraocular inflammation in individual cats. Further work is needed to determine optimal diagnostic tests for documentation of bartonellosis in cats.

ABSTRACT #133

PREVALENCE OF FeLV AND FIV IN NORTH AMERICA. Julie K. Levy, P. Cynda Crawford, College of Veterinary Medicine, University of Florida, Gainesville, FL; Jessica L. Brien, IDEXX Laboratories, Westbrook, ME.

FeLV and FIV are among the most common infectious diseases of cats. Over the past 20 years, prevalence of FeLV has decreased, presumably as a result of widespread test and removal programs and immunization against FeLV. Testing for FIV is less common than for FeLV, and the recently introduced FIV vaccine is not widely used. Whether prevalence of FIV is changing is unknown. Because testing is voluntary and results are not collected into a central database, determining the true prevalence of FeLV and FIV is difficult. A large national study published more than a decade ago reported a prevalence of 13% for FeLV and 7% for FIV in 27,976 diseased and "high-risk" pet cats. In contrast, the prevalence of infection in 1,876 unowned feral cats was reported to be only 4% for each virus. Prevalence was lowest in healthy pet cats in which 1.3% of 1,763 cats recently studied were positive for FeLV and 0.9% of 1,757 cats were positive for FIV. The purpose of this study was to update prevalence data for FeLV and FIV infection in pet cats in North America, including cats of all ages and risk factors.

Veterinary clinics and animal shelters in the United States and Canada were recruited to test kittens and cats for FeLV and FIV using a point-of-care ELISA test (IDEXX SNAP Combo FeLV antigen/FIV antibody) during August to November 2004. Confirmatory tests were not performed as part of the study. Prevalence was calculated as the percent of positive tests in the study population for each virus. The Chi Square test was used to compare prevalence rates between regions. $P < 0.05$ was considered to be statistically significant.

A total of 18,038 cats were tested, of which 446 (2.5%) were positive for FIV and 409 (2.3%) were positive for FeLV. Of these, 58 (0.3%) were coinfected with both viruses. Prevalence was significantly higher in cats tested at veterinary clinics (3.1% FIV, 2.9% FeLV) than at shelters (1.7% FIV, 1.5% FeLV), in mature cats (4.1% FIV, 4.3% FeLV) than in juveniles (1.0% FIV, 1.4% FeLV), in males (3.6% FIV, 2.7% FeLV) than in females (1.4% FIV, 1.9% FeLV), and in diseased cats (6.1% FIV, 6.3% FeLV) than in healthy cats (1.8% FIV, 1.6% FeLV). Owned cats with access to outdoors had higher infection rates (4.3% FIV, 3.6% FeLV) than cats kept

exclusively indoors (0.9% FIV, 1.5% FeLV). For shelter cats, the source (stray, relinquished pet, or feral) had no effect on FeLV infection rate, but feral cats had a higher rate of FIV infection (3.9%) than cats found as strays or relinquished by their owners.

Although the prevalence reported here is lower than in previous reports, it is not possible to compare them to assess changes in infection rates over time because of differences in the study populations. The previous studies each selected a single population for testing: high-risk cats, feral cats, or healthy pets, whereas the present study included cats of all ages, lifestyles, and health conditions, tested contemporaneously under similar conditions and season. Extrapolation of prevalence rates beyond the study population should be made with caution, since cats were not selected randomly from the overall population. Despite increased surveillance for FeLV and FIV and the availability of antiretroviral vaccines, infections with these viruses are common in North America.

ABSTRACT #134

FAILURE OF IMIDOCARB DIPROPIONATE TO CLEAR EXPERIMENTALLY INDUCED *EHRLICHIA CANIS* INFECTION IN DOGS. Susan M. Eddlestone, Mark T. Neer, Stephen D. Gaunt, Richard Corstvet, Amy Gill, Giselle Hosgood, Baton Rouge, LA., Barbara C. Hegarty, and Edward B. Breitschwerdt, North Carolina State University, Raleigh, NC.

The efficacy of imidocarb dipropionate, 6.6 mg/kg, IM, twice, two weeks apart, was evaluated for the therapeutic clearance of *E.canis* from blood and tissues in experimentally induced *Ehrlichia canis* infection in dogs. Fifteen healthy, 6-9 month old, Walker hound-mix bred, dogs weighing 16-29 kgs were obtained from the LSU research colony. Pre-inoculation, antibodies were not detected to *E.canis, Babesia canis* and *Bartonella vinsonii (berkhoffii)* antigens by IFA testing in any dog. Two *E.canis* PCR assays (one performed by the LSU Diagnostic Laboratory and one by the NCSU Vector Borne Disease Diagnostic Laboratory) were performed pre-inoculation in all dogs with no amplification of *E.canis* DNA. Complete blood counts, platelet counts and serum chemistry panels were within normal reference range for all dogs.

All dogs were injected SC with an inoculum of *E.canis* infected canine histiocytic cells. Three weeks post-inoculation (PI), 10 of the dogs were treated with imidocarb and five of the dogs received no treatment. Evaluation of blood *E.canis* PCR and serum *E.canis* IFA titers were performed weekly prior to and for 13 weeks PI. Platelet counts were performed weekly prior to and for 15 weeks PI. In addition, bone marrow and splenic aspirates were evaluated by PCR prior to and 4 weeks PI. Physical examinations were performed weekly and body weight was measured monthly.

There were no clinical signs of illness found in any dog during the study. There were no significant differences in body weight between treated and untreated dogs during pre and PI periods. Platelet counts decreased by three weeks PI for all dogs. In the imidocarb-treated group, platelet counts increased in 8 of 10 dogs during weeks 5 to 7 PI, with two dogs having platelet counts within the reference interval by 8 weeks PI. In the untreated control group, platelet counts increased in four of five dogs during weeks 5 to 13 PI with 3 of 5 dogs having platelet counts within the reference interval by 15 weeks PI. All dogs were PCR negative for blood, bone marrow and splenic aspirates pre-inoculation. All dogs were blood PCR positive (LSU and/or NCSU) between weeks 2-5, PI. Four weeks PI, 12 of 15 dogs were PCR positive on splenic and bone marrow aspirates (LSU). All dogs remained blood PCR positive (NCSU) 13 weeks PI. All dogs produced *E.canis* antibodies ($\geq$ 1:160) by 1 week PI. Titers fluctuated above and below 1:160 during weeks 2 to 4 PI and then remained above 1:1280 after 4 weeks PI in all dogs.

There were no statistical differences in physical exam findings, body weight, platelet counts, PCR results or serum *E.canis* IFA titers between the treated and control groups. In conclusion, imidocarb dipropionate was not effective for the therapeutic clearance of *E.canis* from the blood and tissues of experimentally infected dogs.

ABSTRACT #135

UNIVERSAL BACTERIAL PCR FOR DIAGNOSIS OF CANINE BACTERIAL MENINGOENCEPHALITIS. Jeannette S. Messer, Carley J. Abramson, Curtis A. Barden, and Carmen M.H. Colitz. The Ohio State University College of Veterinary Medicine, Columbus OH.

Canine bacterial meningoencephalitis (BME) is associated with greater than 80% mortality and permanent neurologic deficits in survivors. Bacterial culture of cerebrospinal fluid (CSF) is currently the antemortem diagnostic gold standard, but cultures are positive in less than 20% of histopathologically-confirmed BME. PCR assays using oligonucleotide primers complementary to conserved regions of the 16S ribosomal subunit gene have been used extensively in human medicine to identify bacterial DNA in patients with negative CSF cultures. Universal bacterial (UB) primers replicate both conserved and unique DNA sequences, allowing genetic identification of the bacteria in the sample. The purpose of this study was to develop a universal bacterial PCR assay for canine CSF and to evaluate the sensitivity and specificity of the assay.

Cerebrospinal fluid was obtained from dogs presented to The Ohio State University Veterinary Teaching Hospital with clinical signs and laboratory data consistent with inflammatory central nervous system disease. The CSF was submitted for bacterial culture and analysis consisting of protein concentration, white blood cell (WBC) count, red blood cell count, WBC differential counts and cytology. The remainder of the CSF was used to perform universal bacterial PCR. DNA was extracted using routine phenol-chloroform and Proteinase K procedures. Four PCR reactions were prepared: (1) patient CSF and primers for the glyceraldehyde-3-phosphate-dehydrogenase gene, (2) patient CSF and UB primers, (3) all reagents and primers with no target DNA and (4) known bacteria (*E. coli*, *S. typhimurium, or Enterococcus*) and UB primers. Reactions were amplified in a thermal cycler and reaction products were visualized using a 1% agarose gel stained with ethidium bromide. Amplicons of the appropriate size were purified and submitted to The Ohio State University Plant-Microbe Genomics Facility for direct genetic sequencing. Sequences were then compared to sequences stored in Genbank for bacterial identification.

CSF from 17 patients was analyzed. One bacterial culture and four PCR reactions were positive for bacteria. There were no cases in which bacterial culture and PCR results agreed. There were no significant differences in the number of peripheral WBC, peripheral neutrophils, CSF WBC or CSF neutrophils between dogs positive by bacterial culture or PCR vs. those that were negative.

The universal bacterial PCR assay described in this study appears promising for diagnosis of BME when conventional diagnostic techniques are not sufficiently sensitive. A larger sample size is necessary to evaluate the sensitivity and specificity of the UB PCR assay due to the small number of positive CSF cultures.

ABSTRACT #136

RISK FACTORS FOR THE DEVELOPMENT OF HOSPITAL-ACQUIRED INFECTION IN CRITICALLY-ILL DOGS. SP Shaw, AL Paul, EA Rozanski. Tufts University School of Veterinary Medicine, North Grafton, MA.

Hospital-acquired infections (HAI) represent a source of morbidity and mortality in critically ill dogs. There are numerous reports of epidemic outbreaks of HAI in small animals. Epidemic outbreaks of HAI represent only 5% of all HAI in people. The remainder of the HAI represent endemic infections. Endemic infections are those infections that occur regularly at a low or moderate frequency. The

incidence of endemic HAI and the risk factors for their development in small animals is largely unstudied.

The goal of this study was to prospectively evaluate the incidence of endemic HAI and risk factors for their development in dogs admitted to the Intensive Care Unit at the Tufts University School of Veterinary Medicine. All dogs admitted to the ICU for more than 24 hours from February 1, 2003 to April 30, 2003 were prospectively included in the study. Information collected included number and types of intravenous catheters placed, surgical procedures performed, and the development of signs consistent with the occurrence of a hospital-acquired infection. A hospital-acquired infection was defined as a localized or systemic condition that 1) results from adverse reaction to the presence of an infectious agent(s) or its toxins and 2) was not present or incubating at the time of admission to the hospital.

Data from 307 sequentially admitted dogs representing 1512 hospital days was collected. The incidence of HAI in the study group was 10% (n=30). Nine of 117 (8%) dogs undergoing surgical intervention developed a surgical site infection. Overall, there were eight catheter-related infections/1000 hospital days, 4.6 hospital-acquired pneumonias/1000 hospital days, and 14 urinary tract infections/1000 hospital days. Three of the 307 dogs (1%) admitted to the ICU died as a result of a HAI.

In a multivariate analysis several independent risk factors for the development of HAI were identified. Risk factors included surgical intervention (odds ratio [OR] = 5.04, 2.05-12.4, $p<0.01$), administration of total parenteral nutrition (OR = 21.98, 4.91-98.38, $p<0.01$), administration of metoclopramide (OR = 6.7, 2.28-19.73, $p=0.01$), and the placement of an esophagostomy tube (OR = 1.182, 1.003-1.392, $p<0.01$).

The results highlight the importance of several risk factors in the development of HAI. Further study into strategies to reduce the impact of these risk factors in critically ill dogs is warranted.

ABSTRACT #137

DETECTION OF *CRYPTOSPORIDIUM* SPP. IN FECES OF CATS AND DOGS IN THE UNITED STATES BY PCR ASSAY AND IFA. AV Scorza, MR Lappin. From the Department of Clinical Sciences, Colorado State University, Ft.Collins, Colorado.

In experimentally infected cats, we previously showed a polymerase chain reaction assay (PCR) to be more sensitive than a commercially available, monoclonal antibody-based immunofluorescence assay (IFA; Meridian Diagnostics, Cincinnati, Ohio) for the detection of *Cryptosporidium* spp. infection. The objective of this study was to compare results of the PCR assay and the IFA using feces from client-owned cats and dogs presented for evaluation of diarrhea.

The samples tested in this study are part of a ongoing study of feline and canine cryptosporidiosis in the United States and were collected between October 1999 and November 2004. Referring veterinarians were asked to send a representative fecal sample from cats and dogs with diarrhea to Colorado State University packaged with a cold pack by overnight express. Samples were refrigerated until processed for analysis in the *Cryptosporidium* PCR assay as previously reported and the IFA following manufacturer's recommendations. In this PCR assay, the primers used (awaF-995=5'-TAΓAΓATTΓTAΓTTTΓTTXXT-3' and awaR-1206=5' XTTXXAXXAAXTAAΓAAXΓΓXX-3') amplify *C. parvum* and most of the *C. felis* and *C. canis* strains reported in Genbank.

A total of 292 samples were tested for *Cryptosporidium* spp. by PCR assay and IFA. The data outside and within parentheses are the number of positive samples or the percentage positive, respectively.

All IFA positive samples were concurrently positive by PCR assay. When compared to the results of the PCR assay, the sensitivity and specificity of the IFA were 11.3% and 100%, respectively.

Total Samples (n = 292)		Canine Samples (n = 112)		Feline Samples (n = 180)	
PCR	IFA	PCR	IFA	PCR	IFA
71 (24.3%)	8 (2.7%)	18 (15.1%)	2 (1.8%)	53 (29.4%)	6 (3.3%)

These results document that *Cryptosporidium* spp. infection is common in client-owned cats and dogs with diarrhea in the United States and that the PCR assay utilized here is more sensitive than a commercially available IFA.

ABSTRACT #138

PREVALENCE OF *COXIELLA BURNETII* DNA IN VAGINAL AND UTERINE SAMPLES FROM CATS OF NORTH-CENTRAL COLORADO. K Cairns, M Brewer, MR Lappin Department of Clinical Sciences, Colorado State University, Ft. Collins, CO

Q fever is a world-wide disease of animals including humans that is caused by the intracellular rickettsial organism *Coxiella burnetii.* In humans, respiratory disease is the most common clinical manifestation and infection can be acquired by inhalation of contaminated aerosolized secretions from parturient cats. Antibodies against *C. burnetii* have been detected in serum of some cats in North America but do not necessarily prove current infection. In 1998, the organism was cultured from vaginal samples of nine of 29 cats in Japan. Because the organism is difficult to culture, amplification of *C. burnetii* DNA by polymerase chain reaction assay (PCR) has also been used to document infection. The purpose of this study was to assess the prevalence of *C. burnetii* DNA in vaginal and uterine samples from cats in north-central Colorado using a PCR assay.

The study group was comprised of shelter (n = 50) and client-owned (n = 47), intact female cats presented to the Veterinary Teaching Hospital at Colorado State University between June 2002 and October 2003 for routine ovariohysterectomy. Only one of the cats (a healthy pregnant female in the shelter group) had evidence of vaginal discharge. While anesthetized for surgery, vaginal samples were obtained by Dacron swab, immediately processed for DNA amplification by use of a commercially available kit (QIAGEN QIAamp DNA purification kit, Valencia, CA), and frozen at -70ºC. After ovariohysterectomy, the uterus was collected and frozen at -70ºC. When ready for PCR assay, the uterus was thawed, a 3 by 3 mm piece was cut from the tissues and DNA isolated by use of a commercially available kit (QIAGEN QIAamp DNA purification kit, Valencia, CA). *Coxiella burnetii* DNA (positive control), negative control, DNA from vaginal sample digests, and DNA from uterine sample digests were amplified in an adaptation of a previously reported PCR assay utilizing the following primers targeting the repetitive transposon-like region: 5' primer TAT GTA TCC ACC GTA GCC AGT C and 3' primer CCC AAC AAC ACC TCC TTA TTC. This assay results in a 687 base pair product specific for *C. burnetii*.

DNA was shown to be present in all vaginal sample digests and was assumed to be present in the uterine sample digests because of the large amount of tissue used. *Coxiella burnetii* DNA was not amplified from the vaginal sample of any cat or the uterine biopsy of shelter cats. However, *C. burnetii* DNA was amplified from three of 47 (6.4%) uterine biopsies from client-owned cats.

The results of this study suggest that clinically normal cats in North-Central Colorado can harbor *Coxiella burnetii*. The failure to detect the organism in vaginal swabs could be related to the sensitivity of the PCR assay utilized or the organism was not present at the time of sampling. Care should be taken when attending to parturient cats and direct contact with parturient secretions and uterine tissues should be avoided. Additional studies are indicated to further characterize the role cats play in the transmission of Q fever.

ABSTRACT #139

PREVALENCE OF ZOONOTIC PATHOGENS IN DOGS VISITING HUMAN HOSPITAL PATIENTS IN ONTARIO. S. Lefebvre[1], J. S. Weese[2], D. Waltner-Toews[1], A. Peregrine[3], R. Reid-Smith[1]. Depts. of Population Medicine[1], Clinical Studies[2] and Pathobiology[3] , University of Guelph, Canada.

Visitation of hospitalized humans by dogs and other companion animals is becoming commonplace. While the therapeutic value of such practices has been investigated, the potential health hazards, both to the patients and the dogs, has not. This information is especially important in light of increasing concerns about nosocomial infections in healthcare facilities.

This cross-sectional study measured the prevalence of potential zoonotic pathogens in a group of 102 healthy dogs actively involved in visitation programs in Ontario. A standardized questionnaire was administered to each of the dogs' owners to obtain dog and program information. Fecal samples, aural, nasal, oral, pharyngeal and rectal swabs, as well as hair-coat brushings, were collected from all dogs. *Salmonella* spp was isolated from six fecal samples, vancomycin-resistant enterococci from five, extended-spectrum beta lactamase *E. coli* from six, and *Clostridium difficile* from 58. *Pasteurella multocida* and *P. canis* were isolated from 29 oral swabs. Fecal flotation found two dogs to be shedding *Toxocara canis* and one other to be shedding *Ancylostoma caninum*. Enzyme immunoassays detected *Giardia* spp antigen in seven fecal samples, but failed to detect any *Cryptosporidium* spp. With *C. difficile* excluded, no one dog was found to carry more than one enteric pathogen. Methicillin-resistant *Staphylococcus aureus* was not isolated from any nasal or pharyngeal swabs or from feces. Similarly, *Pseudomonas aeruginosa,* group A streptococci and *Microsporum canis* were not isolated from any aural, pharyngeal or hair samples, respectively.

Only spaying/neutering was identified as a statistically significant protective factor against shedding *Salmonella* (OR 0.10, 95% C.I. 0.29 – 0.69, p = .001). None of the other factors, such as antimicrobial history, animal's diet or degree of interaction with patients, were significant for any organism; however, a few patterns are worth noting. None of the 11 dogs that tested positive for multidrug-resistant bacteria had been hospitalized for anything other than sterilization, and only six of these had prior antimicrobial exposure. In addition, all dogs interacted with other dogs on a regular basis, whether during exercise or as part of a multi-dog household. Follow-up with the ESBL *E. coli*-positive dogs showed at least half of the other dogs (six out of a total of 10) in the multi-pet households were also infected - further evidence of the potential for dog-to-dog spread.

The significance of these findings, particularly the high prevalence of *C. difficile*, warrants cautious consideration. At this point, all that can be said with certainty is that dogs can carry many organisms of potentially pathogenic consequence without displaying clinical signs. In light of this, veterinarians are in a unique position to protect the health of their patients, the owners, and the people they visit through health certification and education programs. Further, veterinarians and physicians should work together to better evaluate the risks of these potential pathogens and develop objective criteria for screening of hospital visitation dogs.

ABSTRACT #140

SEROPREVALENCE OF TICK-TRANSMITTED DISEASE IN SOUTHEASTERN CANADIAN DOGS. Anthony T. Gary[1], Barbara C. Hegarty[2] and Edward B. Breitschwerdt[2]. [1]Colorado State University, Ft. Collins, CO. [2]North Carolina State University, Raleigh, NC.

Infectious diseases transmitted by ticks and other vectors are an important cause of morbidity and mortality in both dogs and people throughout North America. While numerous studies have described the prevalence and geographic distribution of tick-transmitted diseases throughout the United States, little comparable information is available on the veterinary importance of these diseases in Canada. The purpose of this study is to describe the seroprevalence of vector-transmitted diseases in Canada based on samples submitted to the Vector Borne Disease Diagnostic Laboratory (VBDDL) at the North Carolina State University College of Veterinary Medicine.

All available serum samples from southeastern Canadian dogs submitted to the VBDDL between August 9, 2000 and September 19, 2003 were included in the study. Serology for *E. canis*, *R. rickettsii*, *B. canis*, *B. vinsonii berkhoffii*, *B. burgdorferi*, *A. phagocytophilum*, and *B. hensalae* was determined by IFA. *B. burgdorferi* serology was performed using a commercially available test (SNAP® 3Dx™, IDEXX Laboratories, Inc.). Information regarding breed and the city or province from which the sample originated was recorded; however, travel history was unknown for the majority of dogs.

A total of 288 samples were submitted to the VBDDL from southeastern Canada throughout the period of study; serologic results are shown in the table below.

Organism	Negative	Low (≥64-256)	Moderate (≥ 256-2048)	High (>2048)	n	Prevalence
Babesia canis	61	4	1		66	7.58%
Babesia gibsonii	7	1			8	12.50%
Ehrlichia canis	270	1			271	0.37%
Bartonella hensalae	52	3			55	5.45%
Bartonella vinsonii berkhoffi	59				59	0.00%
Rickettsia rickettsii	65	2		1	68	4.41%
Anaplasma phagocytophilum	53				53	0.00%
Borrelia burgdorferi	106	Positive = 2			108	1.85%

The population consisted of 72 different breeds. All positive *B. canis* samples were from Greyhounds; no other breed associations were present. The majority of samples were from the province of Ontario and the cities of Guelph (240/288), Gloucester (14/288), Ottawa (12/288), Toronto (9/288), Blenheim (4/288), Willowdale (3/288), and Orleans (1/288). Five samples were from Quebec and the cities of Montreal (2/288), St Laurent (2/288), and St. Hyacinthe (1/288).

The results indicate that the overall seroprevalence to these tick borne pathogens in southeastern Canada is low. Thus, veterinarians in this region should actively pursue the travel history of dogs visiting endemic areas for evidence of exposure to tick borne infections.

ABSTRACT #141

EXTREME SERUM COBALAMIN CONCENTRATIONS IN DOGS WITH SIGNS OF GASTROINTESTINAL DISEASE AND SERUM COBALAMIN CONCENTRATIONS IN DOGS WITH NEOPLASTIC DISORDERS. JM Steiner[1], JA Anderson[1], KA Hahn[2], CG Ruaux[1], K Griffice[2], S Ryburn[1] and DA Williams[1]. Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Gulf Coast Veterinary Oncology, Houston, TX.

In human patients elevated and sometimes extreme serum cobalamin concentrations have been reported in patients with chronic myelogenous leukemia, promyelocytic leukemia, polycythemia vera, hypereosinophilic syndrome, acute hepatitis, hepatic cirrhosis, hepatocellular carcinoma, and metastatic liver disease. In contrast, no clinical significance has previously been reported for elevated serum cobalamin concentrations in dogs. Therefore, the goal of this study was to identify dogs with extreme serum cobalamin concentrations, record their clinical diagnoses, and also to measure serum cobalamin concentrations in a group of dogs with a variety of neoplastic diseases.

Dogs with serum cobalamin concentrations above the upper limit of the working range of the assay (>1200 ng/L; DPC) were identified from submissions to the Gastrointestinal Laboratory. Serum cobalamin concentrations were re-evaluated at dilutions of 1/20 or 1/50 (for concentrations >24,000 ng/L). Serum samples with serum cobalamin concentrations >12,000 ng/L (10 times the upper limit of the working range) were considered extreme and were recorded. Attending veterinarians for these dogs were called to gather clinical information. For the second part of the study serum samples were collected from 36 patients with a variety of neoplastic diseases presented to Gulf Coast Veterinary Oncology and serum cobalamin was measured in all samples.

A total of 10 dogs with extreme serum cobalamin concentrations were identified. One dog had been supplemented with cobalamin and was removed from the study, leaving nine dogs with extreme serum cobalamin concentrations (mean ±SD: 36,020 ±12,818 ng/L). Four of these dogs had been diagnosed by their veterinarian with inflammatory bowel disease, two with pancreatitis, one with exocrine pancreatic insufficiency (cTLI: 0.9 µg/L), one with gastrointestinal and hepatic neoplasia of unknown type, and one with *Babesia canis* infection. The median serum cobalamin concentration in 36 dogs with a variety of different neoplastic diseases was 491 ng/L (range: 159 – 48,264 ng/L). Two dogs had serum cobalamin concentrations below the lower limit of the reference range (<249 ng/L), while 8 dogs had serum cobalamin concentrations above the upper limit of the reference range (>733 ng/L; serum cobalamin concentrations: 881, 912, 1059, 1081, 1123, 2060, 2074, and 48,264 ng/L). Only one dog had an extreme serum cobalamin concentration. This dog had been diagnosed with a grade II mast cell tumor (MCT).

In conclusion, extreme serum cobalamin concentrations do occur in dogs and, in this study, most dogs affected had IBD. However, the study group was selected from patients with signs of gastrointestinal disease. Also, overall, extreme serum cobalamin concentrations are rare in dogs with IBD. Extreme serum cobalamin concentrations rarely occur in dogs with neoplastic diseases and further studies are needed to evaluate whether dogs with MCTs are predisposed.

ABSTRACT #142

EXPRESSION AND FUNCTION OF TLR2, TLR4, AND Nod2 IN PRIMARY CANINE COLONIC EPITHELIAL CELLS. M. Swerdlow, D. Kennedy, J. Kennedy, P. Henthorn, P. Moore, S. Carding, P. Felsburg, and R. Washabau. Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania; Department of Pathology, School of Veterinary Medicine, University of California-Davis; School of Biochemistry and Molecular Biology, University of Leeds; Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota.

The gut maintains a delicate balance between the downregulation of inflammatory reactions to the commensal bacteria and the capacity to respond to pathogens with vigorous cellular and humoral immune responses. Intestinal epithelial cells (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 (MAMP – LPS, PGN) by pattern recognition receptors (PRR – TLR4, TLR2, Nod2).

We have previously reported (JVIM 2004; 18: 435) that canine IECs could be isolated, cultured, and maintain constitutive expression of TLR4, TLR2, and Nod2. The purpose of the present study was to determine whether TLR2, TLR4, and Nod2 expression in primary IECs is functional and regulated by inflammatory mediators. The methodology employed acute dispersion of colonic epithelial cells (CECs) and growth of primary cultures; assessment of purity using flow cytometry; isolation of total RNA; synthesis of cDNAs using RT-PCR; development of primers for TLR2, TLR4, Nod2, IL-7, and IL-8; and, determination of IL-8 expression following stimulation with either LPS or PGN.

Flow cytometry revealed that the majority (>95%) of the cells were cytokeratin-positive epithelial cells, and that these cells constitutively expressed TLR4, TLR2, and Nod2. TLR4 was up-regulated in canine primary IECs following stimulation with LPS, and TLR2 and Nod2 were up-regulated following stimulation with PGN. Stimulation with either LPS or PGN resulted in the up-regulation of IL-8 expression suggesting that the interaction of the TLR2, TLR4, and Nod2 with their respective MAMPs results in a functional innate immune responses. Canine primary CECs constitutively express IL-7, and IL-7 expression is up-regulated following stimulation with LPS.

This study has demonstrated for the first time the expression of functionally active TLR2, TLR4 and Nod2 by RT-PCR on primary canine IECs. TLR2, TLR4 and Nod2 are expressed at low levels in "non-stimulated" canine primary IECs, however they are rapidly up-regulated in response to challenge with various infectious inflammatory stimuli. In addition, we have shown that stimulation of canine primary IECs with MAMPs specific for TLR2, TLR4 and Nod2 results in the up-regulation of the expression of the pro-inflammatory cytokine IL-8 which is one of the downstream consequences of TLR and Nod2 signaling.

ABSTRACT #143

CHANGES IN ACUTE PHASE PROTEIN CONCENTRATIONS IN CATS WITH EXPERIMENTALLY INDUCED PANCREATITIS. K Fetz[1], JM Steiner[1], CG Ruaux[1], JS Suchodolski[1], N Zavros[2], T Rallis[2], and DA Williams[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Aristotle University of Thessaloniki, Greece.

Acute phase proteins (APPs) are plasma proteins that increase in concentration following tissue damage, microbial infection, trauma, or other inflammatory stimuli. In cats, α_1-acid glycoprotein (AGP), serum amyloid A (SAA), and haptoglobin (Hp) have been reported to be acute phase proteins. In contrast to the situation in humans, serum α_1-proteinase inhibitor (α_1-PI) has not been shown to play a significant role as an acute phase reactant in the cat, but it has never been assessed in parallel with proven APPs in the cat. The aim of this study was to evaluate changes in AGP, SAA, Hp, and α_1-PI over a time period of 48 hours following experimental induction of acute pancreatitis.

Serum samples were obtained from 10 cats, in which acute pancreatitis had been induced by retrograde injection of oleic acid into the pancreatic duct as part of another research project. Samples were collected before and after induction of pancreatitis (8, 24, and 48 hours). Feline AGP was measured by radial immunodiffusion (Cardiotech Services) and SAA by ELISA (Tri-delta diagnostics). Serum Hp was assayed by the hemoglobin binding method, and serum feline α_1-PI by a species specific in-house ELISA. The baseline sample was used as the control value for AGP, SAA, Hp, while serum α_1-PI concentrations were compared to the baseline sample and to an established reference range (0.64-1.4 g/L). Data were analyzed with a statistical software package (GraphPad Prism 4.0) using Friedman's test followed by Dunn's multiple comparison test.

None of the 4 serum proteins investigated showed a significant change in concentration after eight hours. AGP was the only parameter to increase significantly after 24 hours compared to the baseline sample ($p<0.05$). All four serum proteins showed significant increases after 48 hours compared to the baseline sample (Friedman; AGP $p<0.001$; SAA $p<0.05$; α_1-PI $p<0.05$; Hp $p<0.01$). The mean increase after 48 hours was most pronounced for SAA (14.2x) followed by AGP (5x), Hp (1.7x) and α_1-PI (1.4x). Three of 10 cats had increased α_1-PI concentrations after 24 hours compared to the reference range. After 48 hours, α_1-PI concentrations were above the reference range in 6 of 10 cats. Following induction of pancreatitis

SAA, AGP, and α_1-PI showed a significant increase over time ($p=0.0002$, $p<0.0001$, and $p=0.0199$, respectively) while a significant change in serum Hp concentration over time could not be identified following induction of pancreatitis ($p=0.1106$).

In this group of cats, the acute phase reactant that increased earliest after induction of pancreatitis was AGP, while the proportional increase was greatest for SAA. Hp and α_1-PI showed less response to induced pancreatitis. These data suggest that AGP in combination with SAA may be useful for the early detection of feline pancreatic inflammation. Additional studies assessing these inflammatory markers in other feline inflammatory conditions would be of interest.

ABSTRACT #144

EVALUATION OF FECAL α_1-PROTEINASE INHIBITOR CONCENTRATIONS IN CATS WITH INFLAMMATORY BOWEL DISEASE AND CATS WITH GASTROINTESTINAL NEOPLASIA. K Fetz[1], JM Steiner[1], JD Broussard[2], M Alvarez[2], CG Ruaux[1], JS Suchodolski[1], and DA Williams[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX and [2]The Animal Medical Center, New York, NY.

Inflammatory bowel disease (IBD) is a common, poorly understood disorder in cats. The gold standard for the diagnosis of IBD is the detection of inflammatory infiltrates on histological examination of intestinal mucosal biopsies. The aim of this study was to evaluate fecal α_1-PI concentrations in cats with histopathological evidence of gastrointestinal inflammation and cats with gastrointestinal neoplasia.

A single fecal sample was obtained from 20 cats, and matched sera from 18 of these 20 cats (10 male castrated, 10 female spayed; ages ranging from 4 to 17, mean ± SD: 9.9 ± 3.5), as part of an unrelated study at the Animal Medical Center, NY. Prior to sample collection diagnostic endoscopies were performed on all cats and biopsies were analyzed for overall inflammatory severity (grade 1 (G1): mild to moderate; grade 2 (G2): severe; grade 3 (G3): neoplastic). Of 20 cats, 12 cats were initially diagnosed with IBD (G1 or G2), four cats with suspected lymphosarcoma (LSA) (G2 to G3), two cats with LSA (G3), and 2 cats with adenocarcinoma (AC; G3). Based on the histopathological grade of disease, 2 groups of cats were assembled (group 1 (n=8): mild to moderate IBD, group 2 (n=12): severe IBD and neoplastic disease) and statistically analyzed using GraphPad Prism 4.0. Follow-up fecal samples were collected from six cats on two to three additional sampling times, each four weeks apart. Fecal α_1-PI concentrations were measured using an in-house ELISA. Serum concentrations of albumin and total protein were also determined.

Of 20 cats, 19 cats had elevated fecal α_1-PI concentrations, ranging from 1.9 to 233.6 µg/g (normal range: <1.8 µg/g). Group 1 (mild to moderate IBD) and 2 (severe IBD and neoplasia) showed a statistically significant difference in median α_1-PI concentrations (group 1: median: 4.3 µg/g (range: 1.3 to 9.2 µg/g); group 2: median: 20.6 µg/g (4.2 to 233.6 µg/g); Mann-Whitney; $p=0.0049$). Low albumin was detected in 16 of 18 cats, from which sera was available, while 15 of the 16 cats also showed low total protein concentrations. Fecal α_1-PI concentrations measured in follow-up samples in 6 cats were variable.

This study suggests that increased fecal α_1-PI concentrations in association with low serum albumin and low serum total protein are a common finding in cats with IBD and gastrointestinal neoplasia. Furthermore, α_1-PI concentrations appear to be higher in cats with severe IBD or confirmed gastrointestinal neoplasia when compared to cats with mild to moderate IBD.

ABSTRACT #145

EFFICACY OF ORAL LOW-VOLUME SODIUM PHOSPHATE BOWEL PREPARATION FOR COLONOSCOPY IN DOGS. Daugherty MA, Leib MS. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Sodium phosphate (NaP) is a low-volume, over the counter, hyperosmolar, saline laxative that osmotically cleanses the colon by drawing plasma water into the gastrointestinal tract. Although NaP is commonly used as a bowel cleansing preparation in humans prior to colonoscopy, this use of NaP has not been studied in dogs. The goal of this project was to evaluate the efficacy of oral NaP as a bowel preparation for colonoscopy in dogs.

Eight purpose bred mongrel dogs received each of six bowel preparations seven days apart, approximately 18-24 hours prior to colonoscopy. The control preparation (prep A) consisted of a polyethylene glycol solution (PEG) in combination with warm water enemas. The standard NaP preparation (prep B) consisted of NaP 1ml/kg diluted with 2ml/kg water administered via orogastric intubation. A warm water enema 20 ml/kg was administered immediately after each administration of NaP and the following morning prior to colonoscopy. The NaP was repeated four hours after initial administration. Dogs randomly received either preparation A or B during weeks 1 and 2 of the study based on a crossover design. The remaining 4 preparations were variations of prep B. Preparation C had increased water (4 ml/kg to dilute NaP). D was NaP without enemas, E was the same as C with the addition of bisacodyl (10 mg PO 2 hours after each NaP administration), and F was similar to E except enemas were not administered. Preparations C, D, E, and F were administered during weeks 3, 4, 5, and 6 in a crossover design.

Colonoscopy was performed to the level of the cecum on all dogs. A score of 1-4 (one clean colon and ≥3 considered unacceptable preparation) was assigned to each of five regions of the colon: ascending, transverse, and distal, mid-portion, and orad region of the descending colon by a reviewer unaware of the preparation used. The total colon cleansing score (TCS) was defined as the sum of scores from each region.

Regional colon scores and TCS were compared for preparations A and B using ANOVA model for crossover design (significance $p<0.05$). Mean TCS was significantly less (9.4) for prep A then for B (13.6). There was a significant difference ($p<0.05$) at all locations except transverse colon. These findings indicate that the colon cleansing effect of prep A was significantly better than prep B.

Regional colon scores and TCS were compared for preparations C, D, E, and F using ANOVA model for crossover design (significance $p<0.05$). There were no significant differences for any of the variables evaluated. As preparations B and C both consisted of the standard NaP preparation, we conclude that prep A would have better colon cleansing effects than all NaP preparations evaluated in this study. Based on the protocols utilized in this study, we cannot recommend the routine use of NaP for preparation for colonoscopy in dogs.

ABSTRACT #146

INTESTINAL PERMEABILITY AND MUCOSAL ABSORPTIVE CAPACITY TESTING DO NOT CORRELATE WITH CANINE IBD ACTIVITY INDEX OR HISTOLOGICAL SCORING OF INTESTINAL BIOPSIES IN DOGS WITH CHRONIC ENTEROPATHIES. K Allenspach[1], JM Steiner[2], B Shah[2], C Ruaux[2], DA Williams[2], J Blum1, F Gaschen1; [1]Vetsuisse Faculty, University of Bern, Switzerland, and [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX.

Previous studies suggest that histology is not a very sensitive indicator for clinical disease activity in dogs with chronic enteropathies (CE). The objective of this study was to assess mucosal

function by measuring permeability and mucosal absorptive capacity in dogs with CE before and after treatment and to determine whether these variables correlate with clinical activity of disease or histologic scoring of intestinal biopsies.

Eighteen dogs diagnosed with CE were retrospectively grouped into either diet responsive CE (n=10), glucocorticoid-responsive CE (n=6), or dogs that did not respond to either diet or glucocorticoid treatment (n=2). Severity of clinical signs was assessed before and after 10 weeks of treatment using the Canine IBD Activity Index (CIBDAI; Jergens et al 2003). In addition, all biopsies were histologically graded for severity of intestinal infiltration. A second endoscopy was performed in all dogs after treatment. Permeability and mucosal absorptive capacity testing was performed at the time of endoscopy. A solution containing lactulose, rhamnose, xylose, 3-O-methylglucose, and sucrose was administered orally after withholding food for 12 hrs. A 10 ml spot urine sample was collected 6 hours after administration of the sugar solution to determine urinary L/R, X/M, and S/M ratios.

Median CIBDAI in the group of diet-responsive disease significantly decreased from 6.4 before to 1.6 after treatment (p=0.005). Similarly, median CIBDAI decreased significantly in the glucocorticoid-responsive group after treatment (7.8 before treatment; 2.6 after treatment, p=0.02). The median histological severity score of endoscopically collected biopsies did not change with treatment in either of the groups. There was no statistically significant difference after treatment in L/R, X/M, or S/M ratio in either the diet or glucocorticoid-responsive dogs. There was no significant correlation between L/R ratios, X/M ratios, or S/M ratios, when compared with the CIBDAI or with histological scoring.

In conclusion, in this group of dogs with CE testing of intestinal permeability and mucosal absorptive capacity was not a useful indicator of clinical disease activity as assessed by CIBDAI or severity of infiltration assessed by histopathology.

ABSTRACT #147

PHARMACOKINETICS AND CLINICAL EFFICACY OF CYCLOSPORIN TREATMENT IN DOGS WITH STEROID-RESISTANT INFLAMMATORY BOWEL DISEASE. K Allenspach[1], S Rüfenacht[1], S Sauter[1], A Gröne[1], J Steffan[2], GA Strehlau[2], M Kunz[2], F Gaschen[1], Vetsuisse Faculty[1], University of Bern, Switzerland, and the Novartis Centre of Research[2], St-Aubin, Switzerland.

The usual approach of treatment in dogs with inflammatory bowel disease (IBD) consists of therapy with immunosuppressive doses of steroids. Despite this, some dogs will not respond to steroid-treatment and pose a significant challenge to the veterinarian. Cyclosporin A (cyA) has been shown to be effective in steroid-refractory attacks of human IBD. The purpose of this study was therefore to investigate the pharmacokinetics of oral cyA treatment in dogs with steroid-refractory IBD and to assess the clinical efficacy of this drug in severe cases.

Fourteen dogs with IBD that had been unresponsive to immunosuppressive steroid-treatment for at least 10 weeks were prospectively enrolled into the study. All dogs were treated with cyA (Atopica) 5mg/kg po q24hrs for a period of 10 weeks. A score was applied to assess severity of clinical signs (Canine IBD Activity Index, CIBDAI) before and after treatment (Jergens et al 2003). In 9 dogs, a second endoscopy was performed after treatment. In addition, serum concentration of cyA was measured by Fluorescent Polarisation Immunoassay (FPIA) in whole blood EDTA samples in 7 dogs immediately before and at 1, 2, 4, 8, and 24hrs after giving the first dose of cyA to assess the drug pharmacokinetics.

Improvement in clinical signs was seen in 12/14 dogs. Median CIBDAI score after treatment with cyA was significantly reduced (p= 0.01). In addition, a statistically significant gain in body weight after treatment was observed (p= 0.006). In the 9 dogs in which a second endoscopy was performed, the histologic severity of infiltration did not change after treatment. Transient adverse effects attributed to cyA treatment were vomiting or anorexia (4/12), excessive hair loss (1/12) and gingivitis (1/12). Pharmacokinetic assessment of oral cyA administration revealed a mean peak concentration of 765.1 ng/ml (SD 288.19), a mean trough concentration of 37.18 ng/ml (SD 26.49), mean time to maximum level of 1.57 hrs (SD 0.53), area under the estimated concentration ($AUC_{0-\infty}$) of 5255 h x ng/ml (SD 2474.63) and an elimination half life ($t_{1/2}$) of 5.4 hrs (SD 1.16). These data are comparable to published pharmacokinetic data of cyA in healthy dogs.

In conclusion, pharmacokinetics of cyA in dogs with IBD are similar to those of healthy dogs. CyA was effective in reducing clinical signs of severe steroid-resistant IBD in a majority of dogs in this pilot study.

ABSTRACT #148

EVALUATION OF SERUM ALBUMIN CONCENTRATIONS IN CATS AND DOGS WITH SUBNORMAL SERUM COBALAMIN CONCENTRATIONS. K Fetz, JM Steiner, CG Ruaux, JS Suchodolski, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

The determination of serum cobalamin concentrations has been shown to be useful in diagnosing and characterizing intestinal and pancreatic disease in feline and canine patients. Mucosal pathology of the ileum, possibly due to severe chronic inflammation or neoplastic infiltration, leads to defects of the receptors for cobalamin/intrinsic factor complexes in the ileum. The lack of functional receptors subsequently results in malabsorption of cobalamin, utilization of cobalamin body stores, and low serum cobalamin concentrations. Also, exocrine pancreatic insufficiency (EPI) can lead to cobalamin deficiency in cats. Thus, a decrease in serum cobalamin concentration in dogs and cats that do not have EPI indicates the presence of longstanding and severe small intestinal disease. In dogs, low serum albumin concentrations are commonly associated with chronic gastrointestinal disease. In cats, however, subnormal serum albumin concentrations have been rarely described in the context of chronic gastrointestinal disease. The aim of the present study was to evaluate the prevalence of subnormal serum albumin concentrations in cats and dogs with low serum cobalamin concentrations.

Serum samples from 100 client-owned cats and 100 client-owned dogs were selected from accessions to the Gastrointestinal Laboratory at Texas A&M University. Criteria for selection for both dogs and cats included abnormally low cobalamin concentrations (cats: <290 ng/mL; dogs: <249 ng/mL) and the absence of an abnormally low trypsin-like immunoreactivity (TLI; cats: ≥12 µg/L; dogs: ≥5.0 µg/L) concentration. Additional information about these animals in terms of breed, age, clinical history or clinical signs was not assessed. Serum albumin was measured at the Texas Veterinary Medical Diagnostic Laboratory. The lower limit of the reference range for serum albumin is 3.0 g/dL in dogs and 3.2 g/dL in cats.

Seventy-nine of 100 dogs had low serum albumin concentrations, ranging from 0.9 to 2.9 g/dL. The mean ± SD in these 79 dogs was 1.85 ± 0.53 g/dL. In cats, serum albumin concentrations were low in 80 of 100 animals, ranging from 0.3 to 3.1 g/dL. The mean ± SD in these 80 cats was 1.98 ± 0.72 g/dL. There was no significant difference in mean serum albumin concentrations between the 79 dogs and 80 cats with hypoalbuminemia (two-tailed t-test, p=0.203).

These data provide evidence that hypoalbuminemia in association with a low serum cobalamin concentration occurs as commonly in cats as it does in dogs. This finding further suggests that gastrointestinal protein loss might be of similar prevalence in association with chronic gastroenteropathies in both species. Further studies, including studies evaluating hepatic and renal function in patients with hypoalbuminemia, will be of interest.

ABSTRACT #149

ALTERATIONS IN MARKERS ASSESSING THE CANINE SMALL INTESTINAL MICROFLORA IN RESPONSE TO ALTERED HOUSING AND TYLOSIN ADMINISTRATION. CG Ruaux, JS Suchodolski, N Berghoff, K Fetz, A Stoll, U Tress, JM Steiner, and DA Williams. Gastrointestinal Laboratory, College Station, TX.

The difficulty of canine duodenal juice culture limits the ability of clinicians to assess the small intestinal flora in dogs. Serum markers and dynamic tests of the small intestinal microflora have been described, reflecting changes in bacterial metabolic mass (serum unconjugated cholic acid, SUCA, and C^{13}-glycocholic acid blood test, C^{13}-GCBT), bacterial synthetic activity (serum folate concentration), and bacterial competition for substrates (serum cobalamin concentration). The aim of this study was to assess changes in these markers of small intestinal microflora in response to altered housing conditions and administration of a broad-spectrum antibiotic agent (tylosin).

Ten hound-cross laboratory dogs, all intact females, were selected from an in-house colony. The criterion for entry into the study was an elevated serum folate concentration (>13.5 µg/L, reference range 6.5-11.5 µg/L). Dogs were relocated into laboratory dog wards and acclimated for one month. At day 0, following overnight withholding of food, indwelling jugular catheters were placed and baseline sera collected for determination of cobalamin, folate and SUCA. A C^{13}-GCBT was then carried out, using 1 mg/kg C^{13}-glycocholic acid mixed into a standardized meal. The dogs then received 15 mg/kg tylosin per os BID for 28 days. On day 28, jugular catheters were placed again, baseline sera collected, and the C^{13}-GCBT repeated. Serum concentrations of cobalamin, folate and SUCA were determined at screening, and on days 0 and 28. The cumulative percentage of the administered dose recovered (CUMPCD) was determined in blood samples by fractional mass spectrometry. Data were analyzed using repeated measures ANOVA, or Friedman's test if data were not normally distributed.

Alterations in housing and tylosin treatment were associated with significant changes in serum markers of intestinal microflora in these dogs. Serum cobalamin concentrations significantly increased after rehousing ($p<0.05$), but showed no change after tylosin administration. Serum folate concentrations decreased significantly after rehousing ($p<0.05$), then increased significantly after tylosin administration ($p<0.0001$). SUCA was unchanged with rehousing, but increased significantly with tylosin administration ($p<0.01$). Overall bacterial bile acid metabolism as assessed by the C^{13}-GCBT increased significantly ($p<0.0001$) following tylosin administration.

These data suggest that both altered housing conditions and tylosin administration are associated with alterations in the canine small intestinal flora, reflected in alterations in serum markers and dynamic tests of the small intestinal microflora. Increased serum folate, SUCA, and CUMPCD in the C^{13}-GCBT suggest that, in the dogs described here, tylosin administration increased the biomass of organisms carrying out these metabolic functions.

ABSTRACT #150

SERUM PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS (cPLI) IN DOGS TREATED WITH POTASSIUM BROMIDE AND/OR PHENOBARBITAL. JM Steiner[1], P Xenoulis[1], JA Anderson[1], Barr AC[2], and DA Williams[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Texas Veterinary Medical Diagnostic Laboratory, College Station, TX.

Potassium bromide (KBr) is generally considered to be a safe and effective treatment for idiopathic epilepsy in dogs. However, pancreatitis has previously been reported in dogs treated with this drug. In a recent study, serum pancreatic lipase immunoreactivity concentration (cPLI) was shown to be the most sensitive diagnostic tool for canine pancreatitis currently available. The goal of this study was to measure serum cPLI in a large group of dogs treated with either KBr alone or a combination of KBr and phenobarbital and compare them with healthy control dogs.

A total of 182 left-over serum samples submitted to the Texas Veterinary Medical Diagnostic Laboratory for the measurement of serum KBr concentrations was collected. Serum KBr concentrations and, when requested by the veterinarian, serum phenobarbital concentrations were recorded. Samples were divided into two subgroups, subgroup 1 (n=156) for which only KBr measurement had been requested and subgroup 2 (n=26) for which both serum KBr and phenobarbital measurements had been requested. The total numbers of dogs with serum cPLI concentration greater than either the upper limit of the reference range (102.1 µg/L) or the diagnostic cut-off value for pancreatitis (>200 µg/L) were tabulated. The numbers of dogs in subgroups 1 and 2 with serum cPLIs above these limits were also recorded. The data were also analyzed for any correlation of serum cPLI with serum KBr for all dogs enrolled and for both subgroups 1 and 2. Finally, the median cPLI in all dogs, and in dogs of subgroups 1 and 2, was compared to the median cPLI in 74 healthy dogs studied previously.

Serum cPLI was above the upper limit of the reference range in 24 (13.2%) of all 182 dogs, in 23 (14.7%) dogs of subgroup 1, and in one (3.8%) dog of subgroup 2 Serum cPLI was above the diagnostic cut-off value for pancreatitis in 13 (7.1%) of all 182 dogs, in 13 (8.3%) dogs of subgroup 1, and in none (0.0%) of the dogs in subgroup 2. There was no correlation between serum concentrations of cPLI and KBr for all dogs (Spearman $r=0.138$; $p=0.0632$), dogs in subgroup 1 (Spearman $r=0.148$; $p=0.0647$), or dogs in subgroup 2 (Pearson $r=0.178$; $p=0.3844$). Median serum cPLI concentration was significantly different between 74 healthy dogs (16.3 µg/L) and all 182 dogs enrolled (30.1 µg/L; $p=0.0177$) and also the 156 dogs in subgroup 1 (31.3 µg/L; $p=0.0117$). However, median serum cPLI concentration was not significantly different between 74 healthy dogs and the 26 dogs in subgroup 2 (21.6 µg/L; $p=0.5067$).

In conclusion, in this study the median serum cPLI was significantly increased in dogs treated with KBr alone, but not when given in combination with phenobarbital. Also, an increase of serum cPLI above the diagnostic cut-off value for pancreatitis was found in 7.1% of all 182 dogs investigated, further suggesting an increased risk of pancreatitis in dogs treated with KBr.

ABSTRACT #151

BACTERIAL CULTURE RESULTS FROM 251 CASES OF HEPATOBILIARY DISEASE IN DOGS AND CATS. KA Wagner, FA Hartmann, LA Trepanier. University of Wisconsin-Madison, School of Veterinary Medicine, Madison, WI.

Bacterial infection is thought to accompany canine and feline hepatobiliary diseases, although there are few actual studies of common organisms, susceptibilities, or clinical predictors of a positive culture. The purpose of this retrospective study was to characterize the results of bacteriologic cultures of liver and bile in dogs and cats with hepatobiliary disease, referred to the Univ. of Wisconsin VMTH between 1998 and 2003.

In 190 dogs, 7% of hepatic and 28% of biliary cultures were positive for bacterial growth; in 61 cats, 14% of hepatic and 36% of biliary cultures were positive. In patients in which both liver and bile cultures were performed, bile was more commonly positive. Most cats had a single organism identified (82% of feline cultures), whereas most dogs had multiple organisms cultured (61% of canine cultures). The most commonly cultured organisms were Escherichia coli (18% of positives), Enterococcus spp. (16%), Bacteroides spp. (11%), and Clostridium spp. (6%). Coagulase-negative Staphylococcus was a common contaminant. A positive liver culture was correlated with leukocytosis, but not with fever, left shift, toxic

change, hyperbilirubinemia, or degree of increase in ALT or SAP activities. Samples from liver obtained by laparotomy or laparoscopy were more commonly positive (19%) than those obtained by needle biopsy (5%; P = 0.004). Susceptibility profiles indicated that 6/11 E. coli isolates (55%) were susceptible to amoxicillin/clavulanate, 7/11 (64%) to first generation cephalosporins, and 9/11 (82%) to fluoroquinolones; for enterococci, 10/10 (100%) were susceptible to penicillin. Profiles were not generated for anaerobes, which are typically sensitive to amoxicillin/clavulanate or metronidazole, but not fluoroquinolones.

The results of this study indicate that biliary cultures are positive in approximately one third of cases with hepatobiliary disease, and liver cultures are positive in 7-14%. Bile may be a more sensitive site to culture organisms than liver, and surgical biopsy may increase the sensitivity of liver culture over needle biopsy. More than one antimicrobial may be needed for adequate empirical coverage.

ABSTRACT #152

PHARMACOKINETICS OF LIVER TRANSAMINASES IN HEALTHY DOGS: POTENTIAL CLINICAL RELEVANCE FOR ASSESSMENT OF LIVER DAMAGE. O. Dossin, A. Rives, C. Germain, JP. Braun[1] and H. Lefebvre[1]. National Veterinary School of Toulouse and [1]UMR-INRA 181, Physiopathology and Experimental Toxicology - Toulouse, France.

Liver transaminases are routinely used to assess liver damage but their pharmacokinetics are poorly documented in dog. In most textbooks, the plasma half life of these markers is reported to be between 12 and 60 h. Their plasma concentrations are hybrid variables which depend on enzyme production (release from the liver), distribution and elimination. Adequate interpretation of the time course of plasma concentrations of these enzymes requires to determine the basic pharmacokinetic parameters.

Seven healthy adult beagle dogs received a 15,000g supernatant of liver homogenate intravenously and were sampled for 18 days. Alanine transaminase (ALT) and aspartate transaminase (AST) plasma activities were measured on a Vitros 250 (Ortho-Clinical diagnostics, Rochester, NY, USA). Data were analyzed using a non compartmental approach. Plasma clearance (Cl), steady-state volume of distribution (Vss), mean residence time (MRT) and elimination half-life (t1/2) were calculated using classical pharmacokinetic equations.

The basal activity concentration of ALT and AST were 32±11 and 21±3 U/L. The mean concentration of ALT and AST in the liver were 254 and 382 U/g of tissue. The dose of ALT and AST were 226±43 and 151±29 U/kg BW, respectively. The peak plasma concentrations observed at two minutes after dosing were 4100±1028 and 3046±822 U/L for ALT and AST, respectively. The plasma concentrations returned to pre-dosing levels between 14 and 18 days, and between three and five days for ALT and AST, respectively. Cl, Vss, MRT and t1/2 for ALT were 0.017±0.002 mL/kg/min, 77±15 mL/kg, 76±9 h and 59±9 h, respectively. The corresponding values for AST were 0.125±0.032 mL/kg/min, 82±51 mL/kg, 11±5 h and 22±16 h, respectively.

These results indicate that the ability of the body to clear liver enzymes is very poor, which explains the long t1/2 and MRT. The time required to clear most of the enzyme is 5 times t1/2 that is about 16 and five days for ALAT and ASAT, respectively. Vss is small and close to the plasma volume, which indicates that effect of dehydration on plasma ALT and AST concentrations is probably negligible. The differences between ALT and AST pharmacokinetic parameters indicate that both enzymes could be useful for clinico-pathological assessment of liver damage. Although AST is not liver specific it may be relevant when ALT is increased to document the time course of liver damage in the dog.

ABSTRACT #153

MEAL AND MEAL WITH ERYTHROMYCIN PROTOCOLS FOR ULTRASONOGRAPHIC EVALUATION OF GALLBLADDER EJECTION VOLUME IN THE DOG. KL Ramstedt, AE Yeager, SA Center, JF Randolph, HN Erb, Cornell University, Ithaca NY.

Gallbladder (GB) dysmotility may predispose to cholelithiasis, inspissated bile, and cholecystitis in humans and may precede GB mucocele formation in dogs. Confident identification of GB dysmotility may modify clinical recommendations (i.e. elective cholecystectomy) for dogs suspected of developing biliary mucocele. Studies in humans have validated assessment of GB volume dynamics by calculation of the GB ejection fraction; EF% =[(Fasting GB Volume – Postprandial GB Volume)/ Fasting GB Volume] x 100.

Sixteen clinically healthy pet dogs of various ages (median 7.5, 2-11 years) and weights (median 18.3, 4.2-26.9 kg), including eight different pure breeds, were recruited to the study. Each dog was fasted 12-hr before baseline imaging. A standardized meal (100 gm, 1.41 ME kcal/gm [7.4 gm protein, 6.1 g fat per 100 kcal]) was used to stimulate GB contraction. Postprandial meal fed (MF) images were collected at 15, 30, 45, 60, and 120 min (MF protocol). One to five days later, a meal fed-erythromycin (MF-E) protocol was used (erythromycin estolate, 250 mg/ml in cherry flavored syrup; 1.0 mg/kg). GB images were obtained by a single investigator (ATL 3,000 ultrasound system, convex 8-5 MHz,14 mm radius transducer) with dogs in dorsal recumbency. Images were made in longitudinal and transverse axis capturing the widest GB dimension; volume was calculated using the ellipsoid formula. Serial measurements of two dogs recorded on five separate days (fasted condition) and on a single day (10 replicates, fasted condition) determined physiologic variation in GB size or imaging variability. Nonparametric methods investigated GB volume and EF% differences within (Wilcoxon signed rank test) and between (Wilcoxon rank sum test) protocols ($\forall$ = 0.05, two tailed p values); values expressed as median (range). Two-by-two tables evaluated proportions of dogs contracting their GB per imaging interval and time of maximum contraction. Spearman Rank Correlation investigated relationship between weight and maximal EF%.

The CV% of serial measurements ranged from 17.8% to 24.8%. There was no difference between protocols in the interval occurrence of GB contraction or timing of maximal GB contraction. Median EF% at 15, 30, 45, 60, and 120 min for the MF protocol was: 5.3, -18.9, -12.7, -13.6, and -22.3; and for the MF-E protocol was: 0.53, -19.8, -12.3,-21.6, and -22.7. Despite wide variability in EF% among dogs, the 30, 60, and 120 min intervals for each protocol, and the 45 min interval for the MF-E protocol, produced EF% values significantly different from the 15 min interval. Maximal EF% for MF was -37.1 and for MF-E was -31.4 ; 80% of dogs achieved at least a -20% EF%. A significant negative association between weight and maximal EF% was found for MF (smaller dogs had greater EF%); this may have reflected the larger volume of food ingested relative to body size.

This study provides a comparison standard for dogs with suspected GB dysmotility.

ABSTRACT #154

TRANSPLANT OF AUTOLOGOUS CANINE BONE MARROW STROMAL CELLS RETROVIRALLY ENGINEERED WITH CANINE EPO LEADS TO AN INCREASE IN HEMATOCRIT IN HEALTHY DOGS. J. Hernandez[1], F. Fontaine[2], H. Boucher[2], G. Beauchamp[1], N. Eliopoulos[3], J. McLeod[4], J. Galipeau[3], D. Martineau[2], M. Dunn[1]. [1]Dép. Sciences cliniques, [2]Dép. Microbiologie et Pathologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, PQ, Canada. [3]Lady Davis Institute, Montreal Jewish General Hospital, Montreal, PQ, Canada. [4]Baker Institute for Animal Health, Cornell University, Ithaca, NY.

Anemia, a common complication of chronic renal failure (CRF), contributes to decreased quality of life in canine and feline patients. Human recombinant erythropoietin has been successfully used to treat anemia in small animals. Unfortunately, the cost of this drug, production of neutralizing antibodies and the need for multiple weekly injections has limited its use. Gene and cellular therapy are alternative therapeutic approaches. Because gene therapy involves the transfer of genetic material directly to tissues, through viral vectors, the risks involved restrict its use. In contrast, the implantation of autologous cells genetically engineered to release therapeutic proteins in vivo poses fewer risks. The purpose of this study was to observe the change in hematocrit in healthy dogs receiving autologous cells genetically engineered to produce EPO.

Retroparticles coding for canine EPO and Green Fluorescent Protein (GFP) were produced using the 293GPG packaging cell line. Bone marrow was aspirated under general anaesthesia from the humerus of young adult female Beagles. The mononuclear cells were separated, recovered in canine bone marrow stromal cells (BMSC) culture medium and were exposed to retroparticles. Transduced BMSCs were embedded in an FDA-approved collagen-based matrix (Contingen^{TM}) and injected sc in four Beagles. Each dog received 130 x 10^6 autologous EPO-BMSCs divided in 32x200 µl implants on both flanks. Physical examinations were performed and blood pressure (BP), hematocrit (Ht), EPO serum levels, CBC, routine biochemistry profile and total iron binding capacity were measured weekly after implantation. As controls, four healthy adult female Beagles and one dog implanted with autologous BMSCs transduced with empty retroparticles were monitored weekly for Ht, serum EPO levels, CBC and serum biochemistry.

The Ht of all treated dogs increased significantly from 42.5 % (SD: ±4.8) to 53.5 % (SD: ± 2.2) ($p<0.0001$) from days 4 to 35 compared to days 0 and -4 and to the Ht of control dogs. Serum EPO levels of treated dogs were significantly higher at day 7 ($p=0.025$) and marginally higher at day 14 ($p=0.077$) than at day 0. No statistically significant variation was observed for physical examination, BP and serum biochemical findings.

This study demonstrates the feasibility of systemically delivering a therapeutic protein in immunocompetent dogs for a prolonged period without observed side effects. We showed that canine BMSCs are easily transduced with a therapeutic gene using a retroviral vector and that the transduced cells can release functional EPO in pharmacologically significant amounts. We plan to carry out the protocol on anemic canine patient with CRF.

ABSTRACT #155

IMPROVEMENT OF RETINAL FUNCTION IN CANINE PUPPIES FROM MOTHERS FED DIETARY LONG CHAIN N-3 POLYUNSATURATED FATTY ACIDS DURING GESTATION AND LACTATION. KM Heinemann[1], MK Waldron,[2] KE Bigley[1], JE Bauer[1]. Companion Animal Nutrition Lab and Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX. 2. Nestle-Purina PetCare, St. Louis, MO.

Long-chain polyunsaturated fatty acids (LCPUFA) are essential for proper neural and retinal development in many mammalian species. One objective of this study was to investigate the effects of dietary n-3 LCPUFA during maternal gestation and lactation on the fatty acid composition of canine puppy plasma phospholipids (PL) during suckling and early neonatal life. In addition neurologic development as assessed by retinal function of puppies weaned to these same experimental diets were investigated via elecroretinography.

One of two complete and balanced diets varying only in fatty acid composition were fed to six bitches (three dogs in each diet group) from the time of breeding via artificial insemination, throughout gestation, parturition, and lactation. he diets were sufficient in linoleic acid and contained 14 % total fat (weight basis) using either beef tallow or menhaden fish oil as primary fat source. All other components, including total protein, total fat, nitrogen-free extract, vitamins, and minerals were the same. All puppies used in the study were healthy, suckled normally, and ingested colostrum. The puppies were weaned beginning on day 29 to their respective mothers' diets. Prior to sampling, puppies were separated from their mothers for 2-3 hours (days 4, 10, 28) or fasted overnight (day 84). No other source of nutrition was provided to the dams. Bitches' milk supplied sole nutrition for each litter during suckling. As expected, enriching the canine gestation/lactation diet with n-3 LCPUFA using fish oil resulted in statistically significant increases in 20:5n-3 and 22:6n-3 and a decrease in 20:4n-6 both during suckling and after weaning. Plasma PL 22:5n-3 was unchanged. Visual function was assessed via electroretinography (ERG) in 84 day-old puppies. ANOVA revealed significantly better visual performance in the high n-3 LCPUFA diet group. Puppies in this group demonstrated an increased rod response as measured by the amplitude and implicit time of the a-wave. A novel parameter devised in this study was the threshold intensity, which was measured as the initial intensity at which the a-wave was detectable. Again, puppies in the high n-3 LCPUFA diet group responded significantly sooner thereby exhibiting greater rod sensitivity, than the control group. These findings underscore the importance of preformed n-3 LCPUFA in the diet as a means of enriching plasma and neural tissues in DHA during the development. Moreover, dietary DHA appears to be related to improved visual performance in developing canines.

ABSTRACT #156

EFFECTS OF INSULIN RESISTANCE ON REVERSE CHOLESTEROL TRANSPORT, APO A-I KINETICS AND SELECTIVE UPTAKE OF CHOLESTERYL ESTERS IN DOG. François Briand[1,2], Edwige Bailhache[1,2], Patrick Nyugen[2], Michel Krempf[1], Thierry Magot[1], Khadija Ouguerram[1]. Centre de Recherche en Nutrition Humaine, 1: INSERM U539, CHU Nantes, France, 2: Unité de Nutrition et Endocrinologie, Ecole nationale vétérinaire de Nantes, France.

HDL cholesterol (HDL-C) and HDL apolipoprotein A-I (HDL-apo A-I) play a major role in the reverse cholesterol transport. This process includes a hepatic selective uptake of HDL-cholesteryl esters (HDL-CE) and is known to have significant antiatherogenic properties. In humans, insulin resistant states and type II diabetes are characterized by a low plasma concentration of HDL-C and impaired HDL-apo A-I metabolism. In dogs, obesity is a clinically important problem. In this species, as in humans, insulin resistance is associated with a low HDL-C. In humans, this low concentration is explained by an impaired HDL-apo A-I metabolism. The aim of the present report was to study in dogs the changes in the reverse cholesterol transport induced by obesity and associated insulin resistance.

Five healthy male Beagle dogs were overfed with a high-fat diet for 28 ± 2.5 weeks. Obesity was associated with insulin resistance as assessed by the euglycemic hyperinsulinemic clamp technique. Kinetic studies were conducted in dogs, in healthy and insulin resistant states, using a primed constant infusion of [1,2$^{13}C_2$]acetate and [5,5,5-D_3]leucine, as labeled precursors of CE and apo A-I, respectively. Isotopic enrichment was measured by mass spectrometry. A compartmental model was used for the analysis of tracer kinetics data.

HDL-apo A-I did not change with insulin resistance whereas both production and catabolism rates of apo A-I was higher (2 fold, $p<0.05$). HDL-CE was significantly lower with insulin resistance. Production and catabolism rates of HDL-CE were also reduced (2.5 fold for both, $p<0.05$). Activity of cholesterol esterification was lower but not significantly. The selective uptake of HDL-CE was lower (2.5 fold, $p<0.05$).

These results show that insulin resistance dramatically impairs the reverse cholesterol transport in dogs. These changes are at least

similar to those described in humans, especially for the HDL-apo A-I metabolism.

ABSTRACT #157

EFFECTS OF ATORVASTATIN ON THE INTESTINAL CHOLESTEROL ABSORPTION USING STABLE ISOTOPE IN DOG. François Briand[1,2], Khadija Ouguerram[1], Michel Krempf[1], Patrick Nguyen[2]. 1. Centre de Recherche en Nutrition Humaine, Nantes, France ; 2. Unité de Nutrition et Endocrinologie, Ecole nationale vétérinaire de Nantes, France.

Due to their effects on plasma cholesterol, statins are widely used for the treatment of dyslipidemia in humans. Statins decrease plasma lipids but it has been shown that inhibition of cholesterol synthesis could upregulate intestinal cholesterol absorption. Thus, inhibition of both synthesis and absorption could be an emergent therapy for a more efficient cholesterol lowering. The hypothesis of an enhanced cholesterol absorption has only been tested using the measurement of plasma phytosterol as a marker of cholesterol absorption, but labeled cholesterol has never been used. Hypercholesterolemia has been described in dogs. Moreover, as obesity is becoming an important clinical issue, more information on cholesterol absorption and metabolism is needed.The aim of our study was the assessment of intestinal cholesterol absorption in the dog with the dual isotope method of Zilversmit. Then, we tested the hypothesis of an enhanced intestinal absorption with an atorvastatin treatment at high dose.

Seven ovariectomised female beagle dogs were given atorvastatin (5mg/kg/day) for 6 weeks. Before and after treatment, [2,2,3,4,4,6-D6]cholesterol (16mg) was administered orally and [25,26,26,26,27,27,27-D7]cholesterol (8mg) was administered intravenously. By using gas chromatography-mass spectrometry and selected ion monitoring, percent cholesterol absorption was calculated as the plasma ratio of oral/intravenous isotopic tracer three days after administration of labeled cholesterol. Plasma lipids, HDL-cholesterol (HDL-C) and HDL-apolipoprotein A-I (HDL-apoA-I) were also assessed before and after treatment.

Atorvastatin treatment decreased significantly ($p<0.05$) total cholesterol (4.82 ± 0.18 to 3.33 ± 0.23 mmol/l), free cholesterol (1.42 ± 0.12 to 1.08 ± 0.14 mmol/l), cholesteryl ester (3.40 ± 0.07 to 2.25 ± 0.12 mmol/l), phospholipids (4.52 ± 0.16 to 3.53 ± 0.08 mmol/l) and triglycerides (1.03 ± 0.09 to 0.82 ± 0.07 mmol/l). HDL-C also decreased (3.56 ± 0.24 to 2.64 ± 0.15 mmol/l, $p<0.05$) as well as HDL-apoA-I (2.36 ± 0.03 to 1.55 ± 0.04 g/l, $p<0.05$). Percent cholesterol absorption was higher after atorvastatin treatment. (74.9 ± 2.9 to 91.5 ± 2.8 %, $p<0.05$).

This study shows that in dog, an atorvastatin treatment at 5mg/kg/day decreases plasma lipids. However, intestinal cholesterol absorption is upregulated, as it has been suggested in humans.

ABSTRACT #158

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS γ AND α AND UNCOUPLING PROTEIN GENE EXPRESSION IN INSULIN TARGET TISSUES IN OBESE AND INSULIN RESISTANT DOGS. V Leray, C Gayet, B Siliart and P Nguyen; Nutrition and Endocrinology Unit, National Veterinary School of Nantes, Nantes, France.

Visceral adipose tissue, liver and skeletal muscle are insulin target tissues. The insulin resistance of these tissues can lead to perturbations of energy balance, pancreatic function, and lipid storage. In dogs, the expression of peroxisome proliferator-activated receptors PPARγ and PPARα and uncoupling proteins (UCP-1, UCP-2 and UCP-3) are involved in glucid and lipid metabolism. An alteration of their expression could explain the metabolic disorders observed in obese dogs. The aim of our study was to examine PPARγ, PPARα, UCP1, UCP2 and UCP3 gene expression in visceral adipose tissue, skeletal muscle and liver in obese and insulin resistant dogs.

Obesity and insulin resistance were induced in 7 male Beagle dogs (3-9-y old, 8.80-15 kg body weight) by feeding a high-fat (hyperenergetic) diet for 7 months. Adipose tissue, skeletal muscle and liver samples were taken before and after the weight gain. mRNA expression of PPARγ, PPARα, UCP1, UCP2 and UCP3 were quantified by real time RT-PCR by comparison with GAPdH expression as house-keeping gene. The fluorescence cycle threshold (Ct) was calculated to quantify the relative amount of gene expression. Results were expressed according to the $2^{-\Delta\Delta Ct}$ method and the mean level of expression before weight gain was arbitrarily set at 100%.

Expression of UCPs was significantly lower in obese and insulin resistant dogs compared to the same dogs when lean and insulin sensitive. In adipose tissue, UCP1 mRNA levels were four times lower (17 ± 9 vs 100 ± 31 %, $p<0.05$). In hepatic tissue, UCP2 mRNA levels were six times lower (15 ± 5 vs 100 ± 26 %, $p<0.05$). In skeletal muscle tissue, UCP1, UCP2 was three times lower (35 ± 9 vs 100 ± 19 %, $p<0.05$; 30 ± 9 vs 100 ± 23 %, $p<0.05$, respectively) and UCP3 mRNA levels five times (18 ± 5 vs 100 ± 23 %, $p<0.03$).

In parallel, expression of PPARs was significantly lower in obese and insulin resistant dogs compared to the same dogs when lean and insulin sensitive. The expression of PPARα gene was five times lower in hepatic tissue (22 ± 2 vs 100 ± 24 %, $p<0.02$). The expression of PPARγ gene was three times lower in muscle (28 ± 10 vs 100 ± 22 %, $p<0;05$) and four times in adipose tissue (17 ± 9 vs 100 ± 30 %, $p< 0.02$).

The concomitant decrease in PPAR and UCP expression suggests a link between PPAR activation and UCP expression. The decrease of PPAR expression could be responsible for that of UCP expression in obese an insulin resistant dogs, as shown in other species. This modulation of UCP and PPAR expression could contribute to and, at least in part, explain alterations of glucid and lipid metabolism previously observed in these dogs.

ABSTRACT #159

PLASMA CONCENTRATIONS OF ENROFLOXACIN IN CATS AFTER TRANSDERMAL ADMINISTRATION OF A PLO GEL FORMULATION. M Karriker, V Wiebe, K Parsons, S Stanley. Veterinary Medical Teaching Hospital, University of California, Davis, Davis, CA.

Medications formulated in PLO (pluronic lecithin organogel) transdermal gels are compounded and marketed by pharmacies as alternatives to commercially available veterinary products for difficult to dose feline patients. As the popularity of this dosage form increases with both veterinarians and owners, there is growing concern regarding the efficacy of drugs administered transdermally. Previously published studies have shown that medications formulated in a PLO vehicle produce highly variable and often minimal drug levels in veterinary patients. This prospective study was designed to evaluate the serum enrofloxacin (ENRO) levels achieved after dosing healthy cats with ENRO formulated in a transdermal PLO gel.

Four healthy cats (mean age, 7.25 years) were dosed with 0.1ml of ENRO PLO gel 227mg/ml that was rubbed into the hairless, inner skin of the right ear of each cat once daily. In a crossover design, each cat received both a one dose and three dose regimen with a 3-month washout period. For both dosing regimens, a 1 ml whole blood sample was collected via venipuncture at times 0, 30, 60, and 120 minutes around the third or only dose depending on the regimen. Whole blood was centrifuged and the plasma was separated and frozen until analysis.

Plasma samples were assayed for enrofloxacin and ciprofloxacin simultaneously via reverse-phase high-pressure liquid chromatography with florescence detection. ENRO and ciprofloxacin (CIPRO) were extracted from plasma by use of a solid-phase

extraction cartridge. Fifty microliters of each sample was separated using a 5µm, C18 reverse phase column with mobile phase of 90:10 water:acetonitrile with a column flow rate of 0.8ml/min. Plasma standard curves for both ENRO and CIPRO (concentrations 0.005, 0.05, 0.1, 0.5, 1, 10 ug/ml, correlation coefficient 0.998) were made for each assay using pooled feline plasma. The limit of quantification (LOQ) was 0.05µg/ml for both ENRO and CIPRO.

None of the samples analyzed from either dosing regimen showed ENRO or CIPRO levels above the limit of quantification. An independent analytical laboratory further verified the absence of quantifiable drug levels. It can be concluded from these results that neither ENRO nor CIPRO reached detectable levels (>0.05µg/ml) in plasma following transdermal administration in our sample population at the time points tested. As the LOQ was below the minimum inhibitory concentration (MIC) for most pathogens treated with ENRO, the results of this study suggest that a PLO formulation of may not adequately deliver ENRO across the membrane of the feline ear with enough efficiency to achieve plasma levels appropriate to treat any systemic pathogen. This study was not designed to explore alternate dosages or drug concentrations in other body fluids such as urine. Further controlled trials should be performed before administering ENRO, with the intent of achieving appropriate plasma levels, via this route.

ABSTRACT #160

A CLINICAL TRIAL USING A COMMERCIALLY AVAILABLE SUPPLEMENT IMPROVES SIGNS OF AGING IN OLDER DOGS. SD Lauten, TM Smith, SK Cox, RG Bottcher, HN Sanders, LA Sulewski, and JW Bartges, Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine , Knoxville, TN.

A commercially available, canine wellness supplement was evaluated by a six-month, double-blinded, clinical trial. Seventy-eight dogs, ages 7-12 years of age, displaying typical signs of aging but otherwise healthy, were recruited and randomly assigned to receive either a placebo or the supplement. Sixty-seven dogs completed the study. Data were collected at baseline, at two months and at completion of the study (six months). The Comet assay measured changes in DNA damage in lymphocytes. Plasma levels of reduced glutathione (GSH) and oxidized forms (GSSG) were quantified by high performance liquid chromatography. Lipid peroxidation was determined by measuring malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) using a commercial assay kit. Owners filled out behavior questionnaires at each visit, and responses were given numeric scores for analysis. Dogs were also given numeric scores describing coat quality. Body weight was recorded at each visit.

A significant improvement in Comet scores was seen after treatment with supplement ($p<0.001$). Coat condition improved in dogs with coat irregularities at initial presentation ($p<0.001$), while no changes were seen in body weight. No changes were observed in levels of GSH, GSSG, MDA, and HAE. Behavior questionnaires revealed significant improvement in activity levels ($p<0.004$), a decline in restless behavior ($p<0.041$) and improved interactions with family members ($p<0.02$).

Supplementation in accordance with product instructions resulted in a significant reduction in several markers associated with aging in this clinical study population.

ABSTRACT #161

FENTANYL OR MORPHINE DISPOSITION FOLLOWING SINGLE DOSING IN CATS USING A PLO TRANSDERMAL GEL. DM Boothe, E Akin, ML Marsh-Ng, ML, T Smaha, T Finch, M Flair. Auburn University, Auburn AL; Gulf Coast Veterinary Specialists, Houston TX (Marsh- Ng).

The use of a pluronic lecithin organic gel (PLO) for systemic drug delivery continues to be embraced by the veterinary medical community as a potentially effective method of drug delivery, particularly in cats. However, whereas multiple dosing has proven effective for methimazole, no study of single dosing has demonstrated therapeutic drug concentrations of any drug in cats. The purpose of this study was to evaluate the ability of the PLO to deliver in the cat two opioid analgesics that vary in lipophilicity: morphine (MOR) and fentanyl (FEN). Our laboratory previously has demonstrated ineffective delivery of either drug in the dog following PLO TD administration. For MOR (prepared as a 160 mg/ml PLO), four cats were administered 1 mg/kg TD; and four cats were administered 0.1 mg/kg IV as positive control. For FEN (prepared as a 5 mg/ml PLO), six cats received the drug SC (0.005 mg/kg) or TD (0.1 mg/kg) using a randomized cross-over design. Blood was collected intermittently via pre-placed catheters for seven (MOR) or 72 (FEN) hours. Data was subjected to standard noncompartmental pharmacokinetic analysis. Both MOR and FEN were detected using RIA validated in feline plasma; the LOQ for MOR was 4 ng/ml and for FEN, 1 ng/ml. Drug concentrations were measured in the TD gels to be within 20% of the intended concentration.

Following IV administration, MOR C_{max} was 119 ± 62 mcg/ml; elimination half-life (HL) was 7 ± 12 hr and MRT was 21 ± 8 hr. Morphine was detectable but not quantifiable at any time in any cat following TD administration in all six cats. For FEN SC, C_{max} was 397 ± 243 ng/ml at 0.8 ± 0.8 hr; elimination HL was 7.9 ± 5.9 hr and MRT was 13.7 ± 8.9 hr. For TD, FEN C_{max} was 8 ± 5 ng/ml at (T_{max}) 10 ± 19 hr; the elimination HL was 53 ± 22 hr and MRT was 88 ± 31 hr. Bioavailability was 8.1 ± 5.0%. The last detectable concentration of FEN was 4.2 ± 4.7 ng/ml at 61 ± 12 hr following TD delivery compared to 3.8 ± 2.0 at 35 ± 32 hr for SC. Of the two drugs, FEN is much more lipid soluble and thus is more likely to penetrate the stratum corneum and reach systemic circulation. This study does support TD absorption of FEN following TD delivery as a PLO. However, the dose was increased 20 fold compared to SC administration and ability of the drug to achieve effective analgesic concentrations by the TD route using a PLO is not necessarily supported by this study.

ABSTRACT #162

ASSESSMENT OF NONINVASIVE CARDIAC OUTPUT MEASUREMENT BY PARTIAL CARBON DIOXIDE REBREATHING OR TWO-DIMENSIONAL ECHOCARDIOGRAPHY BY COMPARISON TO THE LITHIUM DILUTION METHOD IN ANESTHETIZED NEONATAL FOALS. Steeve Giguère, Eric Bucki, Darcy B. Adin, Alexander Valverde, Amara H. Estrada, Linda Young. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Cardiac output (CO) is the best available parameter to assess overall cardiovascular function. Cardiac output monitoring would be valuable in critically ill and anesthetized neonatal foals. Because measurement of cardiac output is currently considered impractical for routine use in foals, arterial blood pressure is commonly used as an estimate of blood flow. However, arterial pressure is a poor indicator of blood flow in situations when vascular resistance is altered. The objective of this study was to validate and assess various noninvasive methods of measuring CO in neonatal foals by comparison to the lithium dilution method.

Ten healthy neonatal foals were anesthetized and CO was manipulated by varying the depth of anesthesia and infusion of dobutamine. For each foal, measurements were obtained at three separate levels of CO (low, medium and high). Concurrent CO measurements were obtained by lithium dilution (reference method), partial carbon dioxide (CO_2) rebreathing, volumetric echocardiography (Cubic, Teichholz, Bullet, area-length, and single and biplane Modified Simpson formulas), and transthoracic Doppler

echocardiography. For each method, relative bias was calculated as a percentage of the average CO.

Lithium determinations of CO ranged between 3.09 and 11.07 L/min (mean ± SD = 6.39 ± 2.1 L/min), resulting in cardiac indices ranging between 79.0 and 209 ml/kg/min (mean ± SD = 131 ± 35.9 ml/kg/min). Relative bias of Doppler echocardiography significantly increased ($P < 0.05$) whereas that of partial CO_2 rebreathing significantly decreased ($P = 0.03$) with increasing CO. Among methods not influenced by the level of CO, bias of the Bullet method (-4.2 ± 20.9 %; limits of agreement -45.2 to 36.7 %) was significantly lower ($P < 0.05$) than that of each other noninvasive methods evaluated. Volumetric echocardiography using the Bullet method provides an accurate and noninvasive estimate of CO in anesthetized neonatal foals, and warrants investigation in conscious critically ill foals.

ABSTRACT #163

THYROID FUNCTION IN NORMAL, SICK AND PREMATURE FOALS. Babetta A. Breuhaus, and D. Heath LaFevers. Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Thyroid hormones increase metabolism by stimulation of a variety of cell types, and are essential for normal growth and maturation. In many species, fetal serum thyroid hormones increase just before birth and probably play a role in the rapid growth and organ system development that occur in late gestation. Organ systems that are developmentally immature contribute to early morbidity and mortality of premature foals, and decrease the prognosis for long term soundness and athletic potential. This study was designed to test the hypothesis that premature foals experience transient postnatal hypothyroidism.

Serum concentrations of total and free thyroid hormones and thyroid stimulating hormone (TSH), both at rest and in response to thyrotropin releasing hormone (TRH), were measured in normal, healthy neonatal foals that were full term (normal foals), full term neonatal foals that were ill and hospitalized for conditions similar to premature foals (sick foals) and in premature neonatal foals (premature foals) to determine the possible contributions of an immature hypothalamic-pituitary axis and nonthyroidal illness to thyroid dysfunction in premature foals. Normal foals did not receive any medications. Both sick and premature foals received medications routinely used to treat conditions including (but not limited to) failure of passive transfer, sepsis, and perinatal asphyxia syndrome. Blood samples were collected for measurement of baseline concentrations of thyroid hormones and TSH at predetermined ages, and TRH stimulation tests were performed in foals at less than three days of age. Thyroid hormone and TSH concentrations were compared among the three groups of foals by ANOVA. Post hoc comparisons were performed using the Bonferroni correction.

Premature foals had significantly lower serum concentrations of total and free fractions of thyroid hormones than normal foals. Baseline serum concentrations of TSH were not different, but TSH responses to TRH were exaggerated in premature foals compared to normal foals. Serum concentrations of tri-iodothyronine (T_3) and TSH were similar in sick term foals and premature foals, but serum concentrations of thyroxine (T_4) in sick term foals were intermediate between premature and normal foals.

Results suggest that sick term foals experience non-thyroidal illness syndrome, primarily a low T_3 state. Alterations in thyroid function in premature foals may be caused by primary hypothyroidism, decreased peripheral conversion of T_4 to T_3, non-thyroidal illness syndrome, or by a combination of the three. Early thyroid hormone supplementation in premature foals might accelerate organ system maturation, thereby improving short-term survivability and preserving long-term athletic function.

ABSTRACT #164

SERUM BILE ACIDS CONCENTRATIONS IN HEALTHY AND COMPROMISED NEONATAL FOALS. Michelle H. Barton, Natalie Norton, and Bruce LeRoy[1]; Departments of Large Animal Medicine and Pathology[1], College of Veterinary Medicine, University of Georgia, Athens, GA.

Serum bile acids (SBA) are synthesized in the liver, excreted into the bile, and are efficiently reabsorbed into the blood via the enterohepatic circulation. Values for SBA concentrations are well established in healthy adult horses and increased values are indicative of hepatocellular dysfunction or cholestatic disease. The main purpose of this study was to establish the normal range of SBA concentrations in healthy foals from birth to six weeks of age. Blood samples were obtained by venipuncture from ten healthy full term foals immediately after birth, at two days of age, and 1, 2, 3, 4, and 6 weeks of age. Blood samples were also obtained from 33 foals less than one month of age at the time of admission to the Veterinary Teaching Hospital for evaluation of various ailments, including prematurity, failure of passive transfer, septicemia, and colic. SBA concentrations were determined enzymatically based on 3-α-hydroxysteroid dehydrogenase methodology (Trinity Biotech., St. Louis, MO) using an automated analyzer (Hitachi 912, Roche/BMC, Indianapolis, IN). Serum was also analyzed for total and direct bilirubin, and triglyceride concentrations and sorbitol dehydrogenase (SDH) and gamma glutamyltranspeptidase (GGT) activities using commercially available reagents on an automated analyzer. Serum from 38 foals was further analyzed for SBA using a commercially available radioimmunoassay (ICN Diagnostics, Orangeburg, NY). Data were analyzed by ANOVA and correlations were determined by regression.

The mean and range of values by age category are presented in Table 1. Values in the first two weeks of life were significantly greater than values obtained at six weeks of age, which approximated the established normal range of < 15 μmol/L in adult horses. There was a significant correlation ($p < 0.0001$) between the enzymatic assay and the radioimmunoasssay for SBA ($R^2 = 0.63$), though in general, the values were lower in the later assay. When comparing age-matched values between healthy and sick foals, there were no significant differences in SBA. None of the sick foals had a primary diagnosis of hepatic disease. For all foals tested, there was no significant correlation between the SBA concentration and the presence or absence of hemolysis, the bilirubin or triglyceride concentrations, or the GGT activity. There was a significant correlation between increased SBA concentration and SDH activity ($p < 0.0001$, $R^2 = 0.18$).

Table 1. Serum bile acids concentrations (μmol/L) in foals from birth to 6 weeks of age.

	At birth	2 days	1 week	2 weeks	3 weeks	4 weeks	6 weeks
Mean (SD)	41.4 (18.3)	36.8 (13.3)	22.5 (4.7)	20.3 (6.4)	13.6 (3.7)	13.2 (2.6)	12.4 (3.8)
Range	21.7-81.7	26.0-74.3	16.7-29.4	11.3-30.6	7.2-18.4	9.0-17.1	7.4-19.4

In summary, SBA concentrations in foals are significantly greater in the neonatal period. The results of this study underscore the importance of obtaining age-matched controls when evaluating clinical pathology values in the neonatal period.

ABSTRACT #165

NEONATAL ISOERYTHROLYSIS IN 17 HORSE FOALS AND A MULE FOAL: 1988-2003. Ashley G. Boyle, K. Gary Magdesian, Rebecca E. Ruby, University of California, Davis School of Veterinary Medicine; Davis.

Neonatal isoerythrolysis (NI) is an alloimmune disease of foals that results in hemolytic anemia. The purpose of this study was to

examine the clinical, hematologic, biochemical, and blood gas data from foals with NI over the last 15 years. The 18 foals included in the study had a diagnosis of NI: hemolytic anemia with a positive direct antiglobulin test (Coombs' test) and/or identification of anti-erythrocyte antibody in the dam's serum or colostrum. These consisted of five Quarter Horses, eight Thoroughbreds, one Standardbred, one Warmblood-cross, two Paints, and one mule, and 66% were fillies.

Tachypnea was a common physical examination finding (46 ± 31/min). Clinical icterus was present in 11/17 foals at presentation. Total serum bilirubin was increased in 15/16 foals, with increases in both direct (2.85 ± 5.24 mg/dl) and indirect (11.43 ± 5.88 mg/dl) fractions. Sorbitol dehydrogenase was increased in 14/16 foals, reflecting liver disease. Whole blood transfusions were administered to 55.5 % of cases. A variety of blood factors were implicated in 10 foals, including a factor not previously implicated in NI, Dg. The presenting hematocrits and hemoglobin concentrations improved significantly post-transfusion in seven cases (P=0.05). The $P_{CV}O_2$ improved from a presenting low of 21.7 ± 44.5 to 34.2 ± 37.4 g/dl in two cases post-blood transfusion. 83% of the foals survived to discharge.

Whole blood transfusions are successful at increasing oxygen carrying capacity in foals with NI, thereby improving peripheral tissue oxygenation. With appropriate therapy, the prognosis for foals with NI is good.

ABSTRACT #166

EVIDENCE BASED DECISION MAKING IN INITIAL ANTIMICROBIAL SELECTION IN EQUINE NEONATAL SEPTICEMIA. Johanna R. Laine, Benjamin W. Sykes. Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.

Blood culture is an essential part of antimicrobial usage in a septic foal. However, therapy is usually initiated prior to obtaining culture and sensitivity results. Previous recommendations for the treatment of septic foals have been based upon first principles of antimicrobial selection. A key component of these principles is that selection should be based on known patterns of organism distribution and susceptibility. The purpose of this review is to provide evidence based guidelines for the initial antimicrobial therapy in foals with presumed septicemia based on known pathogen distribution and the susceptibility of these pathogens.

Internet data bases and book chapters were reviewed to identify reports of equine neonatal isolates published within the last 20 years. Five studies, each with over 20 isolates, were identified. Two studies also reported the sensitivities of isolated organisms. Data from the five studies was pooled. The frequency of each isolate was determined and multiplied by the likelihood that that isolate would be sensitive to each antimicrobial. The sum of these calculations for each antimicrobial, and thus the likelihood that an unknown organism would be sensitive to a given antimicrobial was determined. Isolate data was compared against both sets of sensitivity data and ranked in order of efficacy.

A total of 691 isolates were included. Gram negative bacteria represented 70.6 %, gram positive bacteria 27.9 % and anaerobic bacteria 1.5 % of all isolates. The frequency of mixed cultures was reported in 3 studies as 12 %, 14.9 %, 55 % and not reported in two. The most frequently isolated organism was *Escherichia coli* (30.4 %). Other frequently isolated organisms were *Streptococcus spp* (12.5 %), *Enterobacter spp* (9.9 %), *Actinobacillus spp* (9.6 %), *Klebsiella spp* (9.1 %), *Staphylococcus spp* (7.8 %) and *Enterococcus spp* (5.8 %).

Using the first set of sensitivity data the following likelihoods of efficacy were determined; Amikacin (68 %), ceftiofur (65 %), gentamicin (62 %), trimethoprim-sulfadiazine (TMS) (56 %), ticarcillin (51 %), ampicillin (48 %) and penicillin (14 %). Using the second set the results were; Enrofloxacin (100 %), amikacin (79 %), chloramphenicol (78 %), tetracycline (73 %), ceftiofur (72 %), gentamicin (67 %), TMS (66 %), ampicillin (65 %) and penicillin (35 %).

Based on this data, the use of ceftiofur combined with an aminoglycoside is recommended. Ampicillin and ticarcillin are reasonable alternatives to ceftiofur but the use of penicillin appears to be of little benefit. The use of chloramphenicol, tetracycline and TMS, which have previously been considered unlikely to be effective, may be justified when concerns of toxicity preclude the use of aminoglycosides, or other factors such as route of administration predominate.

ABSTRACT #167

EVALUATION OF *LACTOBACILLUS PENTOSUS* WE7 FOR THE PREVENTION OF NEONATAL FOAL DIARRHEA. JS Weese, J Rousseau, Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Lactobacillus pentosus WE7, an equine-origin lactic acid bacterium with the good tolerance to acid and bile, aerotolerance, *in vitro* inhibitory effect against *Salmonella, Clostridium perfringens* and *C. difficile*, and the ability to colonize the intestinal tract of healthy foals was evaluated for the prevention of neonatal foal diarrhea in a prospective, randomized, blinded, placebo-controlled study.

160 clinically normal foals from 11 farms in Ontario and Kentucky were enrolled. Foals were randomly assigned to receive either probiotic (approximately 2 x 10^{11} CFU of freeze-dried *L. pentosus* WE7) or placebo (equivalent volume of skim milk powder) once daily for seven days, starting 24-48 hours after birth. Blinded farm personnel performed clinical monitoring for 14 days. Fecal consistency was recorded visually according to pre-determined guidelines.

Of the 160 foals, 153 (96%) completed the study. The remaining seven foals were moved off the farm shortly after foaling and were excluded. 70 foals remained in the probiotic group, with 83 in the control group. Three to 38 foals were enrolled per farm (median 12). There were 80 standardbreds and 73 thoroughbreds. Immunoglobulin G level was assessed in 94 foals. Of these, 90 (96%) had an IgG level greater than 800 mg/dl. The four remaining foals were equally distributed between the probiotic and control groups.

Overall, 78 (51%) foals developed soft feces, 29 (19%) developed diarrhea and 16 (10%) developed diarrhea and other abnormal clinical signs such as anorexia (14), depression (10), weakness (4) and colic (5). Twenty-one (14%) foals required veterinary examination and 15 (9.8%) required treatment. With univariate analysis, probiotic therapy was associated with the development of diarrhea, diarrhea plus additional clinical signs, depression, anorexia, colic and the need for veterinary examination and treatment ($P<0.05$ for each). Probiotic treated foals also had a trend towards more days of diarrhea compared to the control group ($P=0.077$). With multivariate analysis, probiotic therapy was significantly associated with development of diarrhea and diarrhea plus other clinical abnormalities ($P=0.03$ and 0.04, respectively), but not soft feces ($P=0.75$).

This study is the first to demonstrate adverse effects of probiotic therapy in foals, and highlights the need for proper safety and efficacy studies for equine probiotics, something that is currently lacking for most commercial veterinary probiotics.

ABSTRACT #168

ACUTE RENAL FAILURE IN CRITICALLY-ILL NEONATAL FOALS. Corley KTT[1], Axon JE[2], Herron C[2] and Bryant T[2]. 1. Royal Veterinary College, London, UK; 2.Scone Veterinary Hospital, Scone, NSW, Australia.

AIM: This study aimed to describe acute renal failure (ARF) in a group of critically-ill neonatal foals presenting to a major referral institution.

METHODS: A prospectively maintained database of critically-ill foals of less than seven days presenting to Scone Veterinary Hospital between 15th September and 26th November 2004 was examined. Foals that had a creatinine concentration measured at admission were included in the study. Foals greater than 24 hours old on admission were considered to have ARF if their creatinine concentration remained above 2.26mg/dl (200umol/L) following aggressive fluid therapy, unless their final diagnosis included uroperitoneum. For foals less than 24 hours old, those that presented with a creatinine concentration of greater than 2.26mg/dl, which did not halve in concentration in the first 24 hours and did not decrease to less than 2.26mg/dl by 48 hours of life for foals were considered to have ARF. Continuous parameters were compared with the Mann-Whitney U test and non-continuous with Fisher's Exact test.

RESULTS: 65 foals (all Thoroughbred) met the inclusion criteria. Of these foals, eight met the definition for ARF. These foals ranged from 0 to 96 hours old at admission, with a median of 17.25h. There were four colts and four fillies. The final clinical diagnosis for the foals with ARF included perinatal asphyxia syndrome (PAS) for five foals, sepsis for three foals (including two with PAS) and enterocolitis for one foal. In one foal, no underlying disease process was identified. Two foals had very low plasma sodium concentrations (103 and 106mmol/L), both foals exhibited seizure activity. Endogenous creatinine clearance was measured in five foals with ARF, and ranged from 0.07 to 1.48ml/min/kg. It was measured in one foal with a very high admission creatinine (23.3mg/dl) not considered to have ARF, and was 1.59ml/min/kg.

Foals with ARF had greater median creatinine ($p=0.005$) and urea ($p=0.016$) concentrations and higher neurological dysfunction scores ($p=0.004$) than the general population, and lower median chloride concentrations ($p=0.022$). 23 foals had plasma creatinine concentrations greater than 2.26mg/dl on admission, but were considered not to have acute renal failure, because the creatinine concentration rapidly declined with fluid therapy. 21 of these 23 foals were less than 24 hours old at admission, and placental insufficiency may have been a contributing factor to the increased creatinine concentration. These 23 foals had a lower median urea concentration at admission (45mg/dl), when compared to that of the foals with ARF (74mg/dl; $p=0.034$).

All foals were treated with intravenous fluids, adjusted to their electrolyte status Two foals did not receive any additional renal specific treatment. Four foals received dobutamine (one with the addition of noradrenaline) for blood pressure support, three foals received fenoldopam, three frusemide and one dopamine. Seven of the eight foals survived to hospital discharge.

ABSTRACT #169

THE CANINE DAL BLOOD TYPE: A RED CELL ANTIGEN LACKING IN SOME DALMATIANS. Marie-Claude Blais, Donna A. Oakley, and Urs Giger. Section of Medical Genetics and Penn Animal Blood Bank, University of Pennsylvania, Philadelphia, PA.

Based upon alloantibodies produced after sensitizing dogs with transfused blood, more than a dozen blood group systems have been recognized thus far, and some have been classified as Dog Erythrocyte Antigens (DEA). Clinical hemolytic transfusion reactions have been reported in canine patients due to blood incompatibilities associated with DEA 1.1, DEA 4 and a common red cell antigen. We describe here the discovery of a specific alloantibody associated with a presumably new canine red cell antigen, which is lacking in some Dalmatians. Serological tests, including blood typing, crossmatching and Coombs' test, were performed by standard tube techniques, in addition to a novel gel column technology (DiaMed) used in human blood banking.

A DEA 1.1 positive female spayed Dalmatian with chronic renal failure and anemia was referred for renal dialysis. Two crossmatch-compatible DEA 1.1 positive blood transfusions were administered without incident within a two-day period. Because of progressive anemia and failure to respond to recombinant human erythropoietin, the dog required additional transfusions by week 4, but a compatible donor was not readily available; all major crossmatch tests to 50 non-Dalmatian dogs were incompatible. In addition, the two initial donors were now also incompatible, suggesting the development of an alloantibody to a common antigen on the red cell membrane. All auto-controls performed were consistently negative, and a direct Coombs' test at 37ºC was also negative. No siblings of the anemic Dalmatian were available for compatibility assessment. However, 3 of 13 randomly crossmatched Dalmatians were compatible suggesting that they were also missing the same antigen. One of the compatible red cell units was transfused with the expected beneficial effects. The canine patient was found to be DEA 1.1, 3, 4, as well as 5 positive, but DEA 7 negative (reagents to other blood types are currently not available). Further blood typing and crossmatching results did not support an association to any of these known blood types. Thus, the red cell antigen recognized by this antiserum was called Dal. This Dalmatian's serum had a 1:8 anti-Dal titer (37ºC) which, based upon dithiothreitol exposure, was of the IgG class. The three other Dal-negative Dalmatians had not been transfused and their serum contained no detectable anti-Dal alloantibodies.

Based upon the identification of an alloantibody in a Dalmatian, a presumably new blood type named Dal was identified. The Dal red cell antigen seems to be lacking in several Dalmatians. Following sensitization via transfusion, the development of anti-Dal alloantibodies may result in ineffective transfusions or in hemolytic transfusion reactions if Dal positive blood products are subsequently used. Further studies are needed to determine the frequency of the Dal negative blood type in Dalmatians as well as other breeds, and to characterize the Dal red cell antigen and its mode of inheritance. In addition, the clinical importance of anti-Dal antibodies in transfusion medicine must be investigated.

ABSTRACT #170

THALIDOMIDE TREATMENT OF CANINE HEMANGIOSARCOMA. J. Paul Woods, Karol A. Mathews, Alan G. Binnington, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Purpose: Noncutaneous hemangiosarcoma, a malignant neoplasm of vascular endothelium is highly metastatic. Surgery is the primary method of treatment; however, even with adjuvant chemotherapy the prognosis for long-term survival is low. Hence, new therapies are needed. Although thalidomide was withdrawn in the 1960's after recognition of its teratogenicity and association with phocomelia, recently, thalidomide is making a comeback for its immunomodulatory and antiangiogenesis properties to treat inflammatory, infectious, and neoplastic diseases in people. Thalidomide can inhibit the proliferation of blood vessels associated with tumour development, thereby stopping or slowing tumour growth. Therefore, the purpose of this study was to retrospectively report, and to prospectively initiate a study of the use of thalidomide therapy for the treatment of canine hemangiosarcoma.

Materials: Nineteen dogs with histologically diagnosed hemangiosarcoma were retrospectively entered into the study. Dogs were treated at 100-400 mg/day with a median dose of 8.7 mg/kg/day (range 3.7-19.7). Unfortunately, a consistent legal source of

thalidomide for treatment of dogs could not be obtained; therefore, the prospective study was discontinued.

Results: The dogs had a median age of 10 years (range 3-16) and consisted of: eight spayed females, six neutered males, and five males; six Golden retrievers, four mixed breed, two GSD, two standard poodles, five other breeds; with a median weight 35 kg (range 10.5-47.9). Hemangiosarcoma was found in the spleen (15 with six ruptured), right atrium (4), liver (1), and kidney (1). The dogs were staged as: Stage I (nine dogs), II (seven dogs), and III (three dogs). One dog (stage II) was lost to follow up at 1,044 days and one dog (stage I) is alive at 1210 days. The overall median survival for 17 dogs was 160 days (range 34-1087 days).

Tumour Stage	Number of dogs	Survival median (days)	Survival Range (days)
I	7	366	34-969
II	7	165	45-409
III	3	56	36-87

There was statistical difference in survival between Stages I and III ($p = 0.03$) and between Stages II and III (($p = 0.04$).

Conclusions: Only limited efficacy data are available so far to define the clinical utility of thalidomide in canine hemangiosarcoma. However, this pilot study revealed prolonged responses to thalidomide in some patients, which prompts a Phase 2 investigation of thalidomide in canine hemangiosarcoma. The optimal dose and schedule of administration in dogs remains to be determined but absence of myelosuppressive and significant adverse effects suggests thalidomide could be used with combination chemotherapy. We conclude that thalidomide (or its analogues) could open the possibility for novel treatment that targets tumours and their microenvironment.

ABSTRACT #171

EFFECT OF ANTIMICROBIALS ON COAGULATION PARAMETERS IN HEALTHY DOGS. J.A. Webb, D.G. Allen, A. Abrams-Ogg, P. Gentry. Ontario Veterinary College, Guelph, Ontario, Canada.

Antimicrobials are widely used in veterinary medicine. Previous studies in humans and animals have indicated that certain antimicrobials cause a disturbance in hemostasis, including thrombocytopenia, platelet dysfunction and disorders of secondary coagulation. The most commonly implicated antimicrobials include those in the β-lactam family, however disturbances associated with sulfonamides, fluoroquinolones, tetracyclines, imipenem and metronidazole have also been identified. Many of the previous studies have used dosages of antimicrobials far in excess of published therapeutic dosage ranges, and very few in vivo studies assessing the effect of antimicrobials on hemostasis in veterinary medicine have been performed. The purpose of this study was to evaluate the effects of commonly used antimicrobials at therapeutic dosages on primary and secondary coagulation in healthy dogs.

Ten healthy, purpose-bred beagles were administered oral amoxicillin, cephalexin, doxycycline and enrofloxacin in random order at standard therapeutic dosages for seven days. A washout period of sven days (minimum of 14 half-lives) was allowed between administration of each antimicrobial. In addition, four healthy, purpose-bred beagles were maintained as controls. Parameters measured included platelet count, hematocrit, prothrombin time, partial thromboplastin time, fibrinogen level and platelet function. Platelet function was assessed via buccal mucosal bleeding time, aggregometry and closure time in a platelet function analyzer (PFA-100™).

Enrofloxacin administration resulted in a significant decrease in fibrinogen ($p=0.01$), however the post-administration fibrinogen level was still within normal range therefore the change was not interpreted as clinically relevant. Enrofloxacin administration did not result in a significant change in any other measured parameter. There were no significant differences in platelet count, hematocrit, prothrombin time, partial thromboplastin time, fibrinogen level or platelet function after administration of amoxicillin, cephalexin or doxycycline.

In conclusion, administration of selected commonly-used antimicrobials in healthy dogs does not result in hemostatic abnormalities. Although a statistically significant decrease in fibrinogen occurred subsequent to enrofloxacin administration, it is unlikely to be clinically relevant. Further studies that evaluate the effect of antimicrobial administration on hemostasis in animals with underlying disease processes that affect the hemostatic system are warranted.

ABSTRACT #172

PLASMA LIPOLYTIC ACTIVITIES AND POST-PRANDIAL TRIGLYCERIDE FATTY ACID PROFILES OF DOGS FED A DIACYGLYCEROL-RICH MEAL. J.E. Bauer[1]. B. Porterpan[1], K.Bigley[1], D. Nagaoka[1], T Umeda[2], K Otsuji[2], J.E. Bauer[1]. Companion Animal Nutrition Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX. [2]Kao Corporation, Tokyo, Japan.

Digestion, absorption, and re-assembly of dietary fatty acids as triglycerides is a complex post-prandial (P-P) process in which hydrolysis of ingested triglyceride (TG), absorption of free fatty acids, and *de novo* re-synthesis of TG in intestinal mucosal cells occurs prior to chylomicron assembly. We have previously shown in dogs that dietary vegetable oils enriched in 1,3 diacylglyerol (DAG) decrease the extent of P-P hypertriglyceridemia. Studies in rodent species have found increased ∃-oxidation and decreased *de novo* TG re-synthesis in mucosal cells with dietary DAG which help explain these phenomena. It was hypothesized that along with decreased P-P triglyceridemia, feeding a low glycemic index/DAG meal would also modify plasma lipolytic activities. Also, in spite of TG lowering, plasma fatty acid profiles seen with DAG vs. triacylglycerol (TAG) meals of similar fatty acid composition would be equivalent. The objective of this study was to compare the effects of DAG vs TAG meals on plasma hepatic (HL) and lipoprotein lipase (LPL) activities 6 hours after feeding and to evaluate P-P fatty acid profiles of plasma TG and phospholipids (PL) at 2, 3 and 4 hours after a single meal.

Normal adult Beagles were fed meals enriched in dietary DAG (70% DAG, 30% TAG) compared with 100% 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. The meals were fed in random fashion to each of 12 Beagles and P-P blood samples were collected via jugular catheters at 0, 0.5 , 1. ,2 .3, 4 and 6 hours. At 6 hours, 100 I.U. Na heparin was injected and post-heparin plasma collected 15 minute post-injection from the alternate cephalic vein. Plasma TG was determined using an enzymatic technique. Plasma TG and PL were fractionated via lipid extraction, thin-layer chromatography; methyl ester derivatives were prepared for gas chromatography of fatty acids. LPL and HL of post-heparin plasma were assayed by differential radiolableled substrate techniques. No significant differences in LPL were found. However, HL was greater when the high amylase (low glycemic index) meals were fed. As expected, no alteration of fatty acid profiles was found in either TG or PL fractions during the post-prandial period with any of the meals. We conclude that glycemic index modifies HL activity more than dietary acylglycerol type. HL is involved in hepatic lipopoprotein uptake and helps to scavenge circulating TG via hepatic HDL-TG fatty acid uptake with low glycemic index meals. Also fatty acid profiles when DAG meals are fed are equivalent in to typical dietary oil responses.

ABSTRACT #173

D-DIMER CONCENTRATIONS IN HEALTHY AND CLINICALLY ILL CATS. Fox LE, Portillo E, Crum H, Andreasen CB. Iowa State University, College of Veterinary Medicine, Ames, IA.

D-dimer is formed by degradation of cross-linked fibrin by thrombin and plasmin. In human beings and dogs, increased D-dimer concentrations are found with DIC and thromboembolic disease. Using a semiquantitative latex agglutination assay, Nelson et al. found significantly higher median D-dimer concentrations in dogs with either hepatic or thromboembolic diseases when compared with clinically healthy dogs. The purpose of this study was to investigate D-dimer concentrations in healthy cats and clinically ill cats.

Group 1 Healthy Cats (20 healthy cats): The CBC, platelet count, prothrombin time (PT), partial thromboplastin time (PTT), urinalysis, serum biochemical panel, and physical exam were within normal limits.

Group 2 Clinically Ill Cats: (20 sick cats): All cats received CBC, platelet count, serum biochemical panel, PT, PTT, urinalysis, and physical examinations. Additional evaluations, including radiography, ultrasonography, histopathology, and necropsy (4), were used to further evaluate diseases.

Citrated blood samples were collected for D-dimer determination using the Minutex D-dimer-Latex agglutination test for fibrin D-dimer (Biopool International, Sweden) and PT, PTT, platelet estimation.

All Group 1 Healthy Cats had a negative D-dimer concentration (<250 ng/ml). Group 2 Clinically Ill Cats had negative (11 cats-<250 ng/ml) or positive D-dimer concentrations (2 cats-250-500 ng/ml; 4 cats-500-1,000 ng/ml; 3 cats-1,000-2,000 ng/ml) Disorders associated with negative D-dimer concentrations included hypertension (1), neurologic (1), hypertrophic cardiomyopathy (1), inflammatory bowel disease (1), multisystemic disease including cancer (4), cancer (2), and infectious (1). Results of this preliminary data suggest that D-dimer concentrations may not be consistently associated with clinical illness in cats.

ABSTRACT #174

HEMATOLOGICAL ABNORMALITIES AND OUTCOME OF 7 DOGS WITH PRIMARY MYELODYSPLASTIC SYNDROMES. Masaharu Hisasue, Sakurako Neo, Takeshi Ishikawa, Ryo Tsuchiya, Takatugu Yamada. Laboratory of Internal Medicine II, Veterinary Medicine, Azabu University.

Myelodysplastic syndrome (MDS) is a preleukemic disorder and recognized in humans, dogs and cats. MDS is characterized by multi-lineage cytopenia with normo- or hyper cellular marrow, indicating that its pathogenesis should be ineffective hematopoiesis. Additionally, various dysplastic changes are detected in erythroid, myeloid, megakaryocytic cells in bone marrow. MDS is considered to be a clonal hematopoietic disorder in a pluripotent stem cell, since monoclonal hematopoiesis was confirmed by clonality analysis of hematopoietic cell population. Diagnostic criteria of MDS in cats and dogs were delineated (Jain N et al., 1991) based on the French-American-British (FAB) classification system for the human acute myeloid leukemia (AML) and MDS. Furthermore, Weiss et al proposed criteria for subclassification of MDS. But because reports of canine MDS are limited, hematological abnormalities and prognosis have been unclear. We investigated the hematological hematological abnormalities, morphological changes, therapy, and prognosis of seven dogs with MDS.

During 2000 to 2004, seven dogs were diagnosed as having MDS, according to hematological and bone marrow findings at the Veterinary Teaching Hospital, Azabu University. A total of seven dogs with MDS were subclassified into refractory cytopenia. Leukopenia was seen in four dogs (57%), and the mean of WBC count was 5789/μl (Range 2000 to 14800/μl). Anemia was seen in five dogs (71%), and the mean of PCV value was 27.9% (Range 14 to45.3%). Thrombocytopenia was shown in five dogs (71%), and the mean of platelet count was 111.4 x 10^3/μl (Range 0.6 to 2434 x 10^3/μl). In bone marrow findings, four dogs with MDS were normocellular, but other three dogs were hypocellular. The range of ME ratio was 0.36 to 13 (Mean 2.3), predominant of erythroid cell was seen in five dogs (71%). The population of blast cells and myeloblast were low level, blast ratio in all nuclear cells (ANC) was 1.3 to 5.8 %, myeloblast ratio in ANC was 0.9 to 4.8%. Dysplastic changes were shown following as; Megaloblastoid rubricyte (n=3), Abnormal nuclear shape of rubricyte (n=2), Circulating rubricytes without polychromasia (n=2), Multi nuclei rubricyte (n=1), Hyposegmentation (n=3), Psudo-pelger-Huet nuclear anomaly (n=2), Nuclear fragmentation (n=2), Ring shaped nuclear (n=4), Coarse chromachin (n=1), Micromegakaryocyte (n=3), Large mononuclear megakaryocyte (n=2), Large platelet (n=2). Vitamin K_2 (Menatetorenone) (7 cases), predonisolone (3 cases) and cyclosporin (1 case) were used as therapeutic agents. Hematological improvements were shown in five cases (71%) treated with menatetorenone or combination of menatetorenone and predonisolone. The survival durations were between 19 to more than 486 days (Mean=228.7 days), five dogs has been survival within on this study, but two dogs died of immunodeficiency and development of cytopenia, respectively.

In the present study, megaloblastoid rubricyte, hyposegmentation, psudo-pelger-Huet nuclear anomaly, ring shaped nuclear, and micromegakaryocyte were detected frequently, indicating that these dysplastic changes should be important findings to diagnose canine MDS.

Furthermore, the survival durations of the canine MDS with low myeloblast count were relatively longer than those of other previous reports, suggesting that the ratio of myeloblast were one of the important prongostic factors in canine MDS. Furthermore, it was prospected that menatetorenone or combination of menatetorenone and predonisolone therapy can be used as a new effective protocol to treat canine MDS.

ABSTRACT #175

BIOMARKERS OF PLATELET ACTIVATION IN CAVALIER KING CHARLES SPANIELS. D.F. Hogan[1], D.J. Weiss[2], C.A. Thompson[1], M.P. Ward[3]. [1]Purdue University, West Lafayette, IN; [2]University of Minnesota, St. Paul, MN; [3]Texas A&M University, College Station, TX.

Thrombocytopenia (TP) is commonly reported in Cavalier King Charles Spaniels (CKCS) and is frequently associated with macrothrombocytosis. Dogs do not appear to be at increased risk for bleeding and generally have normal platelet function. Most recently, pedigree analysis suggested an autosomal recessive mode of inheritance and similarities to inherited macrothrombocytopenias in humans which are not well described. Mitral regurgitation (MR), secondary to myxomatous valvular disease, is also common in CKCS and has been shown to affect platelets including a reduction in number, increased size, altered aggregation, increased markers of activation and reduced survival time in humans. There also appears to be an increased risk for arterial thromboembolism in humans with MR which may be due, at least in part, to platelet activation.

This study measured leukocyte-platelet aggregates with flow cytometry as biomarkers of platelet activation in 44 normal CKCS (24 male, 20 female) who presented for a cardiac screening clinic. Dogs were divided into two groups (presence of murmur, and TP where platelet count estimated from peripheral smear was <200,000). Logistic regression analysis was used in each group to identify significant associations ($P<0.05$) with biomarkers of platelet activation, age and gender.

Murmurs consistent with MR and TP were noted in 7/44 (16%) and 16/44 (36%) of dogs respectively. There were only 4 dogs (9%) with

platelet counts <100,000. Subjective assessment of enlarged or giant platelets were seen in 12/44 (27%) of dogs with 8/12 (67%) dogs having TP. Risk of having a murmur was a significantly associated with increased age (P<0.001) while the percentage of monocytes with adhered platelets (P=0.012) was significantly associated with the absence of a murmur. Risk of TP was significantly associated with increases in the percentage of monocytes with adhered platelets normalized to platelet count (P=0.005), percentage of neutrophils with adhered platelets normalized to platelet count (P<0.001) and mean fluorescent intensity (MFI) of monocytes with adhered platelets normalized to platelet count (P<0.001), while the risk of TP was significantly associated (P=0.001) with decreased MFI of neutrophils with adhered platelets.

There was no apparent association between MR and platelet count or platelet activation but the study population was small. It is also possible that some dogs without murmurs may have had mild MR as echocardiographic studies were not performed. Evaluation of a larger number of dogs with MR including echocardiographic evidence of MR is warranted. It is also possible that difficult blood collection could have resulted in platelet activation obscuring a difference between groups. The data suggests that some biomarkers of platelet activation may be increased in CKCS with TP which could result in reduced platelet survival and may be a possible explanation for the TP. Further flow cytometric analysis of platelets from CKCS with TP, including surface glycoprotein density, may be helpful in further describing this clinical condition.

ABSTRACT #176

EVALUATION OF PLATELET AGGREGATION USING A POINT-OF-CARE INSTRUMENT IN RETIRED RACING GREYHOUNDS. A Lara Garcia[1], MC Iazbik[1], M Brooks[2], CG Couto[1]; [1]College of Veterinary Medicine, The Ohio State University, Columbus, OH; [2]Comparative Hemostasis Laboratory, Cornell University, Ithaca, NY.

With the increasing popularity of retired rescued Greyhounds, veterinarians are likely to evaluate dogs of this breed more frequently in their practice. Therefore, it is important that they recognize the physiological peculiarities of this breed. For instance, mean PCV, hemoglobin concentration, RBC, and whole blood viscosity are higher, while white blood cell, neutrophil, and platelet counts are lower than in other breeds. In vitro platelet aggregometry has not been extensively studied in Greyhounds due to the difficulty in obtaining platelet-rich plasma, and the bucal mucosal bleeding time has marked inter- and intraobserver variability, so it is not clinically reliable. The PFA-100 is a novel point-of-care platelet function analyzer which is considered one of the most sensitive methods to assess primary hemostasis and to diagnose von Willebrand's disease in humans. This device has recently been evaluated as a rapid method to assess platelet function in dogs. The objectives of this study were to characterize platelet function using the PFA-100 in a group of Greyhounds.

Blood samples were collected from the jugular vein from 46 healthy Greyhounds, ranging in ages from two to eight years old; there were 18 spayed females and 28 neutered males. CBC, biochemical profile, plasma vWF concentration by enzyme-linked immunosorbent assay (ELISA), collagen-binding assay (vWF:CBA), and platelet function assays with PFA-100 using collagen/epinephrine (COL-EPI) and collagen/adenosindiphosphate (COL-ADP) cartridges were performed.

Reference ranges for PFA-100 closure times were 60 to 180 seconds (mean ± SD; 83±37 seconds) using COL/ADP and 80 to 300 seconds using COL/EPI (mean ± SD;139±53 seconds); VWF concentration ranged from 22 to 120% (mean ± SD; 84±23%) and VWF:CBA ranged from 36 to 102% (mean ± SD; 77±18%); platelet count ranged from 144 to 265x10^3/µl (mean ± SD; 192±35x10^3/µl). There was no significant difference in platelet function when compared to a mixed population of 50 non-Greyhound dogs, even though the platelet count was significantly lower (p=0.001).

The PFA-100® constitutes a reproducible, clinically reliable way of evaluating platelet aggregation in Greyhounds. The results of this study support the fact that results of platelet aggregation studies using the PFA-100® in Greyhounds are similar to those in other breeds.

ABSTRACT #177

MOLECULAR CHARACTERIZATION OF HEREDITARY FACTOR VII DEFICIENCY IN THE BEAGLE. MB Callan[1], MN Aljamali[2], ME Griot-Wenk[3], ES Pollak[2], P Werner[1], U Giger[1], KA High[2]. [1]School of Veterinary Medicine, University of Pennsylvania; [2]Children's Hospital of Philadelphia, PA; and [3]University of Bern, Switzerland.

Hereditary factor VII (FVII) deficiency is clinically characterized by a variable bleeding tendency and has been reported in humans, as well as dogs. We describe here the molecular characterization of FVII deficiency in two unrelated Beagles, one from a research colony and the other a clinical patient with a history of hematochezia and persistent prolongation of prothrombin time (PT) following vitamin K therapy for possible anticoagulant rodenticide poisoning. The two affected Beagles had plasma FVII coagulant activity of 4% and 2%, respectively. Other plasma coagulation tests were normal, including the activated partial thromboplastin time (aPTT) and coagulation factor activities of factors XII, XI, IX, VIII, X, and II, and the FVII inhibitor screen was negative.

The cDNA of canine FVII (cFVII) was cloned based on conserved amino acid regions among published FVII sequences from several species. The cFVII cDNA sequence was utilized to search the dog genome database, and the eight exons of the cFVII gene were determined and sequenced based on genomic DNA samples from both affected Beagles. Comparison of these DNA sequences to the normal cFVII DNA sequence revealed a G to A missense mutation in exon 5 in the affected Beagles, resulting in substitution of glycine 96 (GGA) to glutamic acid (GAA) in the second epidermal growth factor-like domain. This novel FVII mutation has not been previously reported in human patients, although a different substitution (glycine to serine) at this residue results in human FVII deficiency. To investigate the effect of this mutation on FVII activity, site-directed mutagenesis was utilized to introduce the same mutation into the normal cFVII cDNA, and both normal and mutant cFVII constructs were transiently transfected into HEK cells. After a 48 hour incubation period, FVII activity in the supernatant from cells producing mutant cFVII was markedly reduced when compared to cells producing normal cFVII in a PT assay.

In order to screen other Beagles for the normal and mutant alleles, a restriction digestion-based screening test was established. The G to A mutation abolishes one restriction site for *Mnl*1 in PCR-amplified exon 5, indicated by generation of a 57 bp fragment that was not present in the digest of the normal allele. Screening of 17 closely related Beagles from a research colony with normal PT values revealed nine heterozygotes in addition to eight normal dogs.

Characterization of the mutation responsible for FVII deficiency in the Beagle and availability of a DNA-based screening test will aid in the identification of dogs that are homozygous or heterozygous for this coagulation defect and determination of the mutant allele frequency in the Beagle breed, allowing breeders and research colonies to eliminate this trait from their lines.

ABSTRACT #178

FELINE SERUM ANTIBODY RESPONSES TO CRANDALL REESE FELINE KIDNEY CELL INOCULATIONS AND CHARACTERIZATION OF TARGET ANTIGENS. JC Whittemore, WA Jensen, JR Hawley, SV Radecki, MR Lappin. From the Department of Clinical Sciences (Whittemore, Hawley, Lappin), Colorado State University, Fort Collins, CO; 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. Experimental cats that have been hypersensitized with CRFK lysates or inoculated with FVRCP vaccines develop antibodies against the cell line but the clinical significance is currently unknown. The objectives of this study were to further characterize the immunodominant CRFK antigens and determine whether these antigens are recognized by antibodies in the serum of client-owned cats.

By use of different gel characteristics, we previously reported the immunodominant CRFK antigens recognized by cats hypersensitized with CRFK lysates to be in the following molecular mass groupings: 46-49 kD, 41-43 kD, 37-39 kD, and 35-37 kD. In the current study, we optimized a western blot immunoassay to clarify antigens in this range of migration. The assay utilized pre-cast mini-gels (NuPage 10% Bis-Tris discontinuous buffer system with MOPS; Invitrogen, Carlsbad, CA), 65µg CRFK lysate per lane, 1:10 serum dilutions, and a 1:250 dilution of goat anti-cat IgG secondary antibody concentration (heavy chain only; Kirkegaard and Perry Laboratories, Gaithersburg, MD). Immunodominant antigens were determined by assaying two serum samples each from 14 cats hyperimmunized with CRFK lysates. Serum samples from 122 client-owned cats were assayed using the optimized CRFK western blot immunoassay.

Based on the numbers of CRFK hypersensitized cat samples recognizing antigens in the optimized western blot immunoassay, the immunodominant CRFK antigens were defined as 46.94 +/- 0.4 kD (n = 18), 40.43 +/- 0.45 kD (n = 11), and 37.51 +/- 0.21 kD (n = 9). Of the 122 client-owned cat sera, 65 recognized 0 antigens, 36 recognized one antigen, 14 recognized two antigens, and seven recognized all three antigens. Overall, 46.7% of the cats had serum antibodies that recognized an immunodominant CRFK antigen.

Results of this study document that antibodies in serum of client-owned cats recognize CRFK immunodominant antigens. Further studies will be necessary to determine whether presence of these antibodies is associated with clinical disease syndromes in cats.

ABSTRACT #179

CLINICAL FINDINGS AND ASSOCIATED CONDITIONS IN DOGS WITH RADIOGRAPHICALLY DIAGNOSED TRACHEAL COLLAPSE: 21 CASES. J Talavera, MJ Fernández del Palacio, A Bayón, A Albert. Veterinary Clinical Hospital. University of Murcia (Spain).

Tracheal collapse (TC) is a syndrome characterized by dorsoventral flattening of the tracheal rings with laxity of the dorsal tracheal membrane. Affected dogs are most frequently middle-aged to old of toy or miniature breeds. Considerable controversy surrounds the precise cause and it is best regarded as a syndrome with multifactorial causes. Investigations of the role played by the secondary factors that initiate the symptomatic state could contribute to a clearer understanding of the syndrome. The aim of this study was to evaluate the influence of associated conditions on clinical manifestations in dogs with TC.

Clinical records of 21 dogs with radiographic evidence of TC were included. Medical information was not considered during the inclusion procedure of the cases. Luminal narrowing of the cervical, inlet or intrathoracic trachea in expiratory or inspiratory lateral radiographic views was the only criteria for selection of cases. Later, corresponding clinical records were reviewed to determine signalment, clinical signs and underlying condition.

Of the 21 dogs included in the study, 11 were male and 10 were female. The median aged of dogs was 9.53 ± 2.69 years (range, 4 to 13.3 years) and weighing 7.74 ± 4.37 kg (range, 2.50 to 20 kg). The Yorkshire terrier was the most frequently represented breed (8/21, 38%). Other breeds presented were: Poodle (4/21, 19%), West Highland White Terrier (2/21, 10%), Cocker Spaniel (1/21, 5%) and Belgian Shepherd dog (1/21, 5%). Five mixed-breed dogs (24%) were also included. The most frequent clinical signs reported by the owner at the time of admission included coughing (18/21, 86%), noisy respiration (5/21, 24%), syncopal episodes (5/21, 24%), dyspnea (4/21, 19%) and exercise intolerance (2/21, 10%); 2 dogs (10%) had clinical signs not related with TC. Chronic mitral valve disease was the most frequently concomitant disease (4/21, 19%); rhinitis and pharyngitis was present in one dog (5%); 6 dogs (29%) presented miscellaneous diseases not related with respiratory system (dermatological, genital, orthopaedic and oncological diseases). TC was the only diagnostic in 11 dogs (52%). Obesity, as subjectively assessed, was reported in 16 dogs (76%). Of the five dogs without over-weight, two had chronic mitral valve disease and in the other three only TC was diagnosed.

In conclusion: (1) obesity is one important factor that precipitates and sustains the clinical syndrome of TC; (2) chronic mitral valve disease is one common concomitant disease that can acts like exciting factor, initiating or exacerbating the clinical signs of TC; (3) some dogs with primary TC can present the clinical syndrome even when secondary-initiating factors are not clinically evident.

ABSTRACT #180

WHOLE-BODY BAROMETRIC PLETHYSMOGRAPHY IN HEALTHY DOGS: INFLUENCE OF JET NEBULIZER AND BIAS FLOW RATE ON THE RESULTS OF A BRONCHOPROVOCATIVE TEST. J. Talavera[1*], F. Bernaerts[1], S. Schuller[1] , N. Kirschvink[2], C. Clercx[1]. [1]Department for clinical sciences B44, [2]Department for functional sciences B41, Faculty of veterinary medicine, University of Liège, Liège, Belgium.[*] Department of Animal Medicine and Surgery, University of Murcia, Spain.

Barometric whole-body plethysmography (BWBP) is a non-invasive pulmonary function test able to monitor airway responses to bronchoprovocative tests (BT). The provocative agent is nebulized into the plethysmograph while a continuous bias flow (BF) maintains the O_2 concentration and prevents CO_2 accumulation. The aims of this study were to assess the influence of type of jet nebulizer and of the BF rate on BWBP response to a BT with histamine.

Three healthy beagle dogs (males, 8 to 9 years, BW 17 to 20 kg) were used. The enhanced Pause (Penh) was used as an index of bronchoconstriction. BWBP variables were obtained before and after nebulization with sterile saline solution and with up to 6 increasing concentrations of histamine (0.1 to 1%) into the plethysmograph. The concentrations of histamine (expressed in %) that increased Penh to 200%, 300% and 400% of baseline (H-Penh200,H-Penh300 and H-Penh400) were obtained by interpolation of the dose-response curve. In each dog, the BT was repeated 6 times at one week interval, according to the type of nebulizer used (N1: Impec®, Aiglon, N2: LC Plus®, Pari and N3: Micron Ultrasonic®, San-up), to the rate of BT used with N1 (2, 8 and 10 L/min) and to the duration of nebulization with N3 (1 or 3 minutes). A one-way repeated measures ANOVA was used to test for differences in H-Penh indices ($p < 0.05$) and when significant, a pairwise multiple comparison procedure (Holm-Sidack) was used. Results are shown in table 1.

Table1: H Penh300 and H Penh400 in function of the nebulizer type and the BF rate

	MALBQ	MALBE	UPC0.5	UPC0.1	UAC100	UAC200
Sensitivity	35.6%	36.9%	4.5%	71.1%	5.3%	2.5%
Specificity	85.4%	91.7%	100%	18.8%	100%	100%

* significantly different from data obtained with N1 and a BF of 10L/min ($p<0.05$)

The present study showed that an important reduction in BF significantly increases the response and reduces H-Penh300 and H-Penh400, and that the type of nebulizer used plays a major role since H-Penh300 and 400 are significantly decreased with an ultrasonic nebulizer.

In conclusion, BT tests using BWPB must be carefully standardized.

ABSTRACT #181

BENEFICIAL EFFECTS OF CARDIAC RESYNCHRONIZATION ON QRS DURATION, DISTANCE WALKED AND LEFT VENTRICULAR FRACTIONAL SHORTENING IN DOGS WITH LONG-STANDING PACING-INDUCED HEART FAILURE—A PILOT STUDY.

Y Nishijima*, P Jenkins*, D Feldman***, J Bonagura*, C Carnes**, R Hamlin*, The Ohio State University College of Veterinary Medicine *, College of Pharmacy**, College of Medicine***, Columbus, OH.

Cardiac resynchronization therapy (CRT) is a treatment for heart failure in people with ventricular dysfunction and widened QRS complex. Rapid ventricular pacing (RVP) from the canine right ventricle leads to left ventricular dysfunction and a widened QRS complex. This pilot study was designed to determine the effects of synchronized atrial and biventricular pacing (CRT) on QRS duration, distance walked (D) in 6 minutes, and left ventricular fractional shortening (FS) in dogs with long-standing heart failure and widened QRS caused by rapid ventricular pacing.

Four dogs received RVP from a single site for up to 2 years. After 15 months of pacing, Heart failure was documented by a >36% reduction in FS, and RVP was continued at a rate of 120/minute to maintain cardiac dysfunction. In two dogs, single-site RVP was continued. In two other dogs RVP was combined with CRT by pacing the right atrium, right ventricular endocardium, and left ventricular epicardium (also 120/minute). Atrioventricular pacing intervals of 110 ms and 90 ms, and right-to-left ventricular pacing intervals of 12 ms and 4 ms respectively, were selected to maximize stroke volume (estimated by aortic flow-velocity integral and tissue Doppler imaging). QRS duration, distance walked (D), and left ventricular FS were measured before and after RVP (n=4), and again following 6 additional months of RVP from a single-site (n=2) or rapid pacing combined with CRT (n=2). Owing to small group numbers, results are presented as mean values without further statistical comparison.

Period	Dogs (n)	QRSd (ms)	D (m)	FS (%)
Baseline (pre-RVP)	4	46	533	38
RVP for 15 months	4	72	288	13
6 months RVP/single-site pacing	2	72	162	9
6 months RVP / CRT pacing	2	64	460	18

Compared to baseline, RVP of 15 months duration lengthened QRS duration, attenuated left ventricular FS, and reduced distance walked in all dogs. In the two dogs receiving CRT for an additional six months, the QRS duration was less prolonged and both FS and D were much higher when compared to dogs paced from a single right ventricular site. CRT appears to confer significant benefits to both physiological (QRS duration, left ventricular FS) and clinical (distance walked) parameters in dogs with heart failure and prolonged QRS caused by RVP. These data may be relevant to comparative studies of CRT, to pacemaker therapy of spontaneous canine bradyarrhythmias, and to advanced management approaches for dilated cardiomyopathy in dogs.

ABSTRACT #182

CATS WITH HYPERTROPHIC CARDIOMYOPATHY DO NOT HAVE ABNORMAL MYOCARDIAL CONTRAST ENHANCEMENT BY CARDIAC MAGNETIC RESONANCE IMAGING COMPARED TO NORMAL CATS.

KA MacDonald[1], ER Wisner[2], PH Kass[3], R Larson[2], MD Kittleson[1]. [1]Dept. Medicine and Epidemiology, [2]Dept. of Surgical and Radiological Sciences, [3]Dept. of Population Health and Reproduction, University of California, Davis.

Objective: Quantify the amount of myocardial contrast enhancement (MCE) of the left ventricle (LV) using cardiac MRI (CMRI) in cats with HCM and normal cats and determine if cats with HCM have different MCE than normal cats.

Animals: Ten normal domestic short-hair cats and 26 Maine Coon and Maine Coon-cross cats with moderate to severe hypertrophic cardiomyopathy (HCM) with no clinical evidence of congestive heart failure.

Methods: Cats were anesthetized with propofol administered as a constant rate infusion, and underwent gradient echo CMRI. Short-axis images of the left ventricle (LV) were acquired extending from the mitral annulus to the apex. Images were acquired before and seven minutes after intravenous gadolinium dimeglumine administration. The LV was divided into four quadrants including the anterior free wall, interventricular septum, posterior free wall, and lateral free wall. Regions-of-interest were manually traced within the quadrants of five mid-LV slices at end-systole. Myocardial contrast enhancement percentage (MCE) was calculated from summed weight-averaged data from all slices. Three-way repeated measures ANOVA was performed to identify whether cats with HCM have different MCE than normal cats. Simple linear regression was used to assess whether MCE was correlated with LV mass, LV mass index (LVMI), or diastolic function assessed by Doppler tissue imaging (DTI). A Student's T test was used to compare the standard deviations of the post-contrast myocardial signal intensity in cats with HCM compared to normal cats in order to evaluate the heterogeneity of the signal intensity of the myocardium.

Results: Only one of 26 cats with HCM and 0 of 10 normal cats had evidence of obvious discrete delayed enhancement on CMRI. There was no difference in MCE between the HCM cat group and the normal cat group. MCE did not correlate with LV mass, LVMI, or DTI. There was no difference in heterogeneity of signal intensities of LV myocardium in HCM cats when compared to normal cats.

Conclusion: Cats with moderate to severe HCM rarely have evidence of DE on cardiac MRI. Delayed enhancement CMRI may not be a useful indicator of diffuse myocardial fibrosis in cats with HCM.

ABSTRACT #183

AN IN VITRO MODEL OF CANINE RESISTANCE ARTERY FUNCTION TO STUDY SIGNALLING PATHWAYS INVOLVED IN VASOCONSTRICTION.

Bailey, SR and Elliott, J. Royal Veterinary College, London, UK.

The purpose of this study was to develop an *in vitro* model of resistance artery function in the dog, using tissues removed during ovariohysterectomy, to study the signalling pathways involved in vasoconstriction. Resistance arteries and arterioles influence peripheral blood pressure and may differ in their function from larger conducting vessels. Rho kinase may influence the response to vasoconstrictors by enhancing the sensitivity to intracellular calcium. Uterine tissue was obtained from healthy female dogs undergoing routine ovariohysterectomy. First and second order uterine arteries (normalised internal diameter 650 ± 98 μm) were dissected from the

tissue and mounted between wires attached to the jaws of a Mulvany-Halpern wire myograph. Resting tension was applied to the blood vessels and after an equilibration period of 30 min, a contractile response was obtained to a standard depolarising Krebs solution (DKS; 118 mM KCl). Following washout and re-equilibration, contractile responses were then obtained to cumulatively increasing concentrations of the α_1-adrenoceptor agonist, phenylephrine (10^{-8} – 10^{-4} M) in the presence and absence of the Rho kinase inhibitor, fasudil (3 μM; 30 min pre-incubation). Tension was continuously recorded by a computerised acquisition system and the data used to construct cumulative concentration-response curves, from which EC_{50} values and maximum responses were calculated by curve fitting software.

The optimum resting tension for these vessels (producing greatest contractions to DKS) was determined to be that producing a vessel circumference equivalent to an internal pressure of 100 mmHg (13.3 kPa), multiplied by a factor of 0.9. Phenylephrine produced concentration-dependent vasoconstriction with a maximum response of 19.64 ± 11.69 mN/mm (mean ± sem; n=4) and an EC_{50} value of 1.41 (1.32-1.51) $x10^{-6}$ M (mean with 95% confidence intervals). In the presence of fasudil, the responses to phenylephrine were inhibited, producing an EC_{50} value of 4.24 (2.4-6.5) $x10^{-6}$ M and a maximum response of 15.92 ± 11.04 mN/mm. These results demonstrate that robust contractile responses may be obtained from resistance arteries dissected from canine uterine tissue. Therefore, this tissue provides a very useful model of resistance vessel function in the dog. α_1-adrenoceptors mediate contraction in these arteries, and the Rho kinase signalling pathway may play a role in altering the degree of contraction attained.

ABSTRACT #184

THE EFFECT OF LONG TERM STORAGE ON THE CONCENTRATION OF BRAIN NATRIURETIC PEPTIDE IN FROZEN PLASMA OF CATS. KA MacDonald[1], TC Klose[1], CJ Munro[2], MD Kittleson[1]. [1]Department of Veterinary Medicine and Epidemiology; [2]Department of Population Health & Reproduction; School of Veterinary Medicine, University of California, Davis; CA.

Plasma BNP concentration ([BNP]) is useful in identifying and assessing severity of cardiac disease in veterinary and human patients. Serial analysis of [BNP] may be useful to assess response to pharmacologic therapy in patients with congestive heart failure. Since there is no rapid point of care assay for feline BNP, plasma samples must be stored and analyzed in batches using a time-consuming radioimmunoassay (RIA). There has been controversy in the literature as to optimization of sample storage, with variable results being reported. No studies have evaluated the effect of long-term storage of plasma with a protease inhibitor on [BNP].

The aim of this study was to determine the stability of brain natriuretic peptide (BNP) in plasma samples stored with a protease inhibitor at -70°C for several months.

Blood was collected from seven normal cats and 18 cats with moderate to severe hypertrophic cardiomyopathy and added to K_3EDTA and aprotinin (a protease inhibitor). Samples were immediately centrifuged and stored in aliquots at –70°C for 3-12 months. Plasma samples were extracted using Sep-Pack columns, and [BNP] was measured using an RIA. Samples were stored from one to seven months prior to the initial assay, and six to 12 months prior to a second assay. There were two subgroups defined by amount of time stored before baseline and second analysis. Group 1 samples were stored for approximately 1.5 months before baseline analysis, and for approximately 6 months before the second analysis. Group 2 samples were stored from approximately 6 months prior to baseline analysis, and approximately 11 months before the second analysis. A Student's paired T-test was used to compare whether [BNP] from baseline analysis was different than [BNP] at the second analysis of all samples, and of samples in Group 1 and Group 2 analyzed separately.

For the entire population, the mean [BNP] was 33 +/- 25 pg/mL at baseline. The mean of the second assay was 22 +/- 18 pg/mL. The average decrease in [BNP] was 30% +/- 44% over 141 days of storage between baseline and second analysis. A paired, two-tailed T-test showed a statistically significance ($p<0.001$) decrease due to storage. As can be seen by the standard deviation, the amount of decrease was highly variable. Group 1 and Group 2 had a comparable average amount of BNP degradation of 29% and 32% respectively.

In conclusion, plasma BNP is significantly degraded after 6-12 months of storage in optimal conditions. A correction factor may be necessary for samples stored for prolonged intervals from populations of cats before batch analysis. However, the variability identified in this study makes any correction factor impossible to identify and use in individual cats.

ABSTRACT #185

DIURETIC EFFICACY OF ORAL SPIRONOLACTONE WHEN USED IN CONJUNCTION WITH FUROSEMIDE IN HEALTHY ADULT GREYHOUNDS. Riordan L, Estrada A. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Spironolactone is an aldosterone-receptor blocker that acts at sites in the distal nephron of the renal tubules. While it is commonly used as a diuretic for the treatment of pulmonary edema, ascites, and for edema associated with nephrotic syndrome, there is no evidence of its diuretic efficacy when used in this manner. In a recent study presented at the 2004 ACVIM forum, spironolactone failed to induce diuresis when used alone in adult healthy dogs. However, because the diuretic efficacy of spironolactone is dependent on sodium delivery to the distal nephron, the use of other diuretics in conjunction with spironolactone may increase its diuretic activity. As spironolactone is most often used in conjunction with furosemide in the treatment of heart failure, it is important to determine its diuretic activity when used in conjunction with furosemide.

This study was conducted to assess the diuretic efficacy of oral spironolactone when used in conjunction with oral furosemide as compared to oral furosemide alone. Six healthy, adult male Greyhounds weighing 32 to 39 kg were used in a randomized, crossover design with a one week washout between treatments. Dogs were randomized to receive either furosemide alone or furosemide in conjunction with spironolactone. Two 24-hour urine collections were performed on each dog via a closed collection system for five dogs and free catch for one dog. Urine output, urine electrolyte concentrations, PCV/TS, serum electrolytes, serum creatinine, BUN, and body weights were determined every 6 hours during the collection period. For the two drug protocol, dogs received a mean spironolactone dose of 2.13 ± 0.22 mg/kg every 12 hours, PO. Because the maximum diuretic efficacy of spironolactone is not achieved until the third day of therapy, the drug was administered for three days prior to the 24-hour urine collection as well as on the day of the collection. Dogs received a mean furosemide dose of 3.05 ± 0.105 mg/kg every 12 hours, PO, on the day of urine collection.

No significant difference was noted between the two treatment groups for total urine volume, daily excretion of electrolytes (sodium, chloride, potassium, calcium), or change in body weight.

Results of this study suggest that spironolactone has no diuretic efficacy at a dose of approximately 2 mg/kg every 12 hours when used concurrently with furosemide in normal healthy male greyhounds. Further investigation, however, is warranted in dogs with heart disease in which activation of the renin-angiotensin-aldosterone pathway may lead to differing results.

ABSTRACT #186

PREVALENCE OF AZOTEMIA IN 124 DOGS WITH MYXOMATOUS VALVE DEGENERATION: A RETROSPECTIVE STUDY (2001-2004). A.P. Nicolle[1], V. Chetboul[1,2], J.-L. Pouchelon[1,2], D. Tessier-Vetzel[1,2], C. Carlos Sampedrano[1], H.P. Lefebvre.[3] [1]Cardiology Unit, National Veterinary School of Alfort, France. [2]INSERM U660, Maisons-Alfort, France. [3]UMR 181 Physiopathologie et Toxicologie Expérimentales INRA-ENVT, National Veterinary School of Toulouse, France.

One of the functional consequences of heart failure in dogs, often reported in veterinary literature, is the development of renal impairment and eventually of chronic renal failure. However this assumption has never been confirmed by original studies in dogs and cats. The primary aim of the present retrospective study performed on dogs affected by myxomatous valve degeneration (MVD) was to assess the prevalence of azotemia in the four different classes of heart failure (NYHA). The secondary objective was to identify any biological differences between azotemic and non-azotemic dogs.

The case records of dogs with MVD presented to the Cardiology Unit of Alfort between 2001 and 2004, were reviewed. The inclusion criteria were: dogs weighing less than 13 kg, with a known azotemia status and with an echocardiographic diagnosis of MVD. Information obtained from the medical records included signalment, initial physical examination findings, results of electrocardiography, blood pressure measurement, thoracic radiographs, echocardiography and, chemistry analysis. Dogs were categorized according to NYHA functional classification (I, II, III, IV).

124 dogs were included (age six to 17 years, weight 2 to 13 kg). The NYHA functional classes I, II, III, and IV comprised 45, 18, 37 and 24 dogs, respectively. No difference in age or body weight was found between the 4 NHYA groups. Half the dogs showed mild azotemia essentially due to an increased plasma urea concentration. The percentage of azotemic dogs rose with the functional class (31%, 50%, 59%, 70% for functional classes I, II, III, and IV, respectively; $p<0.01$). Azotemic dogs were older than non-azotemic dogs ($p<0.001$). No significant difference was found for treatment, or echocardiographic class. Azotemic dogs had higher heart rates (144 ± 34 *vs* 122 ± 38 bpm, respectively; $p<0.05$) and left atrium/aorta ratio (1.7 ± 0.7 *vs* 1.4 ± 0.5, respectively; $p<0.01$).

In conclusion, this study shows that the prevalence of azotemia increases with the NYHA class. The degree of azotemia is however mild. Further studies will be necessary to determine if and to what extent renal function, especially glomerular filtration rate, is altered in heart failure due to MVD.

ABSTRACT #187

DOES IN-HOSPITAL ELECTROCARDIOGRAM (ECG) REFLECT 24 HOUR HEART RATE PARAMETERS ASSESSED BY HOLTER MONITOR IN THE IRISH WOLFHOUND? Sarah A. Zimmerman, Janice M. Bright, E. Christopher Orton. Department of Clinical Sciences, Colorado State University.

Atrial fibrillation (AF) often accompanies and undoubtedly accelerates progression of dilated cardiomyopathy (DCM) in large and giant breed dogs, including Irish wolfhounds. Lone AF is known to be heritable in Irish wolfhounds and is now suspected to cause DCM by unrelenting tachycardia. Medical therapy for AF, whether primary or secondary, is directed at control of tachycardia by decreasing ventricular response rate to control clinical signs and prevent long term complications. Medical therapy is guided by intermittent in-hospital ECG, however periodic measurements may not reflect adequacy of heart rate control over a 24-hour period. Holter monitoring may more accurately reflect the adequacy of rate-control therapy for AF, especially during exercise and excitement. Thus, we compared in-hospital ECG (two minutes) with heart rate data obtained with 24 hour Holter monitor in 32 Irish Wolfhounds with AF. Study participants were evaluated by complete blood count, serum biochemistry, thyroid panel, Doppler echocardiographic examination, standard ECG and 24-hour Holter monitoring. The study population was 63% male and included dogs with lone AF (68%) and concurrent DCM. ECG average HR did not significantly correlate with Holter-determined average HR ($r = 0.302$, 95% CI -.073 to .602), maximum HR ($r = -.013$, 95% CI -.378 to .355), or hours of tachycardia ($r = -.356$, 95% CI -.639 to .013). Mean ECG average HR (157 ± 36 bpm) for all dogs was significantly higher ($p <.0001$) than mean Holter average HR (103 ± 27 bpm). Mean Holter peak HR and hours of tachycardia for all dogs was 262 ± 36 bpm and 9.438 ± 7.845 hours, respectively. In dogs with lone AF verses dogs with DCM there was no significant difference between ECG average HR ($p = .88$), mean Holter HR ($p = .82$), peak Holter HR ($p = .15$), or hours of tachycardia ($p = .40$). In summary, in-hospital average HR tended to overestimate average HR determined over a 24 hour period, and more importantly, did not predict mean HR, peak HR or hours spent in tachycardia. Further, the ventricular response rate of AF was not different in dogs with lone AF and DCM. Thus, in-hospital HR is probably not an adequate guide to medical therapy in Irish wolfhounds with lone AF or DCM and AF.

ABSTRACT #188

ASSESSMENT OF LEFT VENTRICULAR RADIAL AND LONGITUDINAL MYOCARDIAL MOTION BY 2D COLOR TISSUE DOPPLER IMAGING IN HEALTHY MAINE COON CATS. C. Carlos Sampedrano[1], R. Tissier[1,2], A.P. Nicolle[1], V. Gouni[1], J.-L. Pouchelon[1,2], V. Chetboul[1,2]. [1]Cardiology Unit of Alfort, [2]National Institute of Health and Medical Research (INSERM) U660, National Veterinary School of Alfort, France.

Tissue Doppler Imaging (TDI) is a non-invasive technique for evaluating regional left ventricular function. Previous studies have already shown the key role of TDI for detecting pre-clinical HCM before and independently of the development of left ventricular hypertrophy both in human patients and in a rabbit model of hypertrophic cardiomyopathy (HCM).

An inherited form of HCM has been described as a potential cause of heart failure, sudden death and arterial thromboembolism in Maine Coon cats. The early preclinical diagnosis of HCM in Maine Coon cats could have important implications since it should allow the early institution of medical treatment. Reference values for TDI parameters in this breed of cat are however needed for this. The aim of this study was to describe and analyze the left ventricular free wall (LVFW) motion in healthy non-sedated Maine Coon cats using quantitative 2D color TDI.

Twenty-three healthy young Maine Coon cats (age 2.1 ± 0.9 years; weight 5.0 ± 1.0 kg) were involved in the study. All cats were considered to be healthy, on the basis of a complete clinical examination, systemic arterial blood pressure measurement, ECG, and plasma biochemical tests. A standard echocardiographic and Doppler examination was also performed to confirm normal heart anatomy and function. Conventional echocardiography and 2D color TDI examinations were performed by the same trained observer on all cats. Radial LVFW velocities were recorded in endocardial and epicardial segments, and longitudinal velocities in the mitral annulus, and in the basal and apical LVFW segments. Isovolumic contraction and relaxation times were calculated in each myocardial segment and the coefficients of variation (CV, %) were determined for each TDI parameter.

LVFW velocities were higher in the endocardial than in the epicardial layers, and also higher in the basal than in the apical segments ($p<0.001$). Annular velocities were higher than basal myocardial velocities in systole ($p<0.001$) and early diastole ($p<0.05$). CV values were lower for radial velocities particularly in systole, and were also lower for time intervals (16 to 22%) than for myocardial velocities (19 to 62%). No correlation was found between body weight and any TDI parameter (velocities, time intervals). A

significant ($p<0.01$) correlation was observed between the fractional shortening and several systolic velocities (endocardial, basal and apical velocities).

In conclusion, 2D color TDI is a non-invasive technique that could complete conventional analysis of left ventricular function in Maine Coon cats. Further studies in diseased cats are however required to determine the sensitivity, specificity, and clinical relevance of these new cardiac indices.

ABSTRACT #189

NUTRITIONAL EFFECTS OF DIETARY MODIFICATION IN EARLY CANINE CHRONIC VALVULAR DISEASE. LM Freeman, JE Rush, PJ Markwell. Tufts University School of Veterinary Medicine, North Grafton, MA, USA & WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, England.

The purpose of this study was to assess the nutritional effects of a moderately reduced sodium diet enriched with n-3 fatty acids, antioxidants, B vitamins, and certain amino acids (cardiac diet) in dogs with chronic valvular disease (CVD). Dogs with asymptomatic CVD (International Small Animal Cardiac Health Council class 1a or 1b) were studied. After a baseline cardiac evaluation, blood was collected for a biochemistry profile and for measurement of thiamine, amino acids, vitamins C and E, 8-$F_{2\alpha}$-isoprostanes, nitrite/nitrate, and fatty acids. Urine glycosaminoglycans (GAGs) also were analyzed. After baseline measurements in the dogs with CVD were compared to those of healthy control dogs, all dogs with CVD were changed from their usual diets to a run-in diet to ensure that all were eating the same diet before starting the cardiac or placebo diets. After 4 weeks of exclusive feeding of the run-in diet, dogs were re-evaluated and then randomized to receive either the cardiac diet or a placebo diet exclusively in a double-blind fashion. A final evaluation was performed after dogs had eaten the cardiac or placebo diet for 4 weeks. All measurements obtained at baseline were repeated at the 4- and 8-week visits.

At baseline, there were no significant differences between the CVD (n=29) and healthy control groups (n=12) in median age (CVD: 8 yrs, range 4-14 yrs; Controls: 9 yrs, range 5-11 yrs) or median body weight (CVD: 8.9 kg, range 5.7-29.5 kg; Controls: 10.1 kg, range 3.9-21.0 kg). Dogs with CVD had significantly lower baseline serum sodium and chloride concentrations compared to the healthy controls. A number of plasma amino acids, including methionine, cystathionine, and arginine, also were found to be different between the CVD dogs and healthy controls at baseline. After 4 weeks of exclusively eating either the cardiac (n=14) or placebo (n=15) diets, vitamins C and E, cholesterol, and triglyceride concentrations increased significantly more in the cardiac diet group. Thiamine concentrations increased significantly in the cardiac group but not in the placebo group and there was a significant decrease in taurine concentrations for dogs in the placebo group but not in the cardiac diet group. Plasma nitrite/nitrate concentrations increased significantly in both diet groups. These results suggest that dietary modification can alter certain circulating antioxidants and amino acids that may be beneficial in dogs with early CVD.

ABSTRACT #190

CARDIOVASCULAR EFFECTS OF DIETARY MODIFICATION IN EARLY CANINE CHRONIC VALVULAR DISEASE. JE Rush, LM Freeman, PJ Markwell. Tufts University School of Veterinary Medicine, North Grafton, MA, & WALTHAM Centre for Pet Nutrition, Melton Mowbray, Leicestershire, England.

The purpose of this study was to assess the cardiovascular effects of a moderately reduced sodium diet enriched with n-3 fatty acids, antioxidants, B vitamins, and certain amino acids (cardiac diet) in dogs with chronic valvular disease (CVD). Dogs with CVD, classified in either International Small Animal Cardiac Health Council class 1a or 1b were studied. After a complete physical examination, standard 2-D, M-mode, and color-flow Doppler echocardiography, blood pressure (Doppler technique), and a six-lead electrocardiogram were performed. Blood was collected for C-reactive protein (CRP), n-terminal atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and aldosterone. Baseline measurements in the dogs with CVD were compared to those of healthy control dogs, and then all dogs with CVD were changed to a highly sodium-restricted run-in diet to ensure that all were eating the same diet before starting the cardiac or placebo diets. After 4 weeks of exclusive feeding of the run-in diet, dogs were re-evaluated and then randomized to receive either the cardiac diet or a placebo diet exclusively in a double-blind fashion. A final evaluation was performed after dogs had eaten the cardiac or placebo diet for four weeks. All measurements obtained at baseline were repeated at the 4- and 8-week visits.

At baseline, dogs with CVD (n=29) had significantly higher ANP concentrations ($P=0.001$) compared to healthy controls (n=12) but there was no difference in aldosterone, BNP, or CRP concentrations between the two groups. Dogs with CVD had significantly larger hearts than the healthy control group based on several standard and weight-corrected (WC) echocardiographic measurements. After eating the highly sodium-restricted run-in diet for 4 weeks, the 29 dog with CVD had a significant elevation in aldosterone ($P<0.001$) but no change in ANP or BNP. After 4 weeks of eating either the cardiac (n=14) or placebo (n=15) diets, aldosterone decreased significantly in both the cardiac diet and placebo groups but the change was larger in the placebo diet group. There was no significant difference between the cardiac and placebo diet groups in terms of the changes in ANP, BNP, or CRP. Cardiac size (ie, maximal left atrial size, left ventricular internal dimension in diastole, WC left ventricular myocardial area in systole, WC maximal left atrial size, WC interventricular septum in systole, and WC left ventricular outer dimension in systole) decreased significantly more in the cardiac diet group compared to the placebo diet group. These results suggest that dietary modification can alter some neuroendocrine and echocardiographic parameters in dogs with early CVD.

ABSTRACT #191

ECHOCARDIOGRAPHIC EVALUATION OF THE SYSTOLIC FUNCTION IN HEALTHY DOGS UNDERGOING DIPYRIDAMOLE STRESS TEST. Marlos Gonçalves Sousa[1], Daniel Paulino-Júnior[1], Roberta Carareto[1], Gláucia Bueno Pereira Neto[1], Aparecido Antonio Camacho[1; 1] Faculty of Agronomical and Veterinary Sciences, São Paulo State University; Campus of Jaboticabal, São Paulo, Brazil.

Dipyridamole is extensively used in the detection of myocardial ischemia in human beings. However, despite considered a safe and sensitive technique for predicting cardiac events, the lack of data regarding the effect of this drug in animals refrains it from being widely used in veterinary medicine. In dogs, the effects of dipyridamole on systolic function are still not exactly known.

Therefore, the aim of this study was to evaluate the systolic function of healthy dogs undergoing dipyridamole stress echocardiography. For such, six adult female mongrel dogs, with mean weight of 15.93 Kg, were evaluated before (M0) and at 5 (M1), 15 (M2), 25 (M3), 35 (M4), and 45 (M5) minutes after the infusion of dipyridamole (0.5 mg/Kg diluted to a 10-mL volume, intravenously over 4 minutes). Dogs underwent two-dimensional echocardiographic evaluation with simultaneous EKG recording. Left-ventricular short axis images were acquired from the right parasternal view at the pappilary muscles level. We evaluated the left-ventricular end-systolic diameter (LVESD), fractional shortening (FS), ejection fraction (EF), end-systolic left-ventricular volume index (ESLVVI), stroke index (SI), and cardiac index (CI). Care was taken to obtain the same views at every moment of evaluation.

Results are listed in Table 1. When data was submitted to ANOVA, a significant decrease was determined for LVESD and ESLVVI, whereas a significant increase was observed in FS and EF. Such differences were further analyzed by the Tukey-Kramer multiple comparisons test, which showed LVESD to differ from baseline value only at M2 ($P<0.01$). For ESLVVI, M1 ($P<0.05$) and M2 ($P<0.001$) differed from baseline value (M0). For FS, baseline value was statistically different from M1 ($P<0.01$) and M2 ($P<0.001$), while for EF, M1 ($P<0.001$) and M2 ($P<0.001$) differed from M0. Despite not considered significant, a moderate increase in CI and SI was also observed.

Results allowed concluding that dipyridamole stress testing demands an increased systolic function reserve, which is likely to be related to vasodilation and increased myocardial oxygen consumption. However, these changes are reversible and transitory, returning to baseline values about 25 minutes after a single administration of the drug. Since the test was safe and well tolerated by the animals, it could be used to provide and effective screening test in dogs with mild left ventricular dysfunction, which is difficult to detect at rest.

Table 1: Echocardiographic parameters of systolic function from dogs undergoing dipyridamole stress test. Data expressed as mean ± SD.

	M0 Baseline	M1 5 minutes	M2 15 minutes	M3 25 minutes	M4 35 minutes	M5 45 minutes	P
LVESD	2.25 ± 0.31	1.90 ± 0.19	1.73 ± 0.15	2.08 ± 0.20	2.10 ± 0.13	2.32 ± 0.19	0.0002
FS	31.8 ± 3.4	42.5 ± 5.0	46.1 ± 3.6	37.3 ± 4.5	33.3 ± 4.2	30.5 ± 4.0	< 0.0001
EF	61.0 ± 4.4	74.6 ± 5.0	78.5 ± 3.3	68.1 ± 5.4	63.3 ± 5.9	59.0 ± 5.6	< 0.0001
ESLVVI	26.7 ± 5.6	17.6 ± 4.0	14.0 ± 3.8	22.4 ± 6.3	22.5 ± 3.4	29.0 ± 4.1	< 0.0001
SI	42.8 ± 9.9	52.0 ± 10.2	51.3 ± 10.3	49.0 ± 18.3	39.4 ± 7.3	46.9 ± 14.7	0.4521
CI	5.25 ± 1.70	8.74 ± 3.11	7.27 ± 1.44	6.44 ± 3.42	5.09 ± 1.68	5.54 ± 1.65	0.0738

ABSTRACT #192

ASSESSMENT OF ELECTROCARDIOGRAPHIC PARAMETERS IN HEALTHY DOGS SUBMITTED TO DIPYRIDAMOLE STRESS TEST. Marlos Gonçalves Sousa[1], Daniel Paulino-Júnior[1], Roberta Carareto[1], Gláucia Bueno Pereira Neto[1], Aparecido Antonio Camacho[1, 1] Faculty of Agronomical and Veterinary Sciences – São Paulo State University; Campus of Jaboticabal, São Paulo, Brazil.

Dipyridamole stress testing (DST) is a useful alternative technique to exercise testing for evaluating human beings suspected of ischemic heart disease. Due to coronary vasodilation, dipyridamole leads to an increase in coronary blood flow, myocardial oxygen consumption, and cardiac output. In veterinary medicine, DST is still not widely used, especially owing to the lack of data concerning its effects in animals. In dogs it is still uncertain whether dipyridamole can result in arrhythmias or electrical conduction disturbances.

As DST is still in the investigational phase in veterinary medicine, this work was conceived to investigate electrocardiographic features in dogs submitted to dipyridamole stress test. For such, six adult female mongrel dogs, with mean weight of 15.93 Kg, were evaluated before (M0) and at 5 (M1), 15 (M2), 25 (M3), 35 (M4), and 45 (M5) minutes after the infusion of dipyridamole (0.5 mg/Kg diluted to a 10-mL volume, intravenously over 4 minutes). Dogs were placed on a table in right lateral recumbency and a 6-lead computerized EKG was recorded. We evaluated the duration and amplitude of P wave (Pms and PmV), duration of QRS complex (QRSms), R wave amplitude (RmV), PR interval (PRms), QT interval (QTms), and RR interval (RRms). ST segment abnormalities and arrhythmias were also searched.

Results are shown in Table 1. Statistical analysis of the data by ANOVA showed a significant decrease only for RR ($P=0.0082$). After applying Tukey-Kramer test to this parameter, it was seen that only M1 ($P<0.01$) and M2 ($P<0.05$) differed from the baseline value of RRms. Furthermore, no arrhythmias or abnormal deflections were observed during this experiment.

Results allowed concluding that dipyridamole neither change the route of atrial and ventricular depolarization nor leads to the development of malignant arrhythmias. Furthermore, the mild increase in heart rate was short-lived and did not result in ST segment shifts. Thus, DST was concluded to be a safe and feasible stress test to be performed in dogs.

Table 1: Electrocardiographic parameters from dogs undergoing dipyridamole stress test. Data expressed as mean ± SD.

	M0 Baseline	M1 5 minutes	M2 15 minutes	M3 25 minutes	M4 35 minutes	M5 45 minutes	P
Pms	48.8 ± 3.9	49.0 ± 5.8	48.8 ± 8.2	51.0 ± 8.0	47.8 ± 5.9	49.3 ± 5.8	0.9769
PmV	0.23 ± 0.06	0.25 ± 0.07	0.27 ± 0.06	0.24 ± 0.05	0.25 ± 0.05	0.24 ± 0.05	0.8880
QRSms	57.1 ± 6.5	58.3 ± 6.3	57.1 ± 6.2	58.8 ± 4.8	58.3 ± 6.9	59.5 ± 7.8	0.9867
RmV	1.05 ± 0.39	0.95 ± 0.34	1.01 ± 0.40	1.00 ± 0.40	1.03 ± 0.43	1.07 ± 0.44	0.9967
PRms	102.6 ± 13.0	89.0 ± 13.6	92.8 ± 11.6	95.6 ± 13.2	95.5 ± 14.4	103.3 ± 21.3	0.5307
QTms	199.8 ± 25.8	189.0 ± 17.6	190.5 ± 22.2	197.8 ± 20.5	200.0 ± 25.5	206.6 ± 23.0	0.7697
RRms	511.0 ± 109.7	374.3 ± 79.6	401.3 ± 97.4	462.5 ± 127.8	457.5 ± 103.2	500.6 ± 101.5	0.0082

ABSTRACT #193

PHARMACOKINETICS OF A SINGLE INTRAVENOUS DOSE OF DILTIAZEM IN HORSES. C.C. Schwarzwald, R.A. Sams, J.D. Bonagura. Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH.

Atrial fibrillation (AF) is the most common cardiac arrhythmia affecting racing performance in horses. Diltiazem is used for heart rate control in the treatment of AF in other species and its hemodynamic and electrocardiographic effects have been reported in normal horses. The goal of this study was to determine the pharmacokinetics of diltiazem in horses. Diltiazem, 1 mg/kg IV over five minutes, was administered to eight healthy horses. Venous blood samples were collected before and 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 720, and 1440 minutes after administration of diltiazem. Plasma was harvested, snap-frozen, and stored at -80°C until analysis. Plasma concentrations of diltiazem and its major metabolite (deacetyldiltiazem) were determined using high-performance liquid chromatography. Data were analyzed by nonlinear least squares regression with relative weighting ($1/y^2$). A biexponential equation $Cp(t) = A \bullet e^{-\alpha t} + B \bullet e^{-\beta t}$, representing a two-compartment model with first-order drug elimination, best described the data. Pharmacokinetic variables were calculated: Diltiazem concentration at time 0, $C_0 = A+B$; distribution half-life, $t_{1/2\alpha} = 0.693/\alpha$, elimination half-life, $t_{1/2\beta} = 0.693/\beta$; area under the plasma concentration time curve, $AUC = A/\alpha+B/\beta$; area under the first moment curve, $AUMC = A/\alpha^2+B/\beta^2$; total plasma clearance, Cl_t = Dose/AUC; volume of distribution of the central compartment, V_c = Dose/(A+B); volume of distribution during the terminal phase V_t = Dose/(AUC•β); volume of distribution at steady state V_{ss} = Dose•AUMC/AUC^2; mean residence time, MRT = AUMC/AUC; distribution and elimination constants, $k_{21} = (A \bullet \beta + B \bullet \alpha)/(A+B)$, $k_{10} = \alpha \bullet \beta / k_{21}$, and $k_{12} = \alpha+\beta-k_{21}-k_{10}$. The AUC for diltiazem and its major metabolite deacetyldiltiazem were compared using the trapezoid method. Intravenous administration of diltiazem was well tolerated. The median peak plasma concentration was 727 (539-976) ng/mL. The median (min-max) pharmacokinetic variables of diltiazem were: C_0 696 (472-853) ng/mL, $t_{1/2\alpha}$ 12 (6-23) min, $t_{1/2\beta}$ 93 (73-161) min, AUC 63.8 (47.7-86.9) µg•min/mL, AUMC 8,185 (5,147-15,296) µg•min^2/mL, Cl_t 16.0 (11.5-21.0) mL/kg/min, V_c 1.4 (1.2-2.1) L/kg,

V_t 2.2 (1.8-3.2) L/kg, V_{ss} 2.1 (1.6-2.8) L/kg, MRT 126 (100-208) min, k_{12} 0.018 (0.006-0.035) min^{-1}, k_{21} 0.046 (0.022-0.071) min^{-1}, k_{10} 0.010 (0.008-0.014) min^{-1}. The ratio of the AUC of deacetyldiltiazem to that of diltiazem was 0.08 (0.06-0.16). A second metabolite was detected, but could not be identified and quantified due to the lack of an authentic standard. Plasma concentrations were similar to those required in healthy horses to produce consistent hemodynamic and electrocardiographic effects, but higher than therapeutic concentrations for treatment of AF in dogs and humans (60-300 ng/mL). The disposition of diltiazem dosed at 1 mg/kg IV to healthy horses is characterized by a rapid distribution and an elimination phase with a terminal half-life shorter than reported in humans (2–5 h), dogs (2.2–4 h) and cats (2 h). Due to the low plasma concentrations and reported low pharmacologic activity, drug metabolites are not considered clinically important.

ABSTRACT #194

RELATIONSHIP BETWEEN VENTRICULAR PREMATURE CONTRACTIONS (VPC) AND FATTY ACID CONCENTRATIONS IN BOXERS VERSUS DOBERMAN PINSCHERS. LM Freeman, CE Smith, KM Meurs, JE Rush, A Lamb, D Bibus. Tufts University School of Veterinary Medicine, North Grafton, MA, The Ohio State University College of Veterinary Medicine, Columbus, OH, and Lipid Technologies, Austin, MN.

Ventricular arrhythmias are a common problem in certain dog breeds with cardiomyopathy, such as Boxers and Doberman pinschers. These arrhythmias can complicate the underlying cardiac disease and can cause sudden death. There is growing evidence for a relationship between specific types of dietary fat intake and arrhythmia in people and animal models, as well as for the therapeutic use of certain fatty acids in cardiac disease. Although fatty acid abnormalities have been documented in dogs with heart failure, a relationship between fatty acid concentrations and arrhythmia has not been demonstrated in dogs with naturally-occurring disease. This is of particular interest in dogs at high risk for ventricular arrhythmia and sudden death. The purpose of this study was to determine whether 1) there is a difference in fatty acid concentrations between Boxers and Doberman pinschers; 2) there is a relationship between fatty acids and the number of VPCs; and 3) this relationship is different between the two breeds. Boxers and Doberman pinschers being evaluated by 24-hour Holter monitor recording and for which a blood sample was available were included in the study. The total number of VPCs per 24 hours was counted. A circulating fatty acid profile was performed for each dog using gas chromatography. Fatty acid concentrations are reported as the percent normalized concentrations, and were compared between the two breeds using independent t-tests. Spearman correlations were used to compare fatty acid concentrations to the number of VPCs/24 hours.

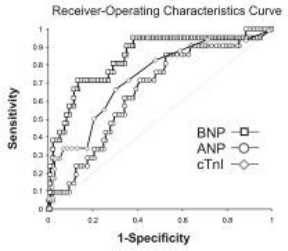
Forty-four Boxers and 18 Doberman pinschers were included in the study. The median number of VPCs/24 hours for all dogs was 21 (range, 0-11,395) but was higher in the Boxers (median=39, range 0-11,395) than in the Doberman pinschers (median=4, range 0-8,873). Compared to the Doberman pinschers, Boxers had significantly higher concentrations of stearic acid (C18:0), γ-linolenic acid (C18:3ω6), eicosanoic acid (C20:0), eicosenoic acid (C20:1ω9), eicosatrienoic acid (C20:3ω9), docosanoic acid (C22:0), and docosatetraenoic acid (C22:4ω6), but had lower concentrations of α-linolenic acid (C18:3ω3) and arachidonic acid (C20:4ω6). Total ω-6 fatty acids were higher in the Doberman pinschers, while total ω-9 fatty acids were higher in the Boxers. There was a significant difference between the two breeds in the relationship between VPC number and total n-3 fatty acids. These data suggest that some fatty acid concentrations differ between Boxers and Doberman pinschers. In addition, the relationship between fatty acid concentrations and the frequency of VPCs may be different in these two breeds. Studies to further investigate these relationships are warranted.

ABSTRACT #195

PROSPECTIVE SCREENING FOR OCCULT CANINE DILATED CARDIOMYOPATHY USING B-TYPE AND ATRIAL NATRIURETIC PEPTIDE AND CARDIAC TROPONIN-I ASSAY. Mark A. Oyama[1], D. David Sisson[2], Phil F. Solter[3]. [1]Department of Veterinary Clinical Medicine, [3]Department of Veterinary Pathobiology, University of Illinois, Urbana, IL; [2]Department of Veterinary Clinical Sciences, Oregon State University, Corvallis, OR.

The detection of occult dilated cardiomyopathy (DCM) in dogs is typically accomplished using ECG, echocardiography, and 24-hour ambulatory ECG monitoring. These procedures are time-consuming, expensive, and require specialized equipment, which makes widespread, routine, or serial screening problematic. The pathophysiology of DCM is characterized by alterations in circulating cardiac neurohormones and biomarkers, and blood-based detection of B-type natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and cardiac troponin-I (cTnI) may provide an attractive alternative to conventional screening. A threshold value of these substances would identify a subset of dogs with a high likelihood of occult disease and trigger the performance of additional diagnostics, such as ECG and echocardiography. Accordingly, we sought to determine the ability of BNP, ANP, and cTnI assay to prospectively detect occult DCM in a population of asymptomatic and ostensibly healthy dogs that belonged to breeds known to be at high risk for development of DCM.

One hundred and eighteen client-owned dogs were examined by ECG, echocardiography, and BNP, ANP, and cTnI assay. Occult DCM was diagnosed if dogs met any of the following criteria: Doberman pinschers, LV end-diastolic diameter >46mm (>49mm if body weight >37kg), LV end-systolic diameter >38mm; Doberman pinschers and Boxers, presence of ventricular premature beat(s) during a 5-minute lead II rhythm strip; all other breeds, fractional shortening <22%. Twenty-one dogs were diagnosed with occult DCM and 97 dogs were considered normal. A BNP level of 6.21pg/ml possessed a sensitivity of 95.2% and specificity of 61.9% for identifying dogs with occult disease. In contrast, ANP and cTnI possessed relatively low predictive value. Blood-based detection of occult DCM can be accomplished using BNP assay and further work should be performed to optimize this test to assist in the screening of canine patients.



ABSTRACT #196

COMPARATIVE ANALYSIS OF CYTOKINE SIGNATURES IN THE CEREBROSPINAL FLUID OF HEALTHY HORSES AND HORSES WITH SELECTED NEUROLOGICAL DISORDERS. N. Pusterla[1], C.M. Leutenegger[1], P.A. Conrad[2], B.C. Barr[3], W.D. Wilson[1]. [1]Department of Medicine and Epidemiology, [2]Department of Pathology, Microbiology and Immunology, [3]California Animal Healthy and Food Safety Laboratory System, School of Veterinary Medicine, University of California, Davis, CA.

The goal of this study was to determine the gene transcription of selected cytokines in the cerebrospinal fluid (CSF) of healthy horses and horses with cervical stenotic myelopathy (CSM), West Nile virus (WNV) encephalitis and spinal cord trauma using TaqMan PCR. The study material consisted of CSF collected at necropsy from 30 horses (12 healthy horses, eight horses with confirmed CSM, four horses

with confirmed WNV encephalitis and six horses with confirmed spinal cord trauma). Total RNA was extracted from the spinal fluid nucleated cells, transcribed to complementary DNA and assayed for equine GAPDH, TNF-α, IFN-γ, IL-2, IL-6, Il-8, IL-10, iNOS and TGF-β by TaqMan PCR. Final quantitation of cytokine transcription was done using the comparative C_T method and was reported as relative transcription or the n-fold difference relative to a calibrator (weakest value across all target genes for normal CSF samples).

The housekeeping gene GAPDH was expressed in all samples, reflecting a successful RNA extraction and cDNA transcription. The cytokine profiles expressed by nucleated cells from the spinal fluid of healthy horses was a balance between pro-inflammatory (TNF-α), anti-inflammatory (IL-6, IL-10) and Th1 (IL-2, IFN-γ) cytokines and growth factor (TGF-β). Horses with CSM expressed elevated TNF-α, absent IL-6 and normal TGF-β, IL-10 and IL-2. The cytokine profile of horses with WNV was characterized by high IL-6, absent TNF-α. Horses with spinal trauma expressed elevated IL-6 and normal IFN-γ and TGF-β. Interleukin-8 was not detected in any CSF sample and iNOS was only expressed by one healthy horse.

Despite the small number of samples for each group, our preliminary results seem to suggest distinct gene signatures expressed by nucleated cells in the CSF of healthy horses and horses with different inflammatory neurological disorders. Cytokine profiles could in the future contribute to the differential diagnosis in situations where conventional laboratory parameters fail to provide diagnostic clues.

ABSTRACT #197

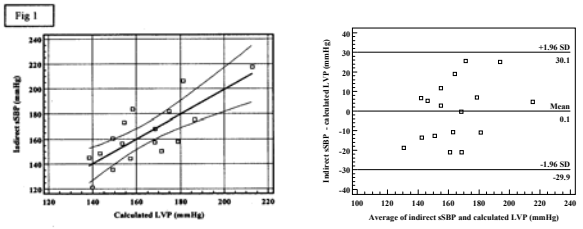
ECHOCARDIOGRAPHIC ESTIMATION OF SYSTEMIC SYSTOLIC BLOOD PRESSURE IN DOGS WITH MILD MITRAL REGURGITATION. SP Tou, DB Adin. University of Florida College of Veterinary Medicine, Gainesville, FL.

Systemic hypertension is likely underdiagnosed in veterinary medicine, as blood pressure (BP) measurement often relies on clinical suspicion. According to Bernoulli's equation ($PG= 4v^2$), mitral regurgitation (MR) velocity should approximate systolic left ventricular pressure (sLVP), assuming a normal LA pressure (LAP), and therefore systolic systemic blood pressure (sSBP) in the absence of aortic stenosis (AS). The use of echocardiography to estimate sSBP using Bernoulli's equation has not been evaluated in veterinary medicine. We hypothesized that echocardiography would accurately estimate sSBP in dogs with mild MR.

Dogs with mild MR secondary to myxomatous mitral valve degeneration were studied. Inclusion criteria were absence of AS, MR with a clear continuous-wave (CW) signal, and LA:Ao of ≤ 1.7 to assume a normal systolic LAP of 8 mmHg. Five simultaneous, blinded CW measurements of maximum MR velocity (V_{max}) and indirect sSBP measurements (using Parks Doppler) were obtained for each dog. Pressure gradient (PG_{max}) was calculated from V_{max} using Bernoulli's equation, averaged and added to an assumed LAP of 8 mmHg to calculate sLVP. The relationship between calculated sLVP and indirectly measured sSBP was determined with simple linear regression and bias was determined by Bland-Altman analysis.

Seventeen dogs met the inclusion criteria. Calculated sLVP was significantly correlated with indirectly measured sSBP within a range of 140-220 mmHg (P= 0.0002, r= 0.78) (Fig 1). Mean ± SD bias was 0.1 ± 15.3 mmHg with limits of agreement of –29.9 to 30.1 mmHg (Fig 2).

Fig. 2



We conclude that calculation of sLVP using MR V_{max} and PG_{max} is significantly correlated with indirectly measured sSBP in dogs with mild MR across the range of BP measurements examined. Further studies are needed to assess correlation over a greater range of BP measurements. Despite the significant correlation, the wide limits of agreement between the methods may limit the clinical utility of echocardiographic estimation of BP and emphasize the clinical need for indirect sSBP measurements.

ABSTRACT #198

CARDIAC TROPONIN I IN DOGS NATURALLY INFECTED BY *EHRLICHIA CANIS*. P.P.V.P Diniz[1], H.S.A. de Morais[2], D. S. Schwartz[1]. [1] School of Veterinary Medicine and Animal Science – São Paulo State University (FMVZ – UNESP), Botucatu, São Paulo, Brazil and [2] School of Veterinary Medicine, University of Wisconsin, Madison.

Ehrlichia canis is a highly prevalent infectious agent in South America causing systemic illness in dogs. Infected dogs may show cardiac involvement, but its importance and prevalence are not known. Circulating cardiac troponin I (cTnI) is a highly specific and sensitive marker for myocardial cell damage in many mammalian species. Thus, we investigated serum cTnI concentration in dogs naturally infected with *E. canis,* clinically ill dogs not infected by *E. canis*, and healthy dogs. One hundred ninety three consecutive dogs seen at a Veterinary Teaching Hospital population in Botucatu, southeast Brazil, were selected. Dogs were included when showing at least three of the following inclusion criteria: presence of ticks, hyperthermia, bleeding, neurological signs, inflammatory ocular disorders, anemia, leukopenia, thrombocytopenia and hyperproteinemia. Patients were excluded if they had a previous diagnosis of heart disease, had received anti-rickettsial medications within 30 days or cardiotoxic drugs, or have undergone cardiopulmonary resuscitation. *Ehrlichia canis* DNA was amplified in 109 dogs by nested PCR. These dogs were negative for *Babesia canis* (semi-nested PCR), *Leishmania chagasi* (indirect fluorescent antibody test) and for eight different serovars of *Leptospira interrogans* (microscopic agglutination test). The remaining 41 clinically-ill dogs were negative for *E. canis*, *B. canis*, *L. chagasi* and *L. interrogans*. Twenty healthy dogs were used as controls. Serum cTnI was measured using human cTnI kits (Dade Behring Dimension RxL clinical chemistry analyzer). Statistical analysis was performed using non-parametric Kruskal-Wallis test. Magnitude of association of risk for myocardial injury was determined by odds ratio (OR) with a 95% confidence interval (CI). Differences were considered significant if $p<0.05$. The cTnI concentration in *E. canis* infected dogs was 0.36 ± 1.11 ng/mL (mean ± SD; median = 0.06 ng/mL, P_{25} = 0.02; P_{75} = 0.18 ng/mL) being elevated (>0.07 ng/mL) in 44% of those dogs. cTnI concentrations in clinically-ill dogs without ehrlichiosis was 0.28 ± 0.93 ng/mL (mean ± SD; median = 0.04 ng/mL, P_{25} = 0.0 and P_{75} = 0.11 ng/mL) being elevated in 32 % of those dogs. The cTnI concentration in controls was 0.02 ± 0.05 ng/mL (mean ± SD; median = 0.0 ng/mL, P_{25} = 0.0 and P_{75} = 0.01 ng/mL). cTnI was higher than in controls in both groups of ill dogs ($p<0.05$). Dogs with ehrlichiosis (OR 7.08; 95% CI 1.57 – 32.03; p = 0.016) are more likely to have increased cTnI than healthy dogs, whereas systemically ill dogs without ehrlichiosis were not (OR 4.18; 95% CI 0.84 – 20.7; p = 0.604). Our results show that almost half of dogs with ehrlichiosis and no apparent heart disease had increased concentration of cTnI suggesting myocardial involvement. They are also seven times more likely to have myocardial injury than healthy dogs. It cannot be inferred if myocardial damage is a direct effect of the organism, a consequence of systemic inflammatory response syndrome or a bias of selection from our inclusion criteria.

ABSTRACT #199

LONG-TERM EFFECTS OF PIMOBENDAN AND BENAZEPRIL ON SEVERAL ECHOCARDIOGRAPHIC AND TISSUE DOPPLER IMAGING VARIABLES IN DOGS WITH ASYMPTOMATIC MYXOMATOUS MITRAL VALVE DISEASE.

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Long-term administration of benazepril (BNZ), an angiotensin-converting enzyme inhibitor, has already been shown to improve quality of life, increase exercise tolerance and extend life expectancy in dogs with naturally acquired NYHA class II-IV heart failure due to myxomatous mitral valve disease (MVD) or dilated cardiomyopathy (DCM). Pimobendan (PIMO), an oral inodilator compound, has been demonstrated to result in significant improvement in heart failure class when added to standard therapy in dogs with DCM and is also registered in many countries for use in canine congestive heart failure due to MVD. The cardiac effects of both BNZ and PIMO on dogs with asymptomatic (class I) MVD are however unknown. The aim of this study was to compare the cardiac effects of long-term monotherapy with PIMO or BNZ in dogs with asymptomatic MVD and mild mitral valve regurgitation.

The study was a blinded, randomized and parallel-group design. Twelve female Beagle dogs (age 6.8 ± 2.1 years) with class I MVD and a grade 1 or 2 left apical systolic heart murmur were selected. They were randomized into two groups of six animals (BNZ and PIMO groups). BNZ and PIMO were administered orally for 206 days at the recommended dosages (0.25 mg/kg sid and 0.25 mg/kg bid for the BNZ and PIMO groups, respectively). All dogs were examined before the start of treatment and then at 15, 49, 124 and 206 days following initiation of therapy (cardiovascular examination, ECG, blood pressure, standard echo-Doppler examination and analysis of the left ventricular myocardial motion via 2D color tissue Doppler imaging).

Some differences between the PIMO and BNZ groups were already detected at day 15. The amplitudes of the differences increased throughout the experimental period and were maximal at day 206. The most striking differences were observed for the following parameters at day 206 for the PIMO and BNZ groups, respectively: heart murmur (increased in average by two grades in the PIMO group), fractional shortening (45.0% *vs* 34.7%, $p<0.001$), systolic endocardial (7.5 *vs* 5.2 cm.s-1, $p=0.001$) and basal velocities (9.4 *vs* 7.0 cm.s-1, $p<0.001$), maximum mitral regurgitant jet area/left atrium area (37.7% *vs* 14.8%, $p<0.001$) and peak velocity of mitral regurgitant jet (6.5 *vs* 2.6 m.s-1, $p<0.001$).

In conclusion, these preliminary results should encourage investigators to perform long-term studies in well-controlled conditions to clearly identify the benefit to risk ratio of the currently recommended treatments for naturally occurring canine MVD.

ABSTRACT #200

URINE ISOPROSTANE CONCENTRATIONS, AN IN VIVO BIOMARKER OF OXIDATIVE STRESS, IN NORMAL DOGS AND DOGS WITH ASYMPTOMATIC CHRONIC DEGENERATIVE VALVE DISEASE IN THE PRESENCE AND ABSENCE OF CHRONIC CARVEDILOL THERAPY.

S.G. Gordon, A.B. Saunders, M.A. McMichael, M.W. Miller, K. Glaze, C.G. Ruaux, J.M. Steiner, and D.A. Williams. Department of Small Animal Clinical Sciences & Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Urine isoprostanes (UIP) are a by-product of free radical action on arachidonic acid and a marker of in vivo oxidative stress. Free radical oxidative stress is associated with the progression of many cardiovascular diseases. Agents that reduce oxidative stress may delay disease progression. Carvedilol (Coreg ®) is a third-generation non-selective beta-blocker with ancillary $alpha_1$-blocking and antioxidant properties.

The purpose of this study was to measure UIP concentrations in normal dogs and dogs with chronic degenerative valve disease (CVD) either not receiving or receiving chronic oral carvedilol therapy at a target oral dose of approximately 1 mg/kg PO q 12 hours for at least 1 month. Normal dogs were matched for signalment and defined as normal based on physical examination, thoracic radiographs, indirect systemic blood pressure, ECG, echocardiogram, complete biochemistry panel, complete blood cell count and urinalysis. The mean age of all dogs was 9.2+/-0.4 years (mean+/-SE) and there was no significant difference in age between groups. UIP was extracted from urine samples by affinity chromatography and UIP were measured using a commercially available enzyme immunoassay kit that has been validated for the measurement of UIP in canine urine (Cayman Chemical, Ann Arbor MI).

The mean +/-SE UIP were as follows; normal signalment matched dogs 5.8+/-0.7 pg/ml/mg creatinine/dl +/-SE (n=18), asymptomatic dogs with cardiomegaly secondary to CVD receiving no carvedilol 5.1+/-0.8 pg/ml/mg creatinine/dl +/-SE (n=15), asymptomatic dogs with cardiomegaly secondary to CVD receiving carvedilol 2.0+/-0.5 pg/ml/mg creatinine/dl +/-SE (n=10). Mean UIP in normal dogs was not significantly different than in dogs with asymptomatic CVD not receiving carvedilol ($p=0.2$). Mean UIP in dogs with asymptomatic CVD not receiving carvedilol was significantly greater than in CVD dogs receiving carvedilol ($p=0.02$).

Chronic treatment with oral carvedilol at clinically relevant doses significantly reduces UIP in dogs with asymptomatic cardiomegaly secondary to CVD providing indirect evidence of the in vivo antioxidant effects of carvedilol. Further evaluation of UIP in spontaneous canine cardiovascular disease stratified by clinical stage and disease etiology may help define the role of oxidative stress in the progression of canine cardiovascular disease.

ABSTRACT #201

SERUM PROCOLLAGEN TYPE III AMINOTERMINAL PEPTIDE, AN IN VIVO BIOMARKER OF FIBROSIS, IN NORMAL DOGS AND DOGS WITH SPONTANEOUS CARDIOVASCULAR DISEASE.

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The major collagen types present in normal and diseased hearts include types I and III. Excessive deposition of collagen resulting in fibrosis causes reduced myocardial compliance and potentiation of arrhythmias. Progressive fibrosis generates an increase in serum products of collagen turnover. Procollagen type III aminoterminal peptide (PIIINP) is one biomarker of this process. Increased serum PIIINP concentrations were initially identified in patients with fibrotic diseases of the liver and lung, osteoarthritis, neoplasia, renal failure, and several cardiac diseases including systemic hypertension, dilated cardiomyopathy (DCM), primary valvular disease, and coronary artery disease. In human heart failure patients, elevated PIIINP correlates with functional heart failure class and is associated with an increased mortality. The purpose of this study was to measure serum PIIINP in normal dogs and dogs with spontaneous cardiovascular disease.

Dogs were defined as normal based on physical examination, thoracic radiographs, indirect systemic blood pressure, ECG, echocardiogram, complete biochemistry panel, complete blood cell count and urinalysis. Normal dogs were divided into two groups for analysis based on age; young dogs (n=18, $\leq$ 4 years of age; mean 3

years) and older dogs (n=29, > 5 years of age; mean 8.6 years). Seven dogs with spontaneous cardiovascular disease were available for analysis and were grouped as follows, asymptomatic heart disease (AHD, n=5) and congestive heart failure (CHF) receiving conventional therapy including spironolactone (n=2). Serum PIIINP was measured using a commercially available radioimmunoassay kit (Orion Diagnostica, Ispoo, Finland). This kit has been previously validated by other investigators for the measurement of canine PIIINP in serum.

The mean +/- SE serum PIIINP were as follows: young normal dogs 16.0 +/- 1.1 ug/L, older normal dogs 10.2 +/- 0.7 ug/L, AHD 12.1 +/- 2.1 ug/L, and CHF+spironolactone 8.5 +/- 4.9 ug/L. There was a significant difference between young and old normal dogs ($p=0.0002$). Differences were not significant between old normal and AHD groups or between AHD and CHF+spironolactone. Serum PIIINP was evaluated as a function of age and demonstrated a significant correlation ($r=0.56$, $p<0.05$). Serum PIIINP was highest in young dogs, declined and stabilized at five years of age with no significant correlation to age thereafter. These data serve as a normal reference range for serum PIIINP in dogs. Future evaluation of PIIINP may offer insight into the pathogenesis of canine cardiovascular disease and provide a scientific rationale for the evaluation of antifibrotic agents.

ABSTRACT #202

SYSTOLIC ANTERIOR MOTION OF THE MITRAL VALVE ASSOCIATED WITH RIGHT VENTRICULAR PRESSURE LOAD IN 8 DOGS. Christopher F. Paige, Jonathan A. Abbott, R. Lee Pyle. Virginia Tech, Blacksburg, VA.

Systolic anterior motion of the mitral valve (SAM) is generally associated with the hypertrophic cardiomyopathy phenotype. Experimental studies and post-mortem findings suggest that abnormal orientation of left ventricular papillary muscles with or without concurrent structural abnormalities of the mitral valve leaflets predispose to SAM. It is believed that SAM results when abnormal drag forces act on the mitral valve causing anterior displacement of leaflet tissue with potential consequences of left ventricular outflow tract (LVOT) narrowing and mitral valve regurgitation. It is established that SAM is labile and factors that provoke or augment SAM include diminished preload, diminished afterload and increased inotropic state.

We retrospectively identified eight canine patients in which SAM was echocardiographically detected in association with disorders that imposed a pressure load on the right ventricle. Five of the patients had severe pulmonic stenosis, two had tetralogy of Fallot and one, cor pulmonale. In four cases, the administration of acepromazine was associated with the recognition of SAM and in one case the finding was associated with probable hypovolemia related to extra-cardiac disease. SAM was recorded in various two-dimensional echocardiographic planes. In all cases, mitral leaflet tissue was displaced dorsocranially during systole and spanned at least 50% of the distance from the region of leaflet coaptation to the interventricular septum. Relative to body-weight, the end-diastolic left ventricular dimension was subnormal in all patients. Hyperdynamic left ventricular systolic performance was identified in all patients. In five of the eight patients, SAM was mild or moderate in degree. Left ventricular outflow tract obstruction and mitral valve regurgitation were documented by Doppler studies in only two of the three patients with marked SAM. However, late systolic acceleration within the LVOT was recorded in two additional patients for which peak velocities were normal.

Hypertrophic cardiomyopathy is considered to be rare in canine patients. In the cases described here, the presence of SAM is likely explained by alterations in left ventricular geometry and function associated with diminished pulmonary venous return together with sympathetic activation resulting from subnormal cardiac output. Although the hemodynamic consequences were apparently minor, the association of SAM with right-sided heart disease might be of interest to those engaged in the practice of veterinary echocardiography.

ABSTRACT #203

CARDIOVASCULAR DEVICE RELATED INFECTIONS. Deborah M. Fine[1], Anthony H. Tobias[2], Alan W. Spier[1]. [1]University of Missouri, Columbia MO; [2]University of Minnesota, St. Paul MN.

Infection of cardiovascular devices is an uncommon and serious complication. Medical records of dogs that were presented to the veterinary teaching hospitals of the University of Minnesota and University of Missouri were reviewed from June 2001 to December 2004. During that period, 27 patent ductus arteriosus (PDA) device occlusion procedures were performed, and 43 transvenous pacemaker systems (PM) were placed. Two (7.4%) PDA occlusion devices and five (11.6%) PM became infected. All dogs received intra-procedural cephalosporin antibiotics.

Both of the PDA occlusion device infections occurred in puppies (16 and 14 weeks of age), and *Pastuerella* spp. were grown from blood cultures (*P. canis* and *P. multocida*, respectively). One puppy developed a seroma at the femoral incision site that resolved with hot-packing. No evidence of an incisional infection was found when the puppy was presented showing lethargy, anorexia, and pyrexia 46 days post-procedure. That puppy was euthanized without treatment, and bacteria were found within the device and in multiple organs at necropsy. The second puppy was presented five days post-procedure showing similar clinical signs as the first. Ticarcillin with clavulanic acid was administered IV for two weeks, followed by oral amoxicillin/clavulanic acid. After one month of antibiotic therapy, that puppy is asymptomatic and has a normal leukogram.

The five dogs with PM infections ranged in age from seven to 12 years. A break in sterile technique occurred in one dog during PM placement, but there were no other known procedural complications. Median time from procedure to presentation for clinical signs of PM infection was 80 days (range: 21 to 419). The most common presenting complaint was swelling at the PM site or jugular vein. Only two of the five dogs were febrile at initial presentation. *Pseudomonas aeruginosa* was cultured from the PM site of two dogs. *Staphylococcus* spp. was cultured from the PM site of 3 dogs, and 2 subsequently developed *Pseudomonas aeruginosa* infections.

Survival following PM infections ranged from 208 to > 940 days. All dogs were initially treated with antibiotics. Two underwent PM removal with replacement following resolution of infection. One of these dogs is alive and has been off all antibiotics for > 890 days. The second dog recovered from PM infection, and was off antibiotics for 190 days, but subsequently died from sepsis following ear canal ablation for chronic *Pseudomonas aeruginosa* otitis. Three dogs were treated with antibiotics alone. One dog died from complications ascribable to chronic PM infection, one receives antibiotics chronically and has persistent draining fistulae from the PM site, and one dog has been infection-free and off all antibiotics for > 320 days.

Optimal therapy of an infected cardiovascular device involves antibiotics and removal of the implant. Whereas some patients may be effectively treated with antibiotics alone, chronic therapy is usually necessary and clinical cure with medical therapy alone is uncommon.

ABSTRACT #204

DILATED CARDIOMYOPATHY IN DOGS WITH CONGESTIVE HEART FAILURE: PROGNOSTIC FACTORS AND INFLUENCE OF ATRIAL FIBRILLATION ON SURVIVAL. J Bronsoiler, MR O'Grady, SL Minors, ML O'Sullivan. University of Guelph, Guelph, Ontario, Canada.

The aim of this study was to determine the impact of breed and atrial fibrillation (AF), as well as additional parameters on survival of

dogs with Dilated cardiomyopathy (DCM) and congestive heart failure (CHF). This 20-year retrospective study involved the medical records from the Ontario Veterinary College. 275 dogs with a diagnosis of DCM were included based on decreased fractional shortening (FS ≤ 15%) and absence of primary valvular disease or other primary cardiac disease, or a DCM diagnosis on necropsy.
Breeds were divided in four categories: giant (dogs >40 kg); large (dogs > 25 kg and < 40 kg); spaniels; and Doberman pinschers. Exclusion criteria included suspect tachycardia or doxorubicin induced DCM. Day of enrolment was taken as the first visit with a diagnosis of CHF requiring furosemide therapy. 49 parameters were investigated. The end-point of the study was taken as the date of death. A multivariable Cox proportional hazard model with censoring was used to identify significant parameters of survival (P<0.05).
Survival in days for dogs with DCM and CHF:

	N	Mean	Median
All Dogs	275	184	59

Breeds	No AF			AF at enrollment			AF after enrollment		
	N	***Mean***	***Median***	***N***	***Mean***	***Median***	***N***	***Mean***	***Median***
Giant	11	113.27	64	29	176.22	29	4	280	347
Large	23	293.85	164	13	492.38	36	1	618	618
Spaniel	17	856.5	736	2	61.5	61.5	0		
Doberman	110	95.71	55.5	41	56.32	9	24	85.08	78.5

A complex model involving breed categories, heart rate, use of ACE inhibitors, presence of AF, age, frequency of ventricular premature beats (VPCs), FS, % increase in LVID-S over expected, and wall stress index in diastole and systole as main effects or as interactions was developed. Parameters that reduced survival when considered as main effects were: absence of ACE inhibitor therapy, AF at onset, reduced FS, and a % increase in LVID-S. A model was developed to construct survival curves for dogs with DCM and CHF using these significant parameters. Enrollment of more dogs is necessary to allow the investigation of additional parameters and improve accuracy of the model.

ABSTRACT #205

Abstract Withdrawn.

ABSTRACT #206

CANINE SECONDARY INTRACRANIAL NEOPLASIA: A RETROSPECTIVE STUDY (1986-2003). Jessica Mikszewski, Lisa Lipitz, Katherine Skorupski, Thomas Van Winkle, Frances Shofer, and Christiane Massicotte. Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

The purpose of this study was to investigate the frequency, location, and clinical findings associated with secondary brain tumors in dogs that presented between the years 1986 and 2003 to the Veterinary Hospital of the University of Pennsylvania. Secondary brain tumors (n=177) outnumbered primary brain tumors (n=173) in dogs during this time period.

Medical records of 177 dogs with secondary intracranial neoplasms confirmed on post-mortem examination were identified. Parameters available were reviewed, including signalment, clinical signs and duration, thoracic radiographs, results of cerebrospinal fluid analysis, and advanced imaging. Locations of the secondary brain tumors as well as other unrelated neoplasms identified on post-mortem examination were recorded.

Of the 177 secondary brain tumors, 51 (29%) were hemangiosarcomas, 44 (25%) were pituitary tumors, 21 (12%) were lymphosarcomas, 20 (11%) were metastatic carcinomas, and 11 (6%) were nasal tumors that invaded the brain. Smaller numbers of histiocytic sarcomas (n=8), malignant melanomas (n=6), and osteosarcomas (n=3) were identified. The average age at diagnosis was 9.6 +/- 3.0 years. Mixed breed dogs were most often represented (n=47; 26%), followed by Golden retrievers (16%) and Labrador retrievers (6%). Most tumors were located within the telencephalon (n=88), and a mentation change (n=77) was the most common presenting clinical complaint, followed by seizures (n=42). Of 144 tumors for which a location in the brain was recorded at necropsy, 44 were multifocal in their distribution in the brain. On postmortem examination, the secondary intracranial tumor was present in the lung in 84 cases (47%), in the kidney in 62 cases (35%), and in the heart in 55 cases (31%). Less common sites of metastasis included the liver (n=52); spleen (n=38); lymph node (n=35); and bone (n=15). Other neoplasms unrelated to the secondary brain tumor were identified on post-mortem examination in 32 dogs (18%). Abnormalities were present in 59 of 108 dogs (55%) that had thoracic radiography performed.

In conclusion, hemangiosarcomas are the most common secondary brain tumors in dogs, followed by pituitary neoplasms. Based on the results of this study, secondary intracranial tumors are more common than primary intracranial tumors in dogs. Signalment and presenting clinical signs are similar for dogs with primary and secondary intracranial neoplasia. Thoracic radiographs and abdominal ultrasonography are indicated to look for metastatic disease or extracranial neoplasia prior to advanced imaging of the brain.

ABSTRACT #207

INFLAMMATORY MYOPATHIES WITH HISTIOCYTIC PREDOMINANCE IN DOGS. CW Dewey[1], PF Moore[2], GD Shelton[3]. [1]Long Island Veterinary Specialists, Plainview, NY; [2] Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis, Davis, CA; [3] Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, CA.

Inflammatory myopathies characterized by extensive infiltration of histiocytes into endomysial and perimysial connective tissue have recently been described in humans. Such disorders have been named macrophagic myofasciitis (MMF) or inflammatory myopathy with abundant macrophages (IMAM). MMF has been linked to the administration of aluminum hydroxide-containing vaccines. IMAM has similar histological features without the vaccine association and is a more aggressive disease. In addition to the characteristic histiocytic infiltrates, there are CD8+ lymphocytic infiltrates and a lack of muscle fiber damage. Similar histological features including lack of myofiber damage and endomysial and perimysial infiltration of dendritic cells expressing CD1c/CD11c/CD4 and macrophages expressing CD11b have recently been identified in five dogs.

Clinical features of MMF and IMAM in people include myalgia, arthralgia, muscle weakness, and occasional fever. Serum creatine kinase (CK) levels and electromyography are often normal, probably because of a lack of direct involvement of muscle fibers by the inflammatory process. The majority of people with MMF respond to immunosuppressive therapy. Five dogs with clinical signs of myopathy and muscle biopsies containing extensive histiocytic infiltrations were evaluated. Three dogs were Rottweilers, one was a Rottweiler mixed breed, and one was a Labrador retriever dog. The average age at presentation was 4.2 yrs (range, 3-6 yrs); there were four female spayed dogs and one male neutered dog. Historical/clinical findings included generalized muscle atrophy (two dogs), lethargy (two dogs), unilateral facial swelling in the temporal (two dogs) and masseter (one dog) regions, jaw pain/difficulty opening the jaw (two dogs), abnormal pelvic limb gait (two dogs), apparent myalgia (two dogs), fever (two dogs), appendicular stiffness/lameness (two dogs), and dysphagia/hypersalivation (one dog). Creatine kinase levels were measured in three dogs; results were normal in two dogs and elevated in one dog. Electromyography was normal in the dog for which it was performed. Two dogs were tested for serum antibody against type 2M fibers; both were negative.

Contrast enhancement of the epaxial and temporalis musculature was demonstrated on MRI in one dog. Four dogs were treated with immunosuppressive doses of prednisone, to which three responded favorably. One of these dogs was switched to oral azathioprine, due to the development of glucocorticoid-associated side effects.

To the authors' knowledge, neither MMF or IMAM has yet been described in dogs. Whether the recently identified histiocytic disorder(s) in dogs is similar to that of humans or a distinct disease is yet to be determined, but warrants further study.

ABSTRACT #208

BIOMARKERS IN CEREBROSPINAL FLUID SHOWING NEURONAL DEGENERATION IN SHIBA DOGS WITH GM1-GANGLIOSIDOSIS. Hiroyuki Satoh, Osamu Yamato, Toyofumi Yamauchi, Tomoya Asano, Masahiro Yamasaki and Yoshimitsu Maede. Hokkaido University, Sapporo, Japan.

GM1-gangliosidosis is a lysosomal storage disease which results from hereditary deficiency of acid ß-galactosidase. In both human and veterinary medicine, there is no cure but symptomatic treatment. Some novel therapies for this disease, such as gene therapy, enzyme replacement therapy, chemical chaperone therapy, or anti-inflammatory therapy, are being developed and need further investigation into their efficacy. Based on clinical, pathological and biochemical observations, canine GM1 gangliosidosis is considered to be an excellent model for human disease.

Since 2000, we have maintained a closed breeding colony of Shiba dogs with GM1 gangliosidosis. The homozygous affected Shiba dogs manifest neurological symptoms of progressive motor dysfunctions starting from five to six months of age and then die by 15 months of age, and the clinical course is associated with the severity of pathological degeneration and the level of GM1 ganglioside accumulation in the CNS.

The aim of this study was to determine biomarkers in CSF to evaluate the pathological degeneration in CNS of these Shiba dogs by using CSF obtained from living individuals. As some possible CSF biomarkers, GM1 ganglioside, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), neuron specific enolase (NSE) and myelin basic protein (MBP) were chosen, and the concentrations or activities of these biomarkers were measured in four affected dogs and three control dogs. In result, these biomarkers were markedly high in the affected dogs compared with the control dogs, and the levels of the biomarkers were also associated with age of the dogs. In additon, preliminarily, an affected dog was treated with prednisolone (0.5 mg/kg EOD PO for eight months) when it started showing symptoms at five months of age. However, this trial did not affect either the clinical course or the CSF biomarkers compared with those of non-treated dogs.

The results in the present study suggested that GM1 ganglioside, AST, LDH, NSE and MBP may be utilized as CSF biomarkers showing CNS degeneration in Shiba dogs with GM1-gangliosidosis. These biomarkers seem to be useful for assessment of new therapeutic methods.

ABSTRACT #209

THE USE OF ULTRASONOGRAPHY TO DIAGNOSE "CAUDAL OCCIPITAL MALFORMATION SYNDROME" IN DOGS – A PROSPECTIVE STUDY IN 12 DOGS.

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Caudal occipital malformation syndrome (COMS) is a developmental abnormality in dogs, and is considered the canine analog of Chiari Type I malformation in humans. The goal of this study was to determine the utility of cervical spinal cord ultrasonography (U/S) to complement magnetic resonance imaging (MRI) for the diagnosis of COMS. In this preliminary report we describe the procedure and compare U/S to MRI findings in dogs with and without COMS.

This study included 12 dogs that presented to TUSVM for signs referable to the cervical spinal cord and/or brain that had craniocervical MRI. The study group consisted of nine dogs that were diagnosed with COMS, and the control group consisted of three dogs that had normal craniocervical MRI or had abnormalities that were not consistent with COMS. All dogs had real-time cervical U/S performed. Sonographic images were obtained by a board certified radiologist with a 5-8 MHz transducer. No anesthesia was required. A dorsal midline acoustic window through the cisterna magnum and a dorsolateral oblique acoustic window through the atlanto-axial intervertebral foramen were used. The reminder of the neck was scanned but no acoustic window was found. Specific attention was made to the presence of cerebellar coning, "medullary kink", syringohydromyelia, and ventricular enlargement. The central canal appeared as faint anechoic line with two parallel echogenic lines. The width of the central canal was measured using sagittal views. All other components were evaluated subjectively.

All study dogs were diagnosed with COMS based on previously described MRI findings. The three control dogs had no MRI findings diagnostic for COMS. U/S findings that were categorized as diagnostic of COMS required measured syringohydromyelia >0.1cm and "medullary kink", and could include cerebellar coning and ventricular enlargement. U/S findings that raised the suspicion of COMS required "medullary kink" and mild to moderate cerebellar coning, and could include measured syringohydromyelia <0.1cm. The absence of these findings was considered a normal study. Based on these categories 4/9 study dogs had U/S diagnosis of COMS, 3/9 study dogs were suspicious of COMS, and 2/9 study dogs were normal. All control dogs were normal.

This preliminary study indicates that cervical spinal cord ultrasound can be useful as a diagnostic aid for COMS. It cannot rule out a diagnosis of COMS, however no false positives were found. To investigate the sensitivity and specificity of this imaging modality blinded U/S examination of large numbers of dogs after MRI evaluation is planned.

ABSTRACT #210

QUANTITATIVE ELECTROMYOGRAPHY IN CANINE MYOTONIC MYOPATHY. Jessica Mikszewski, David Aleman, Patty O'Donnell, Karyn Cullen, and Charles Vite. Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Myotonia congenita has been described in the Miniature Schnauzer and a DNA test is available to diagnose heterozygotes and homozygotes. Previous reports discuss the clinical disease in homozygotes and the subjective clinical and electromyographic response in these dogs to treatment with drugs that block dynamic sodium-conduction channels. The purpose of this project was to describe electromyographic abnormalities in heterozygotes for the condition and to measure the effect of procainamide hydrochloride and phenytoin in homozygotes using quantitative electromyography.

Seven homozygotes and four heterozygotes were available for quantitative electromyography. This study was approved by the Institutional Animal Care and Use Committee and conducted in accordance with guidelines established by the Animal Welfare Act and the NIH. The dogs were anesthetized and measurements were recorded from the thoracic interosseous, pelvic interosseous, and cranial tibial muscles. Additional measurements were taken from homozygotes following 10 mg/kg phenytoin administered intravenously and 40 mg/kg procainamide administered intravenously. Continuous electrocardiography was performed throughout the electrodiagnostic testing, and serum concentrations of

phenytoin and procainamide were obtained following intravenous administration.

Heterozygous dogs had abnormal spontaneous activity (myotonic discharges) on electromyography; however, the mean duration of the myotonic discharge was less for heterozygous when compared to homozygotes. The duration of the myotonic discharge in homozygous dogs was not significantly different following the administration of 40 mg/kg procainamide and 10 mg/kg phenytoin. Both procainamide and phenytoin caused changes in the electrocardiogram at these doses.

In conclusion, heterozygotes for myotonic myopathy in the Miniature Schnauzer show the presence of myotonic discharges on needle electromyography. This is also the case in human heterozygous carriers of recessive myotonia congenita. However, the myotonic discharges in Miniature Schnauzers are shorter in heterozygotes than in homozygotes. In homozygous dogs, the duration of the myotonic discharge is not improved by the administration of procainamide or phenytoin, despite anecdotal evidence of clinical improvement in some patients receiving these drugs.

ABSTRACT #211

THE USE OF ORAL LEVETIRACETAM AS AN ADD-ON ANTICONVULSANT DRUG IN CATS RECEIVING PHENOBARBITAL. CW Dewey[1], G Barone[1], DM Boothe[2], K Smith[1], HJ O'Connor[3]. [1]Long Island Veterinary Specialists, Plainview, NY; [2]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL; [3]Glens Falls Animal Hospital, Queensbury, NY.

The only anticonvulsant known to be safe and effective for use in cats with seizure disorders is phenobarbital (PB). Bromide has been demonstrated to be fairly ineffective as an anticonvulsant drug in this species, and often causes an idiosyncratic allergic pneumonitis. Oral diazepam, though often effective in controlling seizures in cats, occasionally is implicated as the cause of acute fatal hepatic necrosis in this species. Veterinarians are understandably reticent to try some of the newer anticonvulsant drugs currently used for dogs for feline seizure disorders, due to the potential for severe side effects. Levetiracetam (LEV) is a new anticonvulsant drug that does not undergo any hepatic metabolism in people or dogs. It has been shown to be effective as an add-on anticonvulsant in both species, with no apparent side effects at recommended dose regimens. Four cats with seizure disorders that were poorly controlled with PB alone were treated with oral LEV as an add-on drug at a dose regimen of 20 mg/kg body weight, q 8 h. Three of the four cats were suspected to have idiopathic epilepsy. One cat had an intracranial meningioma (removed surgically) and subsequently developed a pancreatic insulinoma. Surgery for the insulinoma was declined; the cat's hypoglycemia was managed with prednisone and diazoxide. The mean PB level prior to LEV was 35.8 ug/ml. After receiving LEV for a minimum of one week, serum samples for LEV concentrations were drawn at times 0, 2h, 4h, and 6h post-administration. These samples were assayed for LEV via HPLC at a commercial laboratory. The seizure frequency after LEV institution was estimated and compared with the estimated pre-LEV seizure frequency for an equal time period. Owners were questioned about side effects, and all patients were re-examined by one or more of the authors at regular intervals. Follow-up CBC and blood chemistry evaluations were performed for all four cats for a minimum of three months. None of the four cats exhibited any side effects attributable to LEV therapy. With the exception of the cat with the insulinoma (mildly increased ALT), bloodwork abnormalities were limited to slightly elevated SAP in two cats. LEV serum concentrations were within the reported therapeutic range for people (5-45 ug/ml) for all samples in all cats. The overall average serum LEV level for all cats was 16.5 ug/ml (range: 6.9-24.3 ug/ml). The median serum half-life (t ½) of elimination for LEV in cats was 5.3 h. Seizure frequency was reduced by an average of 30.5% in 3 cats, and increased by 33.3% in one cat. The results of this pilot study suggest that levetiracetam is a safe drug for cats that may provide some therapeutic benefit when used as an add-on to phenobarbital.

ABSTRACT #212

THE ROLE OF MICROGLIAL ROS GENERATION IN THE PATHOGENESIS OF DEMYELINATION. V.M. Stein[1], W. Baumgärtner[2], A. Zurbriggen[3], M. Vandevelde[3], A. Tipold[1], Department of Small Animal Medicine[1], Institute of Pathology[2], School of Veterinary Medicine, University of Hannover, Germany, Institute of Animal Neurology[3], University of Berne, Switzerland.

Microglial cells are the immune effector elements of the brain. In response to pathological changes in the central nervous system (CNS) they become activated and can exert multiple functions in order to regain homeostasis such as cell to cell interaction, phagocytosis, and generation of nitric oxide and reactive oxygen species (ROS). This microglial response has long been believed to follow a uniform unspecific reaction pattern which is irrespective to the underlying pathology. Various studies point to an important role of ROS generation in the pathogenesis of demyelination. In vitro studies in humans suggest a central role of microglia as effectors and regulators of demyelination.

The aim of our study was therefore to elucidate whether microglial generation of ROS is specific to demyelinating diseases or if it also can occur in other intracranial diseases. The goal was to examine microglia ex vivo to exclude cell culture artefacts. Canine microglia were used to get more information about the pathogenesis of demyelination in the dog. In this species distemper virus infection represents a paradigm for demyelinating diseases and is a well established animal model for demyelination.

Forty-seven dogs were included in our study which suffered from different intracranial diseases such as canine distemper virus infection, tumor, inflammation, idiopathic epilepsy, anomalies, and extracranial diseases. The results of microglial ROS generation in different intracranial diseases were compared to those of dogs with demyelination. The ex vivo examination of microglia was performed by means of flow cytometry and through detection of green fluorescent Rhodamin 123.

As a result microglia isolated from dogs with demyelination showed a significant enhancement of ROS generation compared to microglia from dogs with all other diseases ($p < 0.0001$). This strongly suggests a specific response of microglia in animals with demyelination and underscores the pivotal role of microglia in the pathogenesis of this disorder. Our findings support that canine distemper virus infection in dogs is furthermore a useful animal model to study devastating diseases such as multiple sclerosis and to develop new treatment strategies.

ABSTRACT #213

TRANSCRANIAL MAGNETIC MOTOR EVOKED POTENTIALS IN DOBERMAN PINSCHER DOGS WITH AND WITHOUT CLINICAL SIGNS OF CERVICAL SPONDYLOMYELOPATHY: 32 DOGS. Ronaldo C. da Costa, Roberto Poma, Joane Parent, Gary Partlow, David Holmberg, Howard Dobson, Jonathan LaMarre. Ontario Veterinary College – University of Guelph, Guelph, Ontario, Canada.

Although MRI is an excellent technique to define anatomical lesions, it does not provide functional information. Transcranial magnetic stimulation is a non-invasive technique that provides a functional evaluation of the motor pathways. The transcranial magnetic motor evoked potentials (TMMEP) are more sensitive to spinal cord injury and ischemia and, better predictors of clinical outcome than spinal somatosensory evoked potentials (SSEP) in rats

and dogs. We hypothesized that TMMEPs can be used as a screening electrodiagnostic tool for the detection of cervical myelopathy in clinically normal Doberman dogs, more specifically in Dobermans without clinical signs of cervical spondylomyelopathy (CSM).

In order to test our hypothesis, 32 Doberman dogs were studied, 16 clinically normal and 16 affected with CSM. The diagnosis was based on neurological examination and magnetic resonance imaging (MRI) studies. For the recording of the TMMEPs, sedation with acepromazine and hydromorphone was used. The dogs were positioned in lateral recumbency. The recording electrodes were positioned contra-laterally to the cortical stimulation site in the extensor carpi radialis (thoracic limb) and cranialis tibialis muscles (pelvic limb). The magnetic stimulation was performed using a 9.5 cm focal point coil. Four single stimulations were delivered on each side. The measurement of latencies and peak-to-peak amplitudes were calculated by using the cursors on the oscilloscope. The neuronal path length of each dog was measured from the vertex to the active electrodes of the thoracic and pelvic limbs muscles. ANCOVA (analysis of covariance) was used to analyse the latency and amplitude, as well as the effects of side, location and group. Significant differences were established by the Tukey's test at $p<0.05$.

The results are expressed by means and standard error (SE) and are in milliseconds (ms) for latencies and microvolts (μV) for amplitudes. In the group of normal dogs, the thoracic limb latencies and amplitudes were 14.35 (SE 0.45) and 2975.70 (305.15), respectively. The pelvic limb latencies and amplitudes were 21.86 (0.62) and 1416.32 (170.06). In the group of dogs with CSM, the thoracic limb latencies and amplitudes were 14.52 (0.60) and 2534.23 (319.12), respectively. The pelvic limb latencies and amplitudes were 34.16 (2.06) and 947.74 (229.51), respectively. The results indicated a significant difference in the pelvic limb latencies ($p=0.0002$) and amplitudes ($p=0.036$) between the normal and CSM groups. There was no significant difference for the thoracic limb latencies and amplitudes between groups.

The results of this study indicate that the TMMEPs' latencies and amplitudes for the tibialis cranialis muscle could be used as a screening test for Doberman dogs suspected of having cervical myelopathy.

ABSTRACT #214

ADULT-ONSET DISTAL SENSORIMOTOR POLYNEUROPATHY IN BOUVIER DES FLANDRES DOGS. Ronaldo C. da Costa, Joane Parent, Roberto Poma, Robert Foster, Pari Basrur, G. Diane Shelton. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada and Department of Pathology – University of California San Diego, La Jolla, CA.

Peripheral neuropathies, including those having an adult onset, have been reported in several canine breeds. A number of neuromuscular diseases have been described in Bouvier des Flandres dogs including those associated with laryngeal paralysis, degenerative myopathy and dysphagia-related muscular dystrophy. We report a diffuse polyneuropathy in Bouvier des Flandres dogs.

Two male and two female related adult dogs were examined with a complaint of progressive weakness. The first three dogs were littermates and the fourth dog shared the same sire. Onset of clinical signs in the first three dogs occurred between the ages of 31 to 33 months and at 16 months in the fourth dog. The time between the onset of clinical signs and referral varied from three to 12 months. All dogs shared similar clinical features. Neurological abnormalities included tetraparesis without ataxia, delayed proprioceptive positioning in all limbs, incomplete palpebral closure bilaterally, reduced muscle tone and muscle mass in the distal muscles, absent patellar and cutaneous trunci reflexes, decreased flexor reflexes in all limbs and intact nociception. Plantigrade stance and gait was mild initially but became severe as the disease progressed. There was no evidence of laryngeal paresis. The neurolocalization was a diffuse bilateral symmetrical sensorimotor polyneuropathy (the sensory component was explained by lack of patellar reflex in a walking animal). Complete blood count and biochemistry profile were unremarkable in all dogs. Thyroid profile, thoracic radiographs, abdominal ultrasound and metabolic genetic screening were performed in three dogs and no abnormalities were found. Electromyography and motor nerve conduction velocity were performed in three dogs. Prolonged insertional activity, fibrillation potentials and complex repetitive discharges were observed in the distal limb muscles. Motor nerve conduction velocity of the sciatic nerve in dogs 1, 2 and 3 was 48 m/s, 44 m/s and 45.6 m/s, respectively. Cerebrospinal fluid analysis revealed an increase in lumbar protein concentration in two dogs. One dog was followed for 30 months and had a muscle and nerve biopsy, followed later by necropsy. Two other dogs were euthanized three to 12 months after the diagnosis. Necropsy was performed in two dogs. No abnormalities were observed in brain, spinal cord or internal organs. Myofiber atrophy consistent with denervation and variable degrees of nerve fiber loss within intramuscular nerve branches were prominent in the distal muscles. A decrease in large caliber myelinated fibers with an increased population of smaller caliber fibers, regenerating clusters, and occasional axonal degeneration was found in peripheral nerve biopsies.

In conclusion, this polyneuropathy appears to be a distinct breed-related disease unlike any other previously reported. The pathologic findings suggest that the primary abnormality is an axonopathy affecting the large caliber myelinated fibers. Preliminary genetic studies indicate an autosomal recessive inheritance mode.

ABSTRACT #215

MULTISYSTEM AXONOPATHY IN GOLDEN RETRIEVER DOGS. Ronaldo C. da Costa, Joane Parent, Roberto Poma, Gary Thompson, Alexander de Lahunta, Laura Smith-Maxie. Ontario Veterinary College – University of Guelph, Guelph, Ontario, Canada, and Department of Biomedical Sciences, Cornell University, Ithaca, NY.

Central axonopathies have been reported in Labrador Retrievers, Ibizan Hounds, Boxers and German shepherd dogs but they are considered uncommon diseases. We describe a progressive multisystem axonopathy in three Golden Retriever dogs.

Three Golden Retriever littermates were presented with a history of weakness. The onset of clinical signs was at 6 to 8 weeks in one dog, and at 12 to 14 weeks in the two other dogs. Two were male and one female. The main complaint was pelvic limb weakness, which evolved to tetraparesis. Muscle tremors were also observed. The first dog was examined at three months of age. General physical examination was normal except for severe muscle atrophy in the head and limbs. Neurological abnormalities included tetraparesis, which was worse in the pelvic limbs, slight muscle tremors, which could be induced by palpation, and decreased patellar reflexes. Cranial nerve examination, postural reactions and other spinal reflexes were intact. A neuromuscular disease was suspected. Two other dogs were examined at the age of eight months. Both dogs were extremely weak and could walk only a few steps before collapsing. One dog had severe dyspnea, which had progressed over several weeks. A marked kyphotic posture, possibly related to axial muscle weakness was observed. Proprioceptive positioning and spinal reflexes were intact except for the patellar reflexes, which were decreased. The clinical signs progressed over several months and euthanasia was performed at 6 months in the first dog and at eight months in the other dogs. Complete blood count and biochemistry profile were normal in all dogs. Cerebrospinal fluid analysis was normal in one dog. No abnormalities were found on metabolic genetic screening test in two dogs. Histopathological studies of the spinal cord of all dogs revealed a profound axonal loss at all spinal cord levels. The lesion was

diffuse, but not tract-related. The lateral and ventral funiculi seemed more severely affected but the deeper tracts and the dorsal funiculi were also involved, with lesser severity. Secondary demyelination and extensive gliosis accompanied the distribution of the spinal cord lesions. The axonopathy could be followed into the brain stem along the ascending tracts but it was less evident than in the spinal cord. Neuronal cell body degeneration was observed in the ventral gray column at all levels. A neuropathy, presumably Wallerian degeneration, was seen involving the ventral nerve roots, much more severely than the dorsal roots. The peripheral nerves seemed less affected. Neurogenic muscle atrophy was observed in axial and appendicular muscles.

The clinical and pathological features of this disease have not been observed in any other canine axonopathy. Despite severe diffuse spinal cord axonopathy, no proprioceptive ataxia was observed. The clinical signs reflected the motoneuron loss. Genetic studies are being conducted to determine the inheritance mode of this disease.

ABSTRACT #216

SURVEILLANCE OF CATHETER ASSOCIATED INFECTIONS IN DOGS AND CATS IN AN INTENSIVE CARE UNIT. Michelle L. Marsh-Ng, Derek P. Burney, Jennifer Garcia, James C. Vulgamott, R. Dennis Heald, Chris J. Jones, A. Melissa Garcia-Lacaze; Gulf Coast Veterinary Internists and Critical Care, Houston, TX.

Intravenous (IV) catheter associated infections (CAI) are one of the leading causes of nosocomial infections documented in human patients resulting in increased morbidity, mortality, length of hospital stay and financial costs to medical institutions. IV CAI and potential associated risk factors have been researched and well documented in human medicine leading to the development of practices, protocols and instrument advances aimed at decreasing the risk of CAI. Prospective studies in veterinary patients evaluating CAI and the relation of possible associated risk factors are lacking. The study reported here is a prospective surveillance study aimed to assess the CAI rate in our ICU patients, as well as attempt to identify potential risk factors that might be associated with increased infection rate in veterinary patients. Dogs and cats admitted to the ICU in which a peripheral or central venous catheter (CVC) was placed and maintained for a minimum of 48 hours were included. 16 or 18 gauge 28 cm Venocath® catheters were placed in the jugular or saphenous vein, or 22 and 20 gauge 1 inch or 18 gauge 1½ inch Terumo Surflo® catheters were placed in the cephalic or saphenous vein. Strict protocols were followed regarding catheter placement and removal. Catheter tips were submitted in sterile saline for aerobic and anaerobic culture. Any growth was considered a positive culture. All positive cultures underwent bacterial identification and antimicrobial sensitivity testing. A total of 151 catheter tips were submitted for culture with a positive culture rate of 24.5% (37/151). *Enterobacter* was most commonly cultured representing 45.95% (17/37) of positive cultures. Potential risk factors were statistically evaluated with the chi-squared or Fisher exact test. Risk factors analyzed included catheter use, type of infusate (blood products, TPN, dextrose, hetastarch®, and PPN), corticosteroid administration, catheter location, catheter type, and duration of catheterization, as well as catheter associated complications. Placement of a Venocath® central venous catheter was associated with increased risk of CAI ($P=<0.05$). No other risk factor was statistically significant. Venocath® catheters were then assessed alone. 101 Venocath® catheters were submitted for culture with 24.75 % (25/101) positive culture rate. Each previously mentioned risk factor, as well as multiple risk factors affecting the same catheter were assessed and no statistical significance was found. In conclusion, CAI rate was similar to CAI rates previously reported in human and veterinary medicine. Enteric pathogens were more commonly isolated in our study compared to human studies. Venocath® CVC were associated with increased risk of CAI in cats and dogs in our ICU.

ABSTRACT #217

INTRACRANIAL SUBARACHNOID HEMORRHAGE FOLLOWING LUMBAR MYELOGRAPHY. Packer RA[1], Bergman RL[2], Coates JR[1], Essman SC[1], Weis K[2], Levine JM[1], O'Brien DP[1], Johnson GC[3]. [1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO; [2]Carolina Veterinary Specialists, Charlotte, NC; [3]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO.

Intracranial subarachnoid hemorrhage (SAH) is a rare, but serious complication of lumbar puncture in humans. Possible sequelae include increased intracranial pressure, cerebral vasospasm, or mass effect, which can result in dysfunction or brain herniation. We describe two dogs with intracranial SAH following lumbar myelography. Case 1 was an 8-year-old, neutered male mixed breed dog referred for acute-onset tetraplegia. Nociception and respiratory function appeared normal. Case 2 was a 6-year-old, neutered male Beagle mix referred for severe progressive tetraparesis. Neuroanatomic localization of both cases was to the C1-C5 segment of the spinal cord. Cerebrospinal fluid analysis prior to myelography in Case 1 was within reference range. In both cases, a myelogram was performed by lumbar injection of iohexol [Omnipaque™]. A ventral extradural compressive lesion was identified over the C3-C4 disc space in Case 1, and C4-C5 disc space in Case 2. Both dogs underwent ventral decompressive surgery to remove herniated disc material from the epidural space. Both surgeries were uneventful; however, the dogs failed to recover consciousness or spontaneous respiration following anesthesia. Absence of papillary light reflex, oculocephalic reflex, response to painful stimuli, and brainstem auditory-evoked potentials suggested loss of brainstem function, and the dogs were euthanized.

In Case 1, post-mortem evaluation showed diffuse SAH and clotted blood throughout the length of the spinal cord, brainstem, and ventrum of brain, causing the spinal dura to bulge. The location of the SAH was confirmed histologically. Leptomeningeal petechiae were also present. No inflammation was present. Bacterial cultures were negative, and no organisms or other signs of infection were identified. Coagulation testing was not performed prior to myelography, but there was no clinical evidence of bleeding diathesis, as platelet count [230,000/µL] was within reference range [200,000-500,000/µL], and intra-operative bleeding was negligible.

In Case 2, post-mortem examination was similar to Case 1, with diffuse SAH throughout the spinal cord, and leptomeningeal hemorrhage scattered throughout the subarachnoid space. One subarachnoid vessel in the lumbar region showed mild inflammation. The vasculature was normal in the subarachnoid space of the brainstem, diencephalon, or cerebrum. The diagnosis for cause of death was acute SAH.

Our findings suggest that fatal SAH is a potential complication of lumbar myelography in dogs. The cause of SAH is not known, but may be due to traumatic lumbar tap or idiosyncratic response to contrast medium, with subsequent fatality due to mass-effect and increased intracranial pressure, cerebral vasospasm, or atypical interaction between SAH and contrast medium.

ABSTRACT #218

PREVALENCE OF *BARTONELLA HENSELAE* SPECIFIC ANTIBODIES IN SERUM OF CATS WITH AND WITHOUT CENTRAL NERVOUS SYSTEM DISEASE. LK Pearce, SV Radecki, M Brewer, MR Lappin. From Colorado State University, Ft Collins, CO (Pearce, Brewer, Lappin). Dr. Radecki is a private statistical consultant in Ft. Collins, CO.

Bartonella henselae is the most common cause of cat scratch disease in children and young adults. While neurological complications are thought to be rare, a California study found *Bartonella* spp. to be the most common bacterial agents associated with encephalitis. Additional signs of neurological dysfunction

include seizures, aggression, excitability, myelopathy, neuropathy and meningitis. In some cats experimentally infected with *B. henselae*, neurological signs including seizures, nystagmus, tremors, aggressive behavior and abnormal response to stimuli have been observed. The purpose of this study was to determine if differences exist in the seroprevalence of *B. henselae* antibodies in client-owned cats with and without neurological signs.

The Infectious Diseases Laboratory database was searched between January 2002 and May 2004 for feline serum sample submissions that listed neurological disease as a presenting complaint (Group 1). The neurological complaint was then classified into seizures or other neurological signs. Sera were sequentially selected from cats with a non-neurological presenting complaint (Group 2) and healthy cats (Group 3). Age, breed, sex, and state were recorded for each case. The state was used to classify each cat as high or low risk for *Ctenocephalidies felis* exposure. Samples were stored at -20ºC or -80ºC until thawed and assayed in an ELISA for the detection of antibodies against *B. henselae*. Samples with a titer of $\geq$ 1:64 were considered positive. Relationships between *B. henselae* serological status (positive or negative) and clinical presentations were assessed by Fisher's exact test; logistic regression was used to assess the influence of age and risk of flea exposure. Significance was defined as $P < 0.05$.

Of the 145 cats in Group 1, 63 cats had seizures and 82 cats had other neurological signs. There was no difference in *B. henselae* seroprevalence rates between cats with seizures (37 of 63 cats; 59%) and cats with other neurological diseases (35 of 82 cats; 43%). When the analysis was expanded to include age and risk of flea exposure by logistic regression, neither of these additional factors was significant and the effect of seropositivity was still not significant. Antibodies against *B. henselae* were detected in serum of 104 of 163 Group 2 cats (63.8%) and 68 of 97 of Group 3 cats (70.1%). In the first evaluation, Group 2 cats ($P = 0.0153$) and Group 3 cats ($P = 0.0022$) had a greater seropositive rate than Group 1 cats. When the analyses were expanded to include age and risk of flea exposure by logistic regression, neither of these additional factors influenced the seropositive rate within group and the effect of group was still significant.

In this study, cats with neurological disease were not more likely than other cats to have *B. henselae* antibodies. Presence of *B. henselae* antibodies in serum of individual cats with neurological disease does not prove the clinical signs are related to *B. henselae*.

ABSTRACT #219

CEREBROSPINAL FLUID BETA 2-MICROGLOBULIN LEVELS IN NORMAL DOGS AND DOGS WITH NEUROLOGICAL DISEASE. K.R. Muñana[1], M. Saito[2], F. Hoshi[3]. [1]North Carolina State University, Raleigh, NC; [2]Azabu University, Kanagawa, Japan; [3]Kitasato University, Aomori, Japan.

Inflammatory CNS disorders are a common cause of neurological dysfunction in dogs. Analysis of CSF is instrumental in confirming the presence of inflammation and is considered the mainstay of diagnosis. However, routine CSF analysis provides limited information with respect to the cause of the inflammation, and in many cases a definitive diagnosis is not obtained. Beta 2-microglobulin (B2M) is a protein that is bound to the major histocompatibility complex Class I molecule and is considered a marker of immune activation. Measurement of CSF B2M levels in humans is reported to aid in the diagnosis of specific CNS inflammatory disorders and is helpful for monitoring response to therapy or progression of disease. The purpose of this study was to establish a normal range for CSF B2M concentrations in dogs, and to compare CSF B2M values in dogs diagnosed with inflammatory CNS disease to those obtained from dogs with intervertebral disk disease (IVDD), a primary noninflammatory CNS disorder.

Serum and CSF collected from 10 adult laboratory dogs were used to establish normal values. Serum and cisternal CSF collected during diagnostic testing were obtained from eight client owned dogs diagnosed with encephalomyelitis and from 10 dogs with IVDD. Routine analysis was performed on all CSF samples (WBC count, RBC count, protein concentration). Samples for B2M determination were frozen at -80º C until assayed. Measurements were performed with a standard sandwich ELISA that has been previously described. CSF-serum B2M ratios were calculated for each dog, and mean CSF B2M levels for each group of dogs were compared with a Wilcoxen's rank sum test. Relationship between CSF WBC, RBC and protein levels, and B2M concentration were assessed using a Spearman rank correlation. Significance was established at $p< 0.05$.

Mean CSF B2M concentrations in control dogs were 0.36 µg/ml (SD 0.051, n=10) and 0.40 µg/ml (SD 0.074, n=6) for samples obtained from the cerebellomedullary and lumbar cistern, respectively. There was no statistical difference in values between sites. Dogs with encephalomyelitis had a mean CSF B2M concentration (0.77 µg/ml, SD 0.21) that differed significantly from control values ($p=0.004$). Mean CSF B2M levels in dogs with IVDD (0.46 µg/ml, SD 0.01) also differed significantly from controls ($p=0.002$). Mean CSF B2M values in dogs with inflammatory CNS disease were higher than mean CSF B2M values in dogs with IVDD ($p=.02$). Two dogs with inflammatory disease had B2M CSF:serum ratios >1. A significant correlation was identified between CSF WBC counts and B2M levels ($r_s = 0.73$, $p=0.0006$), This study established normal values for CSF B2M in dogs, and demonstrated that values increase in CNS disorders, with the most notable increases seen with inflammatory disease. Further evaluation of this CSF marker in specific inflammatory CNS disorders is warranted.

ABSTRACT #220

VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN CANINE INTRACRANIAL MENINGIOMAS. S. R. Platt[1], T. Scase[2] V. Adams[2] F. Adamo[3] Sam Long[4]; [1]Centre for Small Animal Studies, Centre for Preventative Medicine, [2]The Animal Health Trust, Newmarket, Suffolk, UK, [3]University of Wisconsin, [4]University of Glasgow, Scotland.

Vascular endothelial growth factor (VEGF) is a regulator of angiogenesis, vasculogenesis and vascular permeability. Compared to normal brain an increased expression of VEGF has been reported in many types of brain tumors. Up-regulation of VEGF has been observed in human meningiomas, and high levels of VEGF expression are predictive of meningioma post-surgical recurrence. The objectives of this study were to evaluate VEGF expression in canine intracranial meningiomas and determine if there is an association between expression and patient outcome.

Tumor tissue from 19 cases of histologically-confirmed WHO type I intracranial meningiomas were surgically obtained. All dogs were subsequently treated with radiotherapy. Immunohistochemistry was performed on 5-micron sections of the paraffin-embedded tumor tissue, using a mouse anti-human VEGF monoclonal antibody (clone JH121) as the primary antibody. The extent, intensity and distribution of VEGF staining for each section were assessed by light microscopy using a semi-quantitative scale. The distribution of positive immunohistochemical staining was defined as diffuse, patchy or multifocal. The intensity of immunohistochemical staining was assessed on a scale of 0-3. The extent of VEGF staining was assessed by estimating the percentage of neoplastic cells exhibiting positive staining for VEGF. Survival analysis was carried out using the Kaplan-Meier procedure. Survival rates among groups were compared using log-rank tests with significance set at P□ 0.05.

VEGF expression was detected in all tumors, with more than 50% of cells staining positively in 17/19 cases. There was a trend for shorter survival times with greater VEGF expression. The median survival time for tumors with □75% of cells staining was 574 days

compared to 434 days for tumors with >75% of cells staining (P=0.1). Intensity and distribution of staining were not significantly associated with survival (P=0.5 and 0.3 respectively). Although there is extensive VEGF expression in canine intracranial meningiomas suggesting a potential future target for therapy, a larger study is necessary to confirm a significant relationship with survival.

ABSTRACT #221

Abstract Withdrawn.

ABSTRACT #222

PROCARBAZINE FOR TREATMENT OF SUSPECTED GRANULOMATOUS MENINGO-ENCEPHALOMYELITIS: 20 CASES (1998-2004). JR Coates[1,2], G Barone[3], CW Dewey[2,3], NM Holloway-Azene[1], CL Vitale[1], JK Sessions[2]. [1]University of Missouri, Columbia, MO; [2]Texas A&M University, College Station, TX; [3]Long Island Veterinary Specialists, Plainview NY.

Granulomatous meningoencephalomyelitis (GME) is an idiopathic inflammatory disease of the central nervous system in dogs and histopathologically characterized by perivascular infiltrates of mononuclear cells in the white matter and meninges of the brain and spinal cord. Recent literature suggests that GME is a T-cell mediated autoimmune disease. Previously reported median survival times in dogs administered glucocorticoids with focal and multifocal GME were 114 and eight days, respectively. (Muñana and Luttgen, 1998) Remission often is short-lived in dogs treated with glucocorticoids. Procarbazine is a potent monoamine oxidase inhibitor that is lipid-soluble and crosses the blood-brain-barrier. Its cytotoxic effects alter DNA, RNA and protein synthesis. The purpose of this retrospective study was to assess the long term treatment effects of procarbazine in dogs with a suspected diagnosis of GME.

Two groups of dogs were available to study: 1) Suspected GME affected dogs administered combined therapies of procarbazine and prednisone (n=20); 2) Histopathologically confirmed GME affected dogs with no treatment (n=11). Dogs with a suspected diagnosis of GME and that received procarbazine were identified retrospectively from medical records of the Texas A&M University Veterinary Medical Teaching Hospital and Long Island Veterinary Specialists between 1998 and 2004. Selection criteria required that all dogs had a neurologic examination, minimum database, cerebrospinal fluid analysis, and brain imaging (computed tomography or magnetic resonance imaging). Procarbazine was administered orally q 24 hours, at a dosage of 25 to 50 mg/m^2. Dogs were monitored for myelosuppression and other side effects.

Age of onset of clinical signs ranged from one to 13 years with a median of six years. Twenty-eight dogs (90%) weighed <10 kg. There were 12 spayed females, eight intact females, eight castrated males and three intact males. Eighteen dogs (58%) were toy breeds. Neuroanatomic localizations included the forebrain (n=14), brainstem (n=27), cerebellum (n=5), spinal cord (n=2) and ocular (n=1). Clinical signs were focal in 5 dogs and multifocal in 26 dogs. Median survival time for all dogs enrolled in the study was 4.5 months. Median survival time for dogs treated with procarbazine was 15.0 months and for those with no treatment 0.62 months. Treatment with procarbazine was significantly associated with survival time (P=<0.001). Procarbazine was the only independent predictor of survival. The prednisone dose was reduced or discontinued in 17 dogs. Side effects associated with procarbazine therapy included myelosuppression in six dogs (30%) and hemorrhagic gastroenteritis in three dogs (15%).

These data suggest that procarbazine treatment for GME may result in a better long term outcome than previously reported with glucocorticoid treatment alone. Dogs treated with procarbazine need to be closely monitored for myelosuppression.

ABSTRACT #223

NECROTIZING MENINGOENCEPHALITIS IN CHIHUAHUA DOGS. Stephanie A. Kube, Peter J. Dickinson, Timothy W. Affolter, Robert J. Higgins. University of California, Davis-Veterinary Medical Teaching Hospital, CA.

A non-suppurative acute to chronic, necrotizing meningoencephalitis was diagnosed in five Chihuahua dogs (four females and one male) aged between one and 10 years. Presenting neurological signs included seizures, blindness and mentation changes from five days to 6.5 months duration. Analysis of cerebrospinal fluid from three dogs indicated total nucleated cell counts ranged from normal to 180/ul and protein levels from 52mg/dL to 72 mg/dL. with a final interpretation, supported by the differential cell count, of an inflammatory disease. Magnetic resonance imaging showed loss of normal grey/white matter demarcation multifocally throughout much of the cerebral cortex, with cortical hyperintensity on T2-weighted imaging. Two dogs were administered anti-inflammatory and immunosuppressive medications (prednisone and/or cytosine arabinoside) with no significant clinical response. All dogs were necropsied. Multifocal asymmetrical areas of necrosis, primarily confined to both grey and white matter of the cerebral hemispheres, were identified grossly in all brains. Overlying gyral collapse was seen with some cavitatory lesions. Microsopically, there was a severe necrotizing non-suppurative meningo-encephalitis sometimes with cystic cavitation. Two dogs also had small lesions in the medulla. Immunophenotyping of inflammatory cells defined consistent populations, but varying in density, of CD18, CD3, CD20, CD45 R and CD 79a positive cells in the lesions. In fresh frozen sections of lesions, there were some $CD1_c$ and $CD11_c$ positive dendritic antigen-presenting cells. Immunocytochemical staining for CDV antigen was negative in all cases. No etiologic agent was identified. The clinical signs, lesion distribution pattern, and type of neuropathologic lesions bear striking similarities to those reported both in Pug dog encephalitis and in necrotizing encephalitis of Maltese dogs.

ABSTRACT #224

ADMINISTRATION OF LOW DOSE ACTH INTRAMUSCULARLY FOR ACTH STIMULATION TESTING IN DOGS. EN Behrend[1], RJ Kemppainen[1], DS Bruyette[2], KA Busch[1], HP Lee[1]. [1]Auburn University College of Veterinary Medicine, Auburn, AL; [2]VCA W. Los Angeles Animal Hospital, Los Angeles, CA.

To date, the only commercially available form of ACTH proven to be effective for stimulation testing in dogs is cosyntropin (Cortrosyn). However, the cost has recently increased dramatically. In order to decrease the expense of the test, use of a reduced dose would be helpful. Although a low dose of 5 mcg/kg has been proven to provide maximal stimulation if given IV, only a dose of 250 mcg/dog has been verified to give maximal stimulation IM. The purpose of the study was to compare adrenal stimulation achieved with 5 mcg/kg cosyntropin given IV versus IM.

Five mixed-breed, healthy dogs were used. ACTH stimulation tests were performed on four occasions, with 4 or 5 dogs being tested on each day. The dogs were randomly assigned to receive ACTH either IM or IV first, and on their second testing day, ACTH was administered by the alternate route. Six days elapsed between testing for each dog. Blood was drawn before (t=0) and 30, 60, 90 and 120 min post-injection. Samples were centrifuged after clotting, and the serum separated and stored at –20C until analysis. Data were analyzed using a repeated measures ANOVA on ranks. Pairwise multiple comparisons were made using the Student-Newman-Keuls

method. Significance was set at $p \leq 0.05$. Values are given as median (range).

Overall, serum cortisol concentration significantly ($p<0.0001$) changed over time after administration of cosyntropin by both routes. Serum cortisol concentrations were not different between the dogs treated IV and IM at any time point. However, in comparison to baseline (t=0), serum cortisol concentration was significantly higher at every time post-ACTH administration. Maximal post-ACTH serum cortisol concentration occurred at t=60-90 when ACTH was administered by either route. Serum cortisol concentration was not significantly different at t=60 vs. t=90, however concentration at t=120 was significantly lower for dogs treated by either route. For dogs treated IV, serum cortisol concentration at t=60 was 297 nmol/L (220-432), at t=90 was 261 nmol/L (222-390) and at t=120 was 180 nmol/L (115-279). For dogs treated IM, serum cortisol concentrations at t=60, 90 and 120 were 289 nmol/L (207-392), 259 nmol/L (155-318) and 174 nmol/L (116-205), respectively.

In conclusion, cosyntropin administered either IV or IM at 5 mcg/kg to normal dogs produces equivalent, and likely maximal, adrenal stimulation. Peak response occurs from 60 to 90 minutes post-ACTH.

ABSTRACT #225

ASSESSMENT OF SUPPRESSION OF THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL (HPA) AXIS AND SYSTEMIC EFFECTS IN NORMAL DOGS TREATED WITH ORAL CONTROLLED-RELEASE BUDESONIDE. S. Stroup, E. Behrend, R. Kemppainen, S. Smith-Carr. Auburn University College of Veterinary Medicine, Auburn AL.

Budesonide is a glucocorticoid used in humans to treat asthma, rhinitis, and inflammatory bowel disease (IBD). Because budesonide undergoes extensive first-pass hepatic metabolism, with 90% of systemically available drug converted to less active metabolites, it theoretically has increased topical activity with minimal systemic effects. A previous veterinary study, however, found that dogs with IBD did have HPA axis suppression when given budesonide. One possible reason for this suppression is that the damaged GI mucosal layer allowed greater systemic drug absorption. The objective of our study was to evaluate the effects of oral budesonide on the HPA axis and other parameters commonly affected by glucocorticoids in dogs with a normal gut mucosal barrier.

Ten healthy dogs, one to six years old, were used. Five dogs received budesonide (3 mg/dog if body weight >9 kg, 2 mg/dog if <9 kg) orally once daily and five control dogs received an empty gelatin capsule once daily for 28 days. Treatment and placebo administration was discontinued Day 28. Serum cortisol concentration pre-and post-ACTH stimulation, glucose concentration (GLU), and ALP and ALT activities, plasma endogenous ACTH (eACTH) concentration, urine specific gravity (USG), and weight were evaluated Days 0, 7, 14, 21, 28, and 35. Data was compared within and between groups for each test day using a repeated measures analysis of variance on ranks. Post hoc comparisons were made using the Student-Newman-Keuls method. Statistical significance was set at $p<0.05$.

No difference existed between groups at baseline for any parameter. Serum cortisol concentration pre- and post-ACTH stimulation was significantly suppressed in the treatment group compared to baseline and to the control group on Days 7, 14, 21, and 28 ($p<0.0001$). One week after discontinuation of budesonide administration (Day 35), pre-ACTH cortisol concentration was not significantly different from baseline, but post-ACTH cortisol concentration was still significantly suppressed. For pre- and post-ACTH cortisol concentrations in the placebo group, no difference existed between baseline and any date. On days 14, 21 and 28, plasma eACTH concentrations were significantly lower in the treatment group in comparison to baseline and to the placebo group ($p=0.0003$). No clinically important differences in weight, USG, GLU, ALP, and ALT were noted between or within groups on any date.

We conclude that oral controlled-release budesonide has suppressive effects on the HPA axis of dogs with normal gastrointestinal integrity. However, other parameters often affected by glucocorticoids were not altered by a four-week treatment course of budesonide at these doses.

ABSTRACT #226

Abstract Withdrawn.

ABSTRACT #227

SERUM IONIZED MAGNESIUM CONCENTRATIONS IN DOGS AND CATS WITH HYPOPARATHYROIDISM. P.A. Schenck. Diagnostic Center for Population and Animal Health, Endocrine Diagnostic Section, Michigan State University, Lansing, MI.

Parathyroid hormone (PTH) production is influenced by the circulating serum concentration of ionized magnesium (iMg), and either a deficiency or excess of iMg may suppress PTH production. In addition, cell membrane receptors may have a decreased sensitivity to serum ionized calcium (iCa) in the presence of low serum iMg concentrations. Some patients with hypoparathyroidism appear refractory to therapy with calcium and vitamin D preparations, and a deficiency or excess serum iMg may in part play a role. The objective was to determine the serum iMg status in dogs and cats with hypoparathyroidism. Hypoparathyroidism was diagnosed in 357 dogs, and in 27 cats over a two year period. A diagnosis of hypoparathyroidism was made based on clinical signs, a low serum iCa concentration, and an inappropriately low PTH concentration in response to hypocalcemia. Dogs and cats already undergoing treatment for hypoparathyroidism were excluded from this study. PTH, iMg and iCa concentrations were determined using the same serum sample. Mixed-breed dogs accounted for 25% of the cases, with 13% Schnauzers, 7% Labrador Retrievers, 5% Dachshunds, 4% Yorkshire Terriers, 4% poodles, 3% Golden Retrievers, and 3% Scottish Terriers. Fifty nine other dog breeds were represented with an incidence of less than 3% each. Of hypoparathyroid cats, 59% were domestic shorthairs, 22% were an unspecified breed, and 15% were Siamese. In dogs with hypoparathyroidism, mean iCa concentration was 0.79 ± 0.19 mmol/L (reference range 1.25 – 1.45 mmol/L), mean PTH concentration was 1.9 ± 1.6 pmol/L (reference range 2 -13 pmol/L), and mean iMg concentration was 0.45 ± 0.09 mmol/L (reference range 0.43 – 0.60 mmol/L). The iMg concentration was below the reference range in 39%, within the reference range in 55%, and above the reference range in 6% of dogs with hypoparathyroidism. Of the 55% of dogs with iMg within the reference range, 69% had an iMg concentration within the lower half of the reference range, and only 31% had an iMg concentration within the upper half of the reference range. In cats with hypoparathyroidism, mean iCa concentration was 0.72 ± 0.14 mmol/L (reference range 1.0 – 1.4 mmol/L), mean concentration was 1.5 ± 0.9 pmol/L (reference range 0 – 4 pmol/L), and mean iMg concentration was 0.47 ± 0.11 mmol/L (reference range 0.43 – 0.70 mmol/L). The iMg concentration was below the reference range in 37%, within the reference range in 59%, and above the reference range in 4%. Of the 59% of cats with iMg within the reference range, 88% had an iMg concentration within the lower half of the reference range, and only 12% had an iMg concentration within the upper half of the reference range. These results suggest that a large number of dogs and cats with hypoparathyroidism have a subnormal or marginal circulating concentration of iMg which may decrease cell membrane receptor sensitivity to iCa and hinder PTH production. Future studies to evaluate the impact of magnesium supplementation in the treatment of hypoparathyroidism should be investigated.

ABSTRACT #228

FASTING GLUCOSE CONCENTRATIONS ARE HIGHER AND GLUCOSE TOLERANCE IS LOWER IN BURMESE CATS COMPARED TO MATCHED NON-BURMESE CATS. R Lederer, JS Rand, J Morton, Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Australia.

Burmese cats in Australia, New Zealand and the UK have an increased risk of developing diabetes, but the underlying pathological cause is unknown. Some predisposing factors in Burmese cats have been identified, such as chronic or recurring medical problems, dental disease, repeated corticosteroid treatment, confinement indoors and lower physical activity. Islet amyloidosis and pancreatitis have been ruled out as primary causes of diabetes in Burmese cats. The aim of this study was to compare glucose and insulin parameters between matched Burmese and non-Burmese cats before and after a glucose challenge, to better understand their predisposition to diabetes.

Clinically healthy Burmese cats (n=8) aged from three to eight years were compared with healthy non-Burmese cats (n=8) matched for age and body condition score. Body condition scores ranged from 5 to 7 on a 9-point scale. Cats were fasted for 24 hours before testing with a simplified intravenous glucose tolerance test, and samples were collected immediately before (0 minutes) and at 10, 60 90 and 120 minutes after a glucose challenge (1g/kg body weight). Serum glucose and insulin concentrations at each of the time points, and areas under the glucose and insulin curves were compared.

Glucose concentrations at 0, 60, 90 and 120 minutes and area under the curve were significantly higher in Burmese than in non-Burmese cats ($p \leq 0.05$). The differences were 1.9, 1.4 (not significant), 3.9, 4.6 and 5,3 mmol/L at 0, 10, 60, 90, 120 minutes, respectively. The largest differences in baseline values were seen between older (6-8 years) Burmese cats and their matches ($p=0.014$). There was a trend for higher baseline insulin concentrations ($p=0.088$) and 120 minute values ($p=0.059$), but lower 10 minute values ($p=0.093$) in Burmese cats, suggesting fasting hyperinsulinemia, and a delayed insulin response.

We conclude that clinically healthy Burmese cats have higher fasting glucose concentrations and lower glucose tolerance than matched non-Burmese cats, and have a trend to fasting hyperinsulinemia, and impaired first phase insulin secretion, with the differences greatest in Burmese cats older than six years of age.

ABSTRACT #229

SYSTEMIC ENDOCRINE EFFECTS OF AN INHALANT GLUCOCORTICOID IN HEALTHY CATS. L Brownlee,[1] B Seguin,[1] K Decile,[1] RW Nelson,[1] LJ Gershwin,[1] CR Reinero[2] . [1]University of California, Davis, [2]University of Missouri, Columbia.

The mainstay of therapy for inflammatory bronchial disease in cats has traditionally been oral or injectable glucocorticoids. While as a species, cats tend to be comparatively resistant to developing side effects with chronic glucocorticoid therapy some cats do not tolerate this drug. Delivery of metered dose inhalant glucocorticoids (IGC) has revolutionized the treatment of human asthma by maximizing local efficacy and minimizing systemic bioavailability. The purpose of this study was to evaluate systemic endocrine effects of an IGC, an oral glucocorticoid (OGC), and placebo in healthy cats. We hypothesized that IGC would have minimal systemic effects on the hypothalamic-pituitary-adrenal axis (HPAA) in healthy pet cats.

A randomized crossover design was used in six clinically normal cats. Drugs administered included inhaled flunisolide (250mcg/puff administered BID), oral prednisone (10 mg/day) and placebo (an empty metered dose inhalant, 1 puff BID). All cats received each treatment for two weeks followed by a one-month washout period. Endocrine effects were evaluated using single early morning cortisol levels, urine cortisol:creatinine ratios (UC:Cr), and ACTH stimulation tests performed pre and post-drug administration for each treatment period.

The mean early morning serum cortisol concentration was lowest for the OGC treatment, but means were not significantly different between treatments: (placebo, 2.8±0.8 ug/dl; IGC, 2.4±0.7 ug/dl; OGC, 1.1±0.9 ug/dl). No significant differences for UC:Cr were noted across treatments. Responses to ACTH stimulation were below the normal range in all cats receiving IGC and in 2 cats receiving OGC. Mean ACTH stimulation peak cortisol levels were significantly lower post-treatment compared to pre-treatment levels in cats receiving IGC (pre, 8.5±2.2 ug/dl; post, 2.9±1.4 ug/dl, $p=0.0004$) but not OGC (pre, 8.0±2.5 ug/dl; post, 6.0±1.9 ug/dl, $p=0.07$). The pre-treatment ACTH stimulation cortisol levels did not significantly differ between IGC, OGC and placebo ($p=0.59$), indicating that the washout period was adequate.

In conclusion, suppression of the HPAA did occur with the relatively high dose of IGC chosen in this study. Surprisingly, the OGC dose used in this study did not cause significant endocrine suppression, although a Type II statistical error may have been committed because of the small sample size.

ABSTRACT #230

CLONING, EXPRESSION, AND PURIFICATION OF FELINE PROINSULIN AND C-PEPTIDE. Z. Caffall, J. Brandao, M. Taylor, D.C. Ferguson, M. Hoenig, College of Veterinary Medicine, University of Georgia, Athens, GA.

Early detection and treatment of diabetes is essential to halt progression of the disease. Specific proinsulin assays are required for the early detection of beta cell dysfunction because a change in the proinsulin secretion, and therefore a change in the ratio of insulin or C-peptide and proinsulin, is one of the earliest markers of the disease process in man. Species- specific proinsulin, insulin, and C-peptide measurements have become available in human medicine and other species. There are no such assays available for the cat to examine changes in the ratio of these hormones to detect early changes in beta cell function.

The goal of this study was to clone, express, and purify feline proinsulin and C-peptide. Primers were designed based on alignment of known proinsulin cDNA sequences from other species. The cDNA was transcribed from feline pancreatic messenger RNA using a non-specific T-tailed primer, then amplified by routine polymerase chain reaction (PCR) with a specific sense primer. The resulting PCR product contained the sequence of the entire coding region for the mature form of feline proinsulin, which encompasses the coding sequence for the A, B, and C chain. This proinsulin sequence was functionally organized similar to other mammalian proinsulins as follows, from the 5' to the 3' direction: 5'-nontranslated region (NTR)-signal peptide coding region with intron, followed by the B-chain coding region, followed by the C-chain coding region with intron, followed by the A chain coding region, followed by a stop codon, followed by 3'NTR. A synthetic feline proinsulin DNA sequence was then prepared to reflect optimization of the codons for the subsequent expression in *E. coli*. After routine PCR of the entire synthetic feline proinsulin coding sequence, the amplified DNA was cloned into an expression vector and transformed into competent cells. Proinsulin was purified as described (Protein Expression and Purification 27:210-219, 2003). Primers for C-peptide were designed based on the known proinsulin sequence. Cloning procedures, and expression in pET vector was similar to that described for proinsulin. The protein was purified using HPLC. Feline proinsulin and human proinsulin have 72 amino acids out of 86 in common, for an overall percentage identity of about 84%. It has the same number of amino acids as human proinsulin, whereas pork and beef proinsulin both have deletions in the C-chain compared to human and feline proinsulin. Feline and human C-peptide differ at 10 positions. Purified feline-specific proinsulin and C-peptide have been used to produce antibodies which will allow the early detection of changes in beta cell function. This work describes for the first time the full

coding sequences of feline proinsulin, insulin, and C peptide and has produced DNA constructs for its *in vitro* expression and purification of the recombinant peptides. These purified recombinant proteins allow the development of standards and immunoassay reagents for the measurement of these peptides to assess pancreatic beta cell function.

ABSTRACT #231

G PROTEINS SHOW NORMAL ACTIVATION IN RESPONSE TO THYROID STIMULATING HORMONE IN FELINE HYPERTHYROID CELLS. Cynthia R. Ward, Darrell G. Dise, Kelli N. Russell, Kenneth J. Drobatz, David Holt. University of Pennsylvania School of Veterinary Medicine. Philadelphia, PA.

The pathogenesis of feline hyperthyroidism is not understood at the cellular or molecular level. Previous studies from our laboratory have identified a specific decrease in expression of the inhibitory G proteins (Gi) in hyperthyroid cells (Hammer et al, *AJVR* 61, 874-879) and have further determined that Gi2 is the specific subset of Gi proteins that is decreased (Ward et al, *JVIM* 15, 298). Moreover, stimulatory G proteins (Gs) were determined to be present in normal levels in hyperthyroid cells. We postulate that this decrease in expression of Gi2 is part of the pathogenesis of feline hyperthyroidism. Although the expression level of Gi2 is decreased, the activity of Gi and Gs proteins has not been investigated. The purpose of this study was to determine whether G protein activity is altered in feline hyperthyroid cells.

Thyroids were harvested from hyperthyroid and age-matched euthyroid cats. They were snap frozen in liquid nitrogen. Membranes were isolated from thawed, homogenized thyroid tissue. G protein activation in the membranes was investigated by measuring specific GTPγ^{35}S binding to the membranes in response to thyroid stimulating hormone (TSH), a natural agonist of the thyroid cell. GTPγ^{35}S binding measures the activation phase of the G protein activation/deactivation cycle. Pertussis toxin (PTX), a specific inhibitor of Gi proteins and cholera toxin (CTX), a specific activator of Gs proteins were used to isolate the activities of Gi and Gs, respectively.

Concentration-response curves were performed to determine the optimal TSH concentration for G protein activation in the thyroid membranes. Normal thyroid (NT) membranes and hyperthyroid (HT) membranes were incubated with concentrations of TSH from 0.1-100 mU/ml. Optimal GTPγ^{35}S binding was found at 1mU/ml TSH for both NT and HT membranes. Basal GTPγ^{35}S binding was 48% lower in the HT membranes (1.69 +/- 0.25 fmoles GTPγ^{35}S binding/mcg protein) than the NT membranes (3.55 +/- 0.86 fmoles GTPγ^{35}S binding/mcg protein). Upon stimulation with TSH, both NT and HT membranes showed a significant ($p<0.05$) increase in GTPγ^{35}S binding over background of 57 +/- 10% and 49 +/- 12%, respectively. PTX treatment of membranes, to inhibit Gi activity and highlight Gs activity, resulted in a significant ($p<0.05$) decrease in TSH-stimulation of NT and HT membranes to 34 +/- 5% and 26 +/- 3%, respectively. This decrease was not significant between the NT and HT groups ($p>0.05$). In membranes treated with CTX, TSH stimulation of GTPγ^{35}S binding was similar ($p>0.05$) between NT and HT cells at 45 +/- 5% and 51 +/-7%, respectively. This stimulation was not significantly different than stimulation seen in untreated membranes ($p>0.05$).

We conclude that G protein activity is not altered in HT cells and that a decreased expression of Gi protein and not an alteration in activity is part of the pathogenesis of feline hyperthyroidism.

ABSTRACT #232

CLONING AND SEQUENCING OF FELINE THYROTROPIN (fTSH): HETERODIMERIC AND YOKED CONSTRUCTS. S Rayalam, LD Eizenstat, M Hoenig and DC Ferguson, University of Georgia, College of Veterinary Medicine, Athens, GA.

Hyperthyroidism is the most common endocrine disorder of elderly cats. However, its early diagnosis and studies of risk factors have been hampered by the inavailability of a feline-specific TSH assay. Although there have been attempts to use available canine TSH immunoassays in cat sera, cross-reactivity is insufficient for distinguishing suppressed values from normal. Feline-specific peptide and antibody reagents are critical for development of a clinically useful immunoassay. In the current study, the genes encoding the mature common pituitary alpha and hormone-specific beta subunits of feline thyroid stimulating hormone (fTSH) were cloned and sequenced. The feline common pituitary alpha gene was cloned from the total RNA extracted from the feline pituitary gland by the reverse transcription polymerase chain reaction (RT-PCR). The gene fragment that encodes mature TSHbeta was cloned from the feline genomic DNA by direct polymerase chain reaction (PCR). Following the experience with improved expression of the human TSH beta subunit, the second intron was included to produce a fTSHbeta mini-gene construct. For both subunits, primers were based on consensus sequences from TSH in other species. The resulting 510 bp PCR product for the alpha subunit included the full coding sequence of the 96 amino acid mature subunit preceded by that of a 24 amino acid signal peptide. The predicted amino acid sequence of the mature α subunit had the following species homologies: to canine (98%), bovine (95%), tiger (97%) and human (69%). The 850 bp sequence of fTSHbeta genomic DNA consisted of two coding exons, an intron of 418 bp and a 60 bp signal sequence. The mature fTSHbeta subunit is homologous to canine (94%), human (88%), bovine (91%) and equine (95%) TSHbeta subunits. An immunoaffinity tag FLAG was added to 3' end of the alpha gene to facilitate detection by Western blot and purification. In human pituitary glycoproteins, single chain or yoked analogues have been shown to have increased stability and bioactivity. In the current study, yoked fTSH (yfTSH) was developed by fusing the nucleotides encoding the C-terminus of the beta subunit to the N-terminus of the alpha subunit by using DNA encoding the C-terminal peptide (CTP) of human chorionic gonadotropin beta subunit of 26 amino acids as a linker peptide. The yoked fTSH construct encoded from N-terminus to C-terminus: beta-CTP-alpha-FLAG. The construct of 1260 bp was cloned and sequence confirmed. This work describes for the first time the full coding sequences of the two subunits of fTSH and has produced DNA constructs for its *in vitro* expression and purification.

ABSTRACT #233

EXPRESSION AND PURIFICATION OF FELINE THYROTROPIN (fTSH): IMMUNOLOGICAL DETECTION AND BIOACTIVITY OF HETERIDIMERIC AND YOKED GLYCOPROTEINS. S Rayalam, LD Eizenstat, RR Davis, M Hoenig, and DC Ferguson. University of Georgia, College of Veterinary Medicine, Athens, GA.

One out of every three hundred cats is now diagnosed with hyperthyroidism. A diagnostic tool that can sensitively detect changes in thyroid status is essential to understand thyroid pathology in the cat and also to diagnose this condition at an earlier stage. Lacking an available source of pituitary-derived fTSH, previously cloned fTSH alpha and beta subunits were ligated into the expression vector PEAK™ and expressed in modified human embryonic kidney cells (PEAK™). As the alpha subunit has two and beta has one N-linked glycosylation sites, expression in a mammalian cell is critical for appropriate post translational modifications and folding. The fTSH alpha and beta subunits were cloned separately downstream of

the EF-1α promoter of the PEAK™ expression vector, and transiently co-transfected into PEAK™ cells. Similarly, a previously cloned and sequenced yoked fTSH (yfTSH) gene was ligated into the PEAK™ vector, transfected into PEAK™ cells with puromycin selection generating a stable cell line expressing yfTSH. The alpha subunit was also stably transfected into PEAK cells to generate a stable cell line expressing the feline pituitary alpha protein. Expression levels of at least 1 µg/ml were achieved for both heterodimeric and yoked fTSH forms. The glycoproteins were purified in one step using anti-FLAG immunoaffinity column chromatography to high purity based upon polyacrylamide gel electrophoresis and silver stain. All glycoproteins were standardized by protein assay. The purified alpha-FLAG glycoprotein had a molecular weight of 20.4 kDa and that of yfTSH-FLAG was 45 kDa. Both were recognized by anti-FLAG monoclonal antibody in Western blot. The glycosylated beta subunit had a molecular weight of 16.2kDa. Both heterodimeric and yoked glycoproteins were recognized by an in-house canine TSH ELISA employing a capture monoclonal antibody previously selected for ovine/canine TSHbeta and a polyclonal antibody generated previously against pituitary canine TSH, as well as by the commercially available canine TSH assay (Diagnostic Products Corp.). However, both assays detected only 33% of the recombinant fTSH as standardized by protein assay. The yoked glycoprotein exhibited parallelism with the heterodimeric form in the in-house ELISA. The recombinant heterodimeric fTSH exhibited 30% of the potency of a pituitary-source bovine TSH (bTSH) standard at inhibiting ^{125}I-bTSH binding by JP09 cells expressing the human TSH receptor [IC50 (ng/ml;mean +/- SD) fTSH: 43±2; bTSH: 13±3]. Discrepancies in immunological detection and biological potency remain to be clarified. This work constitutes the first report of *in vitro* expression and purification of recombinant feline thyrotropin. The demonstration of immunological recognition by antibodies generated against pituitary-source TSH, and of bioactivity confirms that the recombinant glycoprotein may be used to standardize and improve clinical assays for feline TSH.

ABSTRACT #234

MICROALBUMINURIA TESTING IN ASYMPTOMATIC LABRADOR RETRIEVERS NATURALLY EXPOSED TO *BORRELIA BURGDORFERI*. RE Goldstein[1], JL Sandler[2], BA Bellohusen[2] and HN Erb[1] 1. College of Veterinary Medicine, Cornell University, Ithaca, NY. 2. Guiding Eyes for the Blind, Yorktown Heights, NY.

"Lyme nephritis" is a poorly characterized syndrome that has been associated with severe glomerular and tubular renal injury and poor clinical outcome in young to middle aged dogs. In 1 study Labrador and Golden Retrievers were documented to be over represented in a group of dogs suspected to have suffered from this syndrome. The aims of this study were to identify possible associations between natural exposure to *Borrelia burgdorferi*, the causative agent of Lyme disease, using different available diagnostics modalities, and the presence of microalbuminuria (MA), a marker for early renal damage, in asymptomatic young Labrador Retrievers.

Natural exposure to *B. burgdorferi* was evaluated serologically in serum using a Western blot (Cornell University) and in plasma using an in-house Snap® test (3Dx® – Idexx® Labs.) on paired samples in a blinded fashion. Microalbuminuria was assessed using an in-house specific ELISA for canine albumin (ERD® – Heska®). The MA testing was performed in a blinded fashion to the serological results for B. burgdorferi. Non-parametric statistical analyses were utilized. A p value of ≤ 0.05 was considered significant.

Blood samples from 259 asymptomatic dogs (241 Labrador Retrievers, 17 Golden Retrievers, mean age 34 months) were included in the final analysis. Forty five dogs were positive for *B. burgdorferi* exposure on the Snap test. Western blot analysis included 55 dogs considered to be positive for natural exposure to *B. burgdorferi* and 29 dogs positive for vaccinal antibodies. Four dogs that were Snap positive (all low antibody level) were Western blot negative and 14 Western blot positive dogs (10 low, three moderate, one high antibody level) were Snap negative. All dogs with Western blots positive exclusively for vaccinal antibodies were Snap negative. A 93% agreement rate was calculated for the two tests for natural exposure to *B. burgdorferi* ($K = 0.78$, $p<0.0001$). Urine from 16 dogs was positive for MA (9 low, 3 medium and 4 high positive). Of those dogs 3 were positive for B. burgdorferi on a Western blot and 4 on the Snap test. There was no association between MA and positive exposure to *B. burgdorferi* based on results of a Western blot ($p=0.57$) or a Snap test ($p=0.53$).

This study demonstrated excellent agreement between the results of the 3Dx Snap test and the Western blot for antibodies associated with natural exposure to *B. burgdorferi* in asymptomatic young Labrador Retrievers. Additional studies are warranted to assess the value of MA screening for dogs with early Lyme nephritis. We found no association between *B. burgdorferi* exposure and MA in asymptomatic young Labrador Retrievers. Therefore MA screening is likely to be a valuable test for identifying dogs with early "Lyme nephritis". A positive MA result may identify dogs with true renal pathology and will not just be an incidental result of *B. burgdorferi* exposure.

ABSTRACT #235

PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* COLONIZATION IN DOGS ENTERING A VETERINARY TEACHING HOSPITAL. Beth Hanselman, Maureen Anderson, Stephen Kruth, and J. Scott Weese. Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of hospital-acquired infections in human hospitals worldwide and is increasingly being identified as a community-associated pathogen. The structural gene responsible for methicillin resistance, *mec*A, encodes a penicillin-binding protein (PBP) that facilitates decreased affinity to β-lactam antibiotics and MRSA isolates are frequently also resistant to a variety of other antimicrobial classes. MRSA infection is associated with increased morbidity and mortality in humans and there are increasing reports of serious, including fatal, MRSA infections in pets. Transmission of MRSA between pets and owners in households and veterinary clinics has been identified. Although risk factors and transmission patterns for MRSA infection in humans have been heavily studied, there has been minimal investigation into the prevalence or clinical implications of MRSA colonization in dogs. The objective of this study was to determine the prevalence of subclinical MRSA colonization in a canine population that presented to the Veterinary Teaching Hospital (VTH) at the Ontario Veterinary College.

A convenience sample of 203 dogs was consecutively sampled upon admission to the VTH over two months. Cotton swabs moistened with sterile saline were used to collect individual nasal, axillary and rectal samples from each dog and were stored at 4º C until isolation was performed. The swabs were plated directly onto mannitol-salt agar with 2 µg/ml oxacillin and incubated at 35˚C for 48 hours. Swabs were also enriched in 2 ml of a broth consisting of 10 g/l Tryptone T, 75 g/L sodium chloride, 10 g/L mannitol and 2.5 g/l yeast extract, for 24 hours at 35˚C. An aliquot of broth was then inoculated onto mannitol-salt agar with 2 µg/ml oxacillin as above. Isolates were identified as *S. aureus* based on colony morphology, Gram stain appearance, catalase and coagulase reactions and latex agglutination test (LAT). Methicillin-resistance was confirmed via PBP2a LAT and the MIC of oxacillin was determined via Etest.

MRSA was isolated from the nasal passages, but not rectum or axilla, of 2/203 (1%) dogs. In addition, two *S. aureus* isolates were cultured from two other dogs (nasal, axillary sites) that possessed a

borderline-resistant MRSA and grew on MRSA screening agar but did not possess PBP2a and were sensitive to amoxicillin-clavulinic acid, indicating that resistance was based on high-level β-lactamase production. Methicillin-resistant *S. intermedius* (n=2) and *S. schlieferi* (n=1) were isolated from rectal and nasal sites of other dogs. Clinical infections were not present in any dogs carrying methicillin-resistant staphylococci.

Colonization with MRSA was identified in a small percentage of clinically normal dogs. Further study is required to evaluate risk factors for MRSA colonization and implications of MRSA colonization of dogs towards other animals, owners and veterinary personnel.

ABSTRACT #236

ERYTHROCYTE ANTIOXIDANT STATUS AND PLASMA LIPID PEROXIDATION IN ANEMIC DOGS WITH CHRONIC RENAL FAILURE. Marcio D. Lustoza[1]; Márcia M. Kogika[1]; Márcia Y. Hasegawa[1]; Mitika K. Hagiwara[1]; Edimar C. Pereira[2]; Dulcinéia S.P. Abdalla[2]. [1]School of Veterinary Medicine, University of São Paulo; [2] School of Pharmaceutical Sciences, University of São Paulo, Brazil.

The purpose of this study was to investigate the role of oxidative stress in anemia of dogs with chronic renal failure (CRF), as it has been reported in human patients with CRF. Total, reduced and oxidized erythrocyte glutathione, and erythrocyte antioxidant enzymes - glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD), as well as plasma thiobarbituric acid reactive substances (TBARS) were measured in 30 anemic (PCV up to 37%) dogs with CRF and 40 healthy dogs. Erythrocyte concentrations of total and reduced glutathione were determined by high performance capillary electrophoresis, and oxidized glutathione was calculated from the difference between total and reduced glutathione concentrations divided by two. The activities of antioxidant enzymes - GR, GPX and SOD were measured by automatic analyzer. Plasma TBARS was determined by thiobarbituric acid colorimetric assay.

No differences were observed in erythrocytes concentrations of total glutathione, reduced glutathione and in activities of GR and GPX between groups. However, intraerythrocytic concentrations of oxidized glutathione were significantly higher ($P = 0.012$) in dogs with CRF (1.14 ± 0.62 μmol/g of hemoglobin; mean ± SD) when compared to healthy dogs (0.74 ± 0.64 μmol/g of hemoglobin), suggesting that the presence of imbalance of erythrocyte thiols metabolism might be considered, which can be caused by an increase in intraerythrocytic oxidants factors, such as reactive oxygen species (ROS) and an insufficient response in GR activity. Erythrocyte activity of SOD was also significantly higher in dogs with CRF ($2,077 \pm 1,102$ U/g of hemoglobin and $1,562 \pm 621$ U/g of hemoglobin for CRF group and control group, respectively; $P = 0.0062$), which suggests the possibility of enzymatic induction, probably caused by increased concentrations of ROS in intracellular environment; additionally, dogs with CRF presented higher plasma concentrations of TBARS (0.96 ± 0.24 μmol/L) than healthy dogs (0.43 ± 0.24 μmol/L) ($P = 0.0001$), indicating increased plasma lipid peroxidation in CRF dogs. Correlation was detected between serum creatinine and plasma TBARS concentrations ($r = 0.613$; $P = 0.0001$), as well as between PCV and plasma TBARS ($r = -0.634$; $P = 0.0001$), suggesting that increased oxidative stress may be related to the severity of renal dysfunction.

The results obtained in this study indicated an increase in oxidative stress in dogs with CRF, like reported in human patients. However, the increase of erythrocyte superoxide dismutase (SOD) activity, and the maintenance of intraerythrocytic reduced glutathione concentration in those dogs with CRF suggest that erythrocytes are still able to keep some antioxidant defense, and not providing great evidences of the relation between increased oxidative stress and intensification of anemia.

ABSTRACT #237

PLASMA SULPHUR AMINO ACIDS AND TOTAL GLUTATHIONE IN DOGS WITH CHRONIC RENAL FAILURE. Marcio D. Lustoza[1]; Márcia M. Kogika[1]; Helena A. Amaral[1]; Clara S. Mori[1]; Edimar C. Pereira [2]; Dulcinéia S. P. Abdalla [2]. [1] School of Veterinary Medicine – University of São Paulo; [2] School of Pharmaceutical Sciences – University of São Paulo, Brazil.

In order to detect if sulphur amino acids metabolism is abnormal in dogs with chronic renal failure (CRF), plasma methionine, homocysteine, cysteine and total glutathione were determined in 30 dogs with chronic renal failure and in 40 healthy dogs. Sulphur amino acids are important in glutathione synthesis, which is the most widespread cellular thiol compound and an important intracellular antioxidant. Glutathione in plasma may act as a form of storage and transport of cysteine, and possibly as a plasma antioxidant compound.

Sulphur amino acids and total glutathione were measured in plasma by high performance capillary electrophoresis.

No difference was observed in plasma concentrations of methionine and cysteine. Homocysteine ($P = 0.001$) and total glutathione ($P = 0.003$) concentrations were significantly higher in dogs with CRF (7.13 ± 6.76 μmol/L and 1.81 ± 1.12 μmol/L, respectively; mean ± standard deviation) than in healthy group (3.82 ± 3.44 μmol/L and 1.07 ± 0.73 μmol/L, respectively). Significant correlation was detected between serum creatinine and plasma homocysteine ($r = 0.36$; $P = 0.0019$).

In humans with chronic renal failure, increased plasma homocysteine concentration is a common finding and it is associated to endothelial cells dysfunction, thrombosis and reactive oxygen species production, increasing the risks of cardiovascular disease, but the exact mechanism is not completely understood. However, it is known that B-vitamin deficiencies, mainly folic acid, B2, B6 and B12, presence of some genotypes and decreased catabolism of homocysteine by renal tubular cells are involved in hyperhomocysteinemia in chronic renal failure.

Positive correlation between serum creatinine and plasma homocysteine suggests that increased homocysteine concentrations could be also related to renal dysfunction in dogs. Increased plasma total glutathione concentration findings may be attributed to loss of renal tissue, as it is known that kidneys are the main site of glutathione biodegradation.

Data obtained in this study indicate the presence of some unbalance in sulphur amino acids metabolism in dogs with chronic renal failure. Whether the increased homocysteine levels are related to vitamin B complex deficiency or if there is any implication on the cardiovascular system was not studied and deserves a further investigation. Also, the influence of increased total glutathione found in dogs with CRF on the plasma antioxidant status must be elucidated.

ABSTRACT #238

ASSOCIATION OF MICROALBUMINURIA AND THE URINE ALBUMIN:CREATININE RATIO WITH SYSTEMIC DISEASE IN DOGS. JC Whittemore, VL Gill, WA Jensen, SV Radecki, LC Prause, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO and Heska Corporation, Fort Collins CO.

Previous studies have shown microalbuminuria (MALB) without overt proteinuria and the urine albumin:creatinine ratio (UAC) to be excellent predictors of disease, morbidity, and mortality in humans. It is currently unknown whether these relationships are also true for

dogs. The objectives of the current study were to determine the prevalence of systemic disease in urine dipstick (DpP) negative dogs with and without MALB and to determine the diagnostic utility of a semi-quantitative MALB kit (MALBE, E.R.D.-HealthScreen® Urine Test), quantitative MALB assay (MALBQ), and UAC in dogs.

Urine samples from 408 dogs presented to Colorado State University (CSU) and negative on DpP were assessed. Urinalyses were performed at the CSU Clinical Pathology Laboratory. UPC (positive cutoffs of > 0.5 and 0.1), MALBQ and MALBE (positive = values > 1 mg/dl), and UAC values (cutoffs of 100 and 200 mg/gm) were determined. Clinical diagnoses recorded within the months of the urine collection were grouped as follows: healthy, neoplasia, dermatologic and non-dermatologic infection, inflammatory, immune-mediated, urinary and renal, endocrine, cardiologic, orthopedic, neurologic, toxicosis, trauma, and other disease. Sensitivity (Se) and specificity (Sp) were determined for each test using disease status as the standard. The influence of clinical diagnosis, gender, age, BUN, creatinine, blood pressure, urine culture results, temperature, pyuria, hematuria and bacteriuria, on MALBQ, MALBE, or UPC0.1 was evaluated by logistic regression. The small number of dogs positive by UPC0.5, UAC100 and UAC200 precluded any statistical evaluation of these tests.

Sensitivity and Sp for presence of disease for the tests are listed in the following table. Factors predicting MALB included clinical diagnosis, age, BUN, hematuria, urine culture results and bacteriuria, depending on the method of MALB detection.

	MALBQ	MALBE	UPC0.5	UPC0.1	UAC100	UAC200
Sensitivity	35.6%	36.9%	4.5%	71.1%	5.3%	2.5%
Specificity	85.4%	91.7%	100%	18.8%	100%	100%

Based on this study, MALB is associated with the presence of disease. UAC ratios were not useful given their poor sensitivity for disease. The diagnostic utility of MALB tests for identifying occult disease will depend on the prevalence of disease in the study population and the Se and Sp of other screening tests. The Se and Sp of MALB tests for systemic disease were lower than would be anticipated if overtly proteinuric dogs were included in the study.

ABSTRACT #239

PHARMACOKINETICS OF EXOGENOUS CREATININE IN PIGEONS. RA Wagner, HP Lefebvre . Division of Laboratory Animal Resources, University of Pittsburgh, Pittsburgh, PA, Physiopathologie et Toxicologie Expérimentales, National Veterinary School of Toulouse, France.

Plasma creatinine concentration is largely used in clinical pathology for indirect evaluation of renal function (GFR) in mammals. In avian nephrology, use of plasma creatinine as an indicator of renal function is questionable as basal levels are very low, and under the limit of quantitation (LOQ) of routine analyzers; and changes in endogenous creatinine concentrations have not been correlated with renal disease. In contrast to mammals, birds eliminate 90% of their nitrogenous waste by secretion of uric acid, not filtration. Consequently, there are no easily practicable tests for assessment of renal function in birds. Creatinine elimination is primarily via glomerular filtration and is not absorbed by the renal tubules. Possibly exogenous creatinine clearance kinetics can overcome these unique avian renal physiology problems, allowing for a practical renal function test.

Five healthy pigeons, aged between three and 36 months, weighing between 275 and 460 g, were used. Creatinine was dissolved in sterile distilled water to obtain a solution at a final concentration of 40 mg/mL. Creatinine was administered by IV bolus at a dose level of 40 mg/kg. Blood was sampled just before administration of the test article. After bolus iv administration, 0.2 mL blood was sampled at 2, 4, 8, 15 and 30 minutes, 1, 1.5 and 2 hours. Blood was collected in heparinized tubes and immediately centrifuged. Plasma was stored at 4 °C until assay. Plasma creatinine was assayed using a dry chemistry analyzer, the limit of quantitation being 0.5 mg/dL. Pharmacokinetic analysis was performed by a non-compartmental approach using WinNonLin software. Data are expressed as mean±SD.

Creatinine was not quantifiable in the plasma before dosing. The peak plasma creatinine concentration observed at two minuteswas 10.3±2.8 mg/dL. Two hours later, the plasma creatinine was quantifiable in only three birds with value lower than 0.79 mg/dL. Plasma clearance (Cl), steady state volume of distribution (Vss), mean residence time (MRT), and elimination half-life (t1/2) were 13.4±2.7 mL/kg/min, 602±102 mL/kg, 46±10 min and 37±9 min.

These results indicate that the plasma clearance of creatinine in pigeons is about fourfold the plasma clearance of creatinine observed in dogs, explaining consequently the very low basal level. The volume of distribution of creatinine is very close to total body water volume, as previously observed in mammals. Use of exogenous plasma creatinine clearance test could offer a practical way for assessing renal function in pigeons, but requires further investigations to check the sensitivity of such an approach and to improve the practicability.

ABSTRACT #240

COMPARISON OF URINARY ALBUMIN EXCRETION NORMALIZED BY CREATININE CONCENTRATION OR URINE SPECIFIC GRAVITY. HM Syme and J Elliott, Royal Veterinary College, London, UK.

Survival of both azotaemic and non-azotaemic cats is inversely related to urine albumin to creatinine ratio (UAC). Semi-quantitative test kits for the measurement of urine albumin concentration (E.R.D.-HealthScreen™ Feline Urine Test, Heska Corporation) normalize the urine albumin concentration with urine specific gravity (USG) instead of urine creatinine concentration (UC). This approach has potential advantages in that it reduces expense, the volume of urine required and the sample turn-around time (if, as a result, samples do not need to be sent to an outside laboratory). The purpose of the present study was to determine if urinary albumin normalized to USG (UA/USG) predicts survival of cats as well as UAC does, and investigate the correlation between the two methods for normalization.

Cats were included in the study if they had been diagnosed with renal failure or were apparently healthy (on the basis of history, physical examination and routine biochemical analysis). In addition the following data had to be available from the medical record: the age of the cat, urine albumin and creatinine concentrations, USG, plasma creatinine concentration and survival time. Urinary albumin concentration was measured using a sandwich ELISA assay previously validated for feline urine. 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). Variables that were included in the analyses were age, plasma creatinine concentration and either log UAC or log UA/USG (in separate models due to co-linearity of the variables). The measures of protein excretion were log transformed for statistical analysis. Pearson's correlation coefficient was calculated to describe the relation between the two measurements of albumin excretion.

Data were available from 124 cats, 54 of which died during the period of follow-up. Age ($p=0.03$) and plasma creatinine concentration ($p<0.001$) were predictive of survival in both models. Log UAC ($p<0.001$) and log UA/USG ($p<0.001$) were both highly predictive of reduced survival time. Correlation between UAC and UA/USG was strong (Pearson correlation coefficient 0.97, $p<0.001$).

Normalization of urine protein measurements to USG or to urine creatinine concentration yields similar prognostic information. The use of either method is acceptable for clinical practice.

ABSTRACT #241

MOLECULAR CHARACTERIZATION OF TRICHOMONADS FROM FECES OF DOGS WITH DIARRHEA. Jody L. Gookin, Adam J. Birkenheuer, Victoria St. John, Michelle Spector‡, Michael G. Levy North Carolina State Univ., Raleigh, NC; ‡Fred Hutchinson Cancer Research Center, Seattle, WA.

Trichomonads are occasionally observed in feces of dogs with diarrhea. On the basis of superficial morphological appearance, these infections have been attributed to opportunistic overgrowth of the commensal, *P. hominis*. However, molecular characterization of canine trichomonads has never been reported. This study was performed to determine, by means of rRNA gene sequence analysis, the identity of trichomonads observed in feces from dogs with diarrhea. Total DNA was extracted from fecal samples obtained from a three-month-old mixed breed dog and litter of German Shepherd pups having profuse liquid diarrhea containing numerous trichomonads. Total DNA was subject to PCR amplification of rRNA genes using species specific and universal primers respectively. PCR products were cloned and bidirectionally sequenced. The three-month-old mixed breed dog had clinical signs of profuse watery diarrhea, dehydration, weight loss, and lethargy. The dog was born into a closed research colony and had no history of contact with cats, swine or cattle. In addition to copious numbers of trichomonads, fecal samples tested positive for *Giardia* sp. antigen, and contained numerous *Clostridium* sp. spores and low numbers of inflammatory cells. Feces were negative for *Cryptosporidium* antigen and helminth ova. A 642 base pair product of the 18S rRNA gene was amplified and cloned from DNA extracted from feces. The sequence shared 100% identity with *T. foetus* (AY754332). The pup was treated with metronidazole and fenbendazole. Diarrhea gradually resolved and feces were negative for *T. foetus* by PCR 15-mos after diagnosis. The litter of German shepherd pups (n=6) were four weeks of age and had clinical signs of acute-onset profuse liquid diarrhea, weight loss, and weakness. Feces contained numerous motile trichomonads and *Isospora canis* ova and were negative for *Giardia* sp. and parvoviral antigen. An 1864 base pair product of the 5.8S, ITS1, ITS2, partial 18S and 28S rRNA genes was amplified and cloned from DNA extracted from feces. The sequence shared 100% identity with *P. hominis* (AY758392). The pups were treated with fenbendazole and four of six died within four days. For the remaining two pups, diarrhea gradually resolved and the dogs were reportedly healthy six months later. The present study is the first to establish the molecular identity of trichomonads infecting dogs with diarrhea. These studies validate the longstanding assumption that canine trichomoniasis may be attributed to *P. hominis*. Importantly, these studies additionally recognize that canine trichomoniasis may be caused by infection with *T. foetus*. This report is consistent with prior studies in suggesting that trichomonads flourish in young dogs having diarrhea associated with co-existing intestinal infection. Whether there is any prognostic significance to the identity of trichomonads or whether trichomoniasis contributed directly to diarrhea in the present cases cannot be resolved by the present study.

ABSTRACT #242

PREVALENCE OF SELECT INFECTIOUS DISEASE AGENTS IN CATS FROM ARIZONA. JM Eberhardt, K Neal, T Shackelford, MR Lappin. Colorado State University (Eberhardt, Lappin), Santa Cruz Humane Society, Nogales, AZ (Neal), and North Phoenix Spay and Neuter Clinic, Phoenix, AZ (Shackelford).

A number of different infectious diseases cause morbidity and mortality in the domestic feline population with some infectious agents being of zoonotic concern. Infected cats can serve as reservoirs and directly transmit infectious agents or can carry infected flea and tick vectors into contact with other animals including humans. Fleas and ticks are common in Arizona. An *Ehrlichia canis*-like organism is known to infect cats, but documented cases in the literature are few. Because *E. canis* infection is common in dogs in Arizona (12% seroprevalence rate), we hypothesized that it would be a logical state in which to perform a prevalence study in order to identify more *E. canis* infected cats to study. The objective of this study was to determine the prevalence of *Ehrlichia sp*., *A. phagocytophilum, Mycoplasma haemofelis*, '*Candidatus* Mycoplasma haemominutum*',* and *Bartonella sp.* DNA in blood of cats in Arizona.

Blood (1.5 ml) was collected from feral and relinquished cats (n = 112) by veterinarians in Phoenix and Nogales, Arizona, placed into 1.5 ml EDTA tubes, and stored at -20ºC. Samples were collected between March 2004 and July 2004, times when environmental tick and flea loads are expected to be highest in this region. The samples were batched until shipped on cold packs by overnight express to Colorado State University. On arrival, the samples were stored at -20ºC until assayed. Previously published PCR assays for amplification of DNA of *Ehrlichia spp.*, *A. phagocytophilum*, *Neorickettsia risticii*, *M. haemofelis,* '*Candidatus* Mycoplasma haemominutum', and *Bartonella sp.* were utilized.

DNA from one or more of the organisms was amplified from 31 of 112 blood samples (27.7%). DNA consistent with *Bartonella clarridgeiae* (15 samples; 13.4%), *B. henselae* (14 samples; 12.5%), '*Candidatus* M. haemominutum' (nine samples; 8.0%), and *M. haemofelis* (5 samples; 4.5%) was detected in the blood of some cats. DNA of *Ehrlichia spp.*, *A. phagocytophilum*, or *N. risticii* was not amplified from the blood of any cat.

Our results indicate that cats from these regions of Arizona are exposed to *M. haemofelis,* '*Candidatus* Mycoplasma haemominutum', *B. henselae,* and *B. clarridgeiae*. Failure to amplify DNA of *A. phagocytophilum* may relate to the fact that the tick vector, *Ixodes pacificus,* is not known to be present in these regions of Arizona. Failure to amplify DNA of *Ehrlichia* spp. suggests that cats were not exposed, were exposed but not infected, or were infected but the DNA was not amplified by the PCR assay used in this study.

ABSTRACT #243

EFFECTS OF A SINGLE DOSE OF AN INTRANASAL FELINE HERPESVIRUS 1, CALICIVIRUS, AND PANLEUKOPENIA VACCINE ON CLINICAL SIGNS AND VIRUS SHEDDING AFTER CHALLENGE WITH VIRULENT FELINE HERPESVIRUS 1. MR Lappin, RW Sebring, M Porter, SJ Radecki, J Veir. Colorado State University, Fort Collins, CO (Lappin, Veir), and Heska Corporation, Fort Collins CO (Sebring, Porter, Radecki).

Feline herpesvirus 1 (FHV-1) infection is very common in cats, is extremely contagious between cats, and frequently results in severe clinical disease. In a recent prevalence study at a humane society in north central Colorado, FHV-1 DNA was amplified from throat swabs or nasal discharges collected from 52 of the 61 cats (85.2%) tested. Thus, it is important to induce immunity in kittens by vaccination quickly, particularly in populations at high risk for exposure. The purpose of this study was to determine whether a single administration of commercially available intranasal FVRCP vaccine (Feline UltraNasal™ FVRCP Vaccine, Heska Corporation, Fort Collins, CO) to kittens lessened clinical signs and FHV-1 viral shedding when compared to unvaccinated control kittens after FHV-1 challenge.

Three groups of 10 unvaccinated kittens were administered one dose of the vaccine two, four, or six days before challenge, respectively and one group was maintained as unvaccinated controls. FHV-1 challenge was then induced following USDA protocols and the kittens were observed for clinical signs of disease for 14 days. Throat swabs were collected from the group of kittens vaccinated on day -6 and the group of kittens used as controls on days -6, -3, 0, 4, 6, 8, and 12. Swabs were placed in sterile saline, incubated for two

hours at room temperature, and frozen at -70ºC until transported on dry ice for assay. FHV-1 and feline GAPDH DNA were amplified from each sample by use of a previously described fluorogenic PCR. Results for the fluorogenic assay for FHV-1 DNA were compared to cell count derived from a standard curve for GAPDH/cell to ensure an adequate cell count was present on the swab for analysis.

When vaccinated kitten results were compared to control kitten results, cats vaccinated six or four days prior to challenge had significantly lower clinical scores ($P < 0.05$) than control cats. FHV-1 shedding was lower in kittens vaccinated six days prior to challenge than in control cats on day 6 after challenge ($P < 0.05$).

Administration of this vaccine within several days prior to exposure lessened clinical signs of disease and FHV-1 shedding compared to unvaccinated cats.

ABSTRACT #244

ANALYSIS OF *BABESIA GIBSONI* RECURRENCE AFTER ATOVAQUONE TREATMENT. Aya Matsuu, Hiromi Ikadai, Shozo Okano, Seiichi Higuchi. Kitasato University, Towada, Japan.

This study was performed to examine the sensitivity of recurring *Babesia gibsoni* to atovaquone in experimentally infected dogs that were initially treated with this drug. Changes in the *B. gibsoni* mitochondrial DNA sequence for cytochrome b, which binds atovaquone, were also studied. Three dogs were experimentally infected with *B. gibsoni* isolated from naturally infected dogs in Aomori Prefecture, Japan. Once parasitemia reached 10%, atovaquone was administered orally (30 mg/kg twice daily for seven days). Although the parasite disappeared from blood smears within two days of atovaquone treatment, by 33 ± 2 days after the last treatment, parasites reappeared in blood smears. An *in vitro* sensitivity test was performed using peripheral blood collected from two dogs at the time of recurrence. For the original parasites, complete growth inhibition occurred at 1000 nM of atovaquone, whereas the recurring parasites were inhibited by only 39.5 ± 8.3% and 31.3 ± 8.1% of this concentration after 48 hour of incubation, respectively. DNA isolated from recurring *B. gibsoni* in the three dogs was sequenced for the cytochrome *b* gene and compared with that from the original parasite. A single nucleotide mutation resulting in one amino acid replacement (methionine to isoleucine) was common to all three dogs. This mutation was seen only in recurring parasites after atovaquone treatment, but not in pretreatment parasites, using restriction length fragment polymorphism analysis. These results indicated that the use of atovaquone therapy alone allows the recrudescence of parasites and decreases parasite susceptibility to this drug. Point mutations in the sequence for mitochondrial cytochrome *b* might be associated with this.

ABSTRACT #245

FELINE LYMPHOCYTE BLASTOGENESIS IN RESPONSE TO FELINE HERPESVIRUS 1 ANTIGENS AND CONCANAVALIN A AFTER VACCINATION WITH FIVE FVRCP VACCINES. MR Lappin, J Veir, R Sebring, SV Radecki. Department of Clinical Sciences, Colorado State University, Fort Collins, CO (Lappin, Veir) and Heska Corporation, Fort Collins, CO (Sebring, Radecki).

Feline herpesvirus 1 (FHV-1) infection is common in cats and disease manifestations can be severe. Protection against FHV-1 is mediated in part by cell-mediated immune responses. A FVRCP vaccine (Feline UltraNasal™ FVRCP Vaccine, Heska Corporation) for intranasal administration (FVRCP-IN) was recently licensed in the United States. The purpose of this study was to assess lymphocyte blastogenesis (LBT) in response to FHV-1 antigens and the non-specific mitogen concanavalin A (Con A) in cats after vaccination with five different FVRCP vaccines.

FHV-1 negative kittens (n = 50) were purchased and randomly divided into five groups of 10. On days 0, 28, and 56, each group of kittens was administered the FVRCP vaccine for IN administration or one of four FVRCP vaccines for SQ administration. Blood was collected into heparin on days 67, 81, and 180. Whole blood LBT assays were performed using either FHV-1 UV inactivated antigens (equivalent to 400, 40, or 4 $TCID_{50}$ per well) or concanavalin A (5µg/ml, 10µg/ml, or 20µg/ml per well) to stimulate cell division. Responses (stimulation indices) to Con A and FHV-1 antigens were log transformed prior to statistical analysis to normalize the residuals. Geometric means by group were calculated and data were analyzed using a repeated measures experiment (the MIXED procedure in SAS, SAS Institute, Cary, NC, Version 9.1). The statistical model included vaccine group, time, and the interaction between time and group as fixed effects. If the time by group interaction was statistically significant ($P < 0.05$), within time group effects were evaluated. Where within group effects were significant, group means were compared using Fisher's least significant difference test (LSD) in a pair-wise fashion.

Group mean stimulation indices for both FHV-1 antigen and Con A were > 1 for each cat group on all collection days. A group by time interaction was detected for both Con A and FHV-1 ($P < 0.05$). Within time group effects were detected at 10 and 12 ($P < 0.05$), but not 26 ($P > 0.05$), weeks for each of these responses. At 10 weeks, cats vaccinated with the FVRCP-IN vaccine had significantly greater responses to Con A and FHV-1 as compared to cats vaccinated with each of the SC vaccines. At 12 weeks, cats vaccinated with the FVRCP-IN vaccine had significantly greater responses to both Con A and FHV-1 than cats in two of the four groups vaccinated with FVRCP vaccines SQ.

These results suggest that cats administered the FVRCP-IN vaccine have greater or comparable cell mediated immune responses to FHV-1 antigens and Con A as cats administered FVRCP vaccines SQ for the first several months after vaccination. Challenge studies will be needed to further characterize how these findings relate to vaccine-induced FHV-1 immunity.

ABSTRACT #246

CYTAUXZOON FELIS: MOLECULAR CHARACTERIZATION AND DIAGNOSTIC TEST DEVELOPMENT. A. Birkenheuer, H. Marr, J. Le, A. Valensizi and E. Breitschwerdt. North Carolina State University College of Veterinary Medicine Raleigh, NC.

Cytauxzoonosis is an emerging infectious disease in North America. There currently are no specific serologic or molecular tests available to diagnose *C. felis* infections. Therefore, our first specific aim was to characterize *Cytauxzoon felis* 18S rRNA gene sequences from organisms causing naturally occurring cases of fatal cytauxzoonosis, and compare these sequences to those reported in Genbank. Our second specific aim was to develop a rapid, sensitive and specific polymerase chain reaction (PCR) test for the diagnosis of *C. felis* infections in feline whole blood.

Full-length 18S rRNA genes were amplified by PCR using primers designed to amplify nearly all piroplasms. These amplicons were cloned into plasmid vectors and sequenced bi-directionally using an automated DNA sequencer. These sequences were determined to have >99% sequence identity with the *C. felis* sequences reported in Genbank. These finding confirmed that the 18S rRNA genes sequences from organisms causing fatal and non-fatal infections were not different.

Based on these sequences a *C. felis* specific primer pair was developed to amplify a 285 base pair fragment of the 18S rRNA gene. This primer pair amplified the appropriate size product from the four naturally infected *C. felis* samples, but produced no amplicons from non-infected feline blood samples or other pathogens including *Babesia canis* (all subspecies), *B. gibsoni* (both genotypes), *Theileria annae, Toxoplasma gondii, Rickettsia, Ehrlichia, Mycoplasma* or *Bartonella*. The 285 base pair amplicon was cloned, sequenced and confirmed to be the appropriate *C. felis* 18S rRNA

gene fragment. Test sensitivity was established by using serially diluted plasmid in either water or non-infected feline whole blood. The test was able to detect concentrations as little as 1 plasmid copy/µl. This sensitive and specific test will be useful to rapidly diagnose clinical *C. felis* infections and perform molecular epidemiologic studies of *C. felis* in wildlife and domestic cats.

ABSTRACT #247

BABESIA GIBSONI CYTOCHROME B GENE ANALYSIS. A. Birkenheuer and H. Marr. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Babesia gibsoni (Asian genotype) is an emerging infectious disease in dogs in North America. Atovaquone and azithromycin combination therapy has been recently shown to be the most effective therapy for *B. gibsoni*. Despite these recent advancements, rare treatment failures have been reported. It is possible that these failures are secondary to drug resistance. *Babesia gibsoni* and other protozoal parasites have been show to develop resistance to atovaquone, and this resistance in some protozoa is associated with point mutations in the cytochrome B gene. Our specific aim was to characterize the cytochrome B gene sequences from multiple *B. gibsoni* isolates to determine the degree of conservation between isolates.

Partial cytochrome B genes were amplified from 3 *B. gibsoni* using degenerate oligonucleotide primers designed to amplify *Babesia* and *Theileria* cytochrome B genes. The amplicons were cloned into a plasmid vector and sequenced bidirectionally using an automated DNA sequencer. These partial gene sequences were translated to determine the putative amino acid sequences. The resulting amino acid sequences were 226 aa long. This partial gene sequence codes for the N-terminus of the cytochrome B protein representing 50% of the complete protein and includes one of the putative atovaoquone binding sites that is associated with resistance. A multiple sequence alignment of the putative amino acid sequence determined that the 3 *B. gibsoni* partial cytochrome B sequences shared >99% homology with each other and 83.6% homology with *B. bovis*. The single amino acid difference detected in one of the *B. gibsoni* isolates was not located in the atovaquone binding site.

These cytochrome B genes characterized from *B. gibsoni* will be used for at least two functions in the future. First PCR-RFLP and or gene sequencing can be used to screen *B. gibsoni* isolates for mutations that could result in resistance to atovaquone. Secondly cytochrome B is present with *Babesia* organisms as a multi-copy extrachromosomal molecule with some species having over 100 copies/organism. Making it an attractive target for molecular diagnostic test development that should provide improved sensitivity over the currently available tests.

ABSTRACT #248

THE USE OF PRE-ENRICHMENT MEDIA TO ENHANCE DETECTION AND ISOLATION OF *BARTONELLA SPP*. FROM DOGS. Michael Wood, Ricardo Maggi, and Edward Breitschwerdt. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

When considering bacteria as a cause or cofactor in a disease state, culturing one or more organisms from tissue samples is frequently used to support causation. Unfortunately, certain bacteria, such as *Bartonella*, fail to grow consistently with traditional culture techniques. This is particularly true with *B. henselae (Bh)*, which has only been isolated once from a dog. Currently, *Bartonella* presence in dogs is determined by serology and amplification of bacterial DNA in blood samples. The following study demonstrates that these methods may not be sufficiently sensitive to detect *Bh* infection in dogs, possibly because of low bacteria/mL of blood. By utilizing a pre-enrichment *Bartonella* alpha *Proteobacteria* growth medium (BAPGM) followed by real-time PCR (RT-PCR), dogs initially lacking serological and PCR evidence of *Bh* infection were found to be bacteremic. BAPGM is a modified insect growth media formulated by our laboratory to enhance the growth of alpha *Proteobacteria*, which includes all species of *Bartonella*. A convenience sample of serum and EDTA-anticoagulated blood submitted to the NCSU Vector Borne Disease Diagnostic Laboratory (VBDDL) between August and November 2004 was used in this prospective study. The only inclusion criterion was the attending clinician's request for *Bartonella* serology and/or PCR. Seven samples from dogs with endocarditis, polyarthritis, thrombocytopenia, cyclical fever and general malaise were tested. Serum from 5/7 patients was used for detection of *Bh* and *B. vinsonii berkhoffii (Bvb)* IgG antibodies using immunofluorescence assays (IFA). Concurrently, for all cases, one aliquot of EDTA anti-coagulated blood was used for RT-PCR targeting the *Bartonella* 16S-23S intergenic spacer region. A second aliquot was inoculated into BAPGM and maintained at 35ºC for seven days, at which time an aliquot was sub-inoculated onto blood agar plates and another aliquot was again processed for RT-PCR. If bacterial growth was obtained on the plate, colonies were cloned and sequenced. *Bh* IFA titers ranged from undetectable to 1:1024. All seven samples tested negative for *Bartonella spp.* DNA when initially screened by RT-PCR. After seven days of pre-enrichment, 4/7 samples were RT-PCR positive for *Bartonella spp*. DNA. Bacterial colonies grew from only these four pre-enrichment samples following sub-inoculation. Based upon DNA sequencing, two isolates were *Bh,* whereas the molecular identification of two isolates is pending. In only 3/5 dogs did the evidence for exposure to *Bh* or *Bvb* via serum titers correlate with the RT-PCR results post enrichment.

This study highlights potential diagnostic limitations of *Bartonella* serology and RT-PCR for the identification of *Bartonella spp*. bacteremia in dogs. Increased diagnostic sensitivity can be achieved by pre-enrichment growth of *Bartonella* organisms in a clinical sample. To our knowledge, the use of BAPGM facilitated the first successful growth and isolation of *B. henselae* from the blood of sick dogs. Improvements in diagnostic techniques may revolutionize our ability to diagnose and treat patients with previously undetectable infections.

ABSTRACT #249

ESCHERICHIA COLI RESISTANCE IN CANINE URINARY TRACT. Dawn M. Boothe[1], Timothy J. Smaha[1], Terri L. Hathcock[2], Brenda M. Bixler[2]. [1] Auburn University College of Veterinary Medicine Anatomy, Physiology, and Pharmacology and [2] Bacteriology- Mycology Laboratory.

This study examined the incidence of antimicrobial resistance to selected *E. coli* isolates associated with first time urinary tract infection (UTI) in dogs in a Veterinary Teaching Hospital setting. Further, an attempt was made to identify factors which might contribute to the advent of resistance in these patients. Antibiograms (n=175) performed by the Clinical Microbiology Laboratory (CML) at Auburn University (following guidelines of the National Committee of Clinical Laboratory Standards) were collected retrospectively for the dates of May 2002 through December 2004. Samples collected by free-catch were excluded. Medical records of these patients were reviewed. The proportion of organisms resistant to each antimicrobial (n=17) on the susceptibility panel was determined. Additionally, each *E coli* isolate was scored as either susceptible ("S") or resistant ("R") for its antibiogram, with "R" indicating at least 9 of the 17 drugs on the susceptibility panel were designated by the CML as "R" or "I" (intermediate). This designation was then used to determine the proportion of resistant *E coli* isolates for each of the following factors: signalment (age; weight, gender, neutered status); medical (overweight, previous antimicrobial therapy, immune suppressive drugs, endocrine disease) or hospitalization (ward, duration of hospitalization, and method of

collection). Proportional comparisons were made using Chi square analysis.

Overall resistance to all 17 antimicrobials tested was 31%. No isolate was resistant to amikacin or nitrofurantoin. The antimicrobials with resistance less than or equal to 10% were ceftazidime (7%) and tobramycin (10%). Those antimicrobials to which E *coli* was most resistant were cephalothin, piperacillin, ciprofloxacin, and enrofloxacin (44% each), ticarcillin (47%), carbenicillin (48%) and ampicillin (50%). Antimicrobials with resistance levels between 10% and 40% included ceftiofur (20%), gentamicin (23%), chloramphenical (28%) amoxicillin-clavulanic acid, tetracylcine (39%) and sulfadimethoxine trimethoprin (40%). Those factors associated with a greater percent resistance ($p \geq 0.0005$) were gender (males), previous antimicrobial therapy (within 30 days), immune suppressive drugs (within 30 days), and days of hospitalization (≥ 5days).

This retrospective study suggests an incidence of E. *coli* resistance higher than clinically anticipated. This, coupled with identification of factors associated with higher incidences of resistance, suggests urine culture and susceptibility testing - rather than empirical choice- is the basis for antimicrobial selection in selected cases.

ABSTRACT #250

COMPARISON OF GRAM NEGATIVE ANTIMICROBIAL RESISTANCE PATTERNS AT FOUR UNIVERSITIES. Dawn Boothe[1], Timothy Smaha[1], Heather Davis[1], Frank Austin[2], Joshua Daniels[3], and Jeff O'Kelley[4]; [1]Auburn University, Auburn, AL; [2]Mississippi State University, Mississippi State, MS; [3]Washington State University, Pullman, WA; [4]University of Florida, Gainesville, FL.

The purpose of this study was to compare the incidence of antimicrobial resistance at four veterinary medical colleges to selected microbial organisms cultured from the urine of dogs. This study was stimulated by the higher-than-expected incidence of resistance to *E coli* cultured from dogs with urinary tract infections by the Clinical Microbiology Laboratory (CML) at Auburn University (AU) (see accompanying abstract). Other participating CMLs included Mississippi State University (MSU), University of Florida (UF) and Washington State University (WSU). Each CML followed guidelines as set forth by the National Committee on Clinical Laboratory standards (NCCLS). Data from three of the four CMLs was based on tube dilution, with the fourth (MSU) reflecting disc agar diffusion. Antibiograms were collected retrospectively from isolates recovered from the urine of dogs; the study period was May 2003 to May 2004. The percent of isolates resistant to each drug was determined for each CML. The mean of percent resistant isolates to all drugs was determined for each organism and each CML. Resistance of each CML was then compared based only those drugs common to each (reported as range and median).

For *E. coli*, resistance to all drugs tested by each CML was highest for AU (n= 175; 31±17%) followed by MSU (n = 14; 18± 13%), UF (n=64; 14± 9%) and lowest for WSU (n=56; 10±10%). For comparison of CML based on six antimicrobials tested in common, AU was consistently the highest, whereas, WSU was the lowest save ticarcillin (TIC; lowest for MSU). The incidence of resistance for each drug among the CMLs was (range; median): amoxicillin clavulanic acid (AMXC: 2 to 40%; 13%), ampicillin (AMP; 27-50%; 32%), cephalothin (4 to 45%; 30%), enrofloxacin (7 to 46%; 15%), sulfamethoxazole-trimethoprim (SXT: 9 to 38%; 22%) and TIC (7 to 46%; 15%). For two organisms, only two CML provided data. For *Proteus mirabilis* (n=11 each for AU and UF), the incidence of resistance was the same for both CML and 10 antimicrobials with resistance to tetracycline being the highest at 100%. Patterns also did not differ for *Klebsiella* spp (n= 10 for UF, 8 for AU) except for TIC which was much higher at AU (86%) compared to FL (0%). For both CML, resistance to AMP was highest (86%). This pilot study suggests marked differences occur in the incidence of resistance reported among the CML; differences may reflect true geographical differences or differences in methodology among the CML, although the latter should be precluded if CML adhere to NCCLS guidelines and interpretive standards.

ABSTRACT #251

EFFICACY OF HUMAN RECOMBINANT ALPHA-2B INTERFERON AND FELINE RECOMBINANT OMEGA INTERFERON AGAINST FELINE HERPESVIRUS REPLICATION *IN VITRO*. Nicola Siebeck[1], David J. Hurley[2], Maricarmen Garcia[3], Craig Greene[4], Roberto Köstlin[1], Ursula Dietrich[3]; [1]Department of Small Animal Surgery, Ludwig-Maximilians University of Munich, Germany, [2]Department of Large Animal Medicine, [3]Department of Avian Medicine/Virology, [4]Department of Small Animal Medicine & Surgery, University of Georgia, Athens, GA.

The use of human recombinant alpha Interferon (rHuIFN-α) and of recombinant feline omega Interferon (rFeIFN-ω) have been reported for treatment of FHV-1 in cats. Minimal data is available concerning the efficacy of these treatments. Recombinant HuIFN-α and rFeIFN-ω are type I interferons, which act as mediators of the innate (nonspecific) immune response. Anti-proliferative and immune modulating properties, and direct antiviral effect are considered major functions of type I IFN. The purpose of this study was to evaluate the antiviral efficacy and to compare the inhibitory effects of rFeIFN-ω (Virbagen® Omega) and rHuIFN-α2b (Intron®-A) on FHV-1 replication *in vitro*.

Viral titers were assessed by plaque reduction assay. Briefly, confluent monolayers of Crandell feline kidney cells in 24-well culture plates were treated with either rFeIFN-ω or rHuIFN-α2b over concentrations ranging from 100U/ml to 500,000U/ml. A reduction in the number and size of plaques were used as indicators of antiviral activity. Assays were performed in duplicate and repeated. An MTT assay of cellular enzyme function was used to exclude possible cytotoxic effects of IFN that could confound interpretation of the antiviral effects. A one-way ANOVA and Dunnett's test were performed to assess the significance of the data.

A significant reduction in plaque number was observed for rFeIFN-ω at 100,000U/ml (54.7%) and at 500,000U/ml (59.8%) ($p<0.05$). Plaque size was significantly reduced by 47.5% at 100,000U/ml, by 81% at 250,000U/ml and by 70.5% at 500,000U/ml ($p<0.05$) rFeIFN. Recombinant HuIFN-α2b treatments did not produce any significant reduction in the number of plaque. However, significant reduction in plaque sizes was measured 56 % at 100,000U/ml, by 75.7% at 250,000U/ml and by 69% at 500,000U/ml ($p<0.05$). None of the high-dose treatments of either interferon caused significant cellular toxicity in the MTT assay ($p<0.05$).

Both interferons were shown to have anti-FHV-1 effects at high concentrations. Treatment with rFeIFN-ω was more effective than treatment with rHuIFN-α2b. The results showed for the first time that antiviral efficacy for interferon can also be measured in terms of a reduction of plaque sizes indicating the ability to inhibit viral cell-to-cell spread. This ability may be relevant for clinical applications such as the topical treatment of FHV-1.

ABSTRACT #252

MEASUREMENT OF CONJUNCTIVAL CYTOKINE RESPONSES DURING *CHLAMYDOPHILA FELIS* INFECTION IN THE CAT. R. Dean, C. Helps, T. Gruffydd-Jones, R. Harley; School of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, UK.

Chlamydophila felis is a common cause of conjunctivitis in the cat but little is known about the immune response to infection. Cytokines

are known to play an important role in mucosal humoral and cell mediated immunity. A pilot study was performed to assess if it was feasible to detect mRNA encoding feline cytokines on ocular swabs taken from cats infected with *C.felis.* Quantification of cytokines using real-time reverse transcriptase PCR was then performed to detect trends in cytokine levels before and during infection.

Eight specific-pathogen-free cats were bilaterally inoculated with 3 $x10^3$ units of a field isolate of *C.felis* onto the conjunctiva (day 0). Conjunctival swabs were taken at regular intervals prior to infection and following infection. Four cats were followed for 11 days post-infection (dpi), 1 cat for 35 dpi and 3 cats until day 42dpi.

Following extraction of RNA from the conjunctival swabs, reverse transcriptase real-time PCR was performed using gene specific primers and probes for G3PDH and IL-2, -4, -5, -6, -10, -12p35, -12p40 and -18, TNFα, TGFβ and IFNγ mRNA.

All swabs were positive for G3PDH mRNA. The amount of mRNA present for each cytokine was quantified relative to the amount of G3PDH mRNA on the same swab. Each swab was then assigned a relative copy number (RCN) for each cytokine and trends in RCNs observed.

IL-2**,** IL-6, TGFβ and IFNγ were undetectable in most cats prior to infection. RCNs of these cytokines increased and peaked at 4-14 dpi. IL-2, IL-6 and IFNγ levels then decreased by 10-1000 fold, and in some cats IL-2 and IL-6 became undetectable once more. However, TGFβ RCNs tended to remain high, close to peak levels. IL-4 was intermittently detected in three cats prior to infection, and post infection only low RCNs were detected in seven cats. IL-12p40 and TNFα were intermittently detectable in all cats prior to infection, and IL-10 was detectable in four cats prior to infection. Following infection RCNs of these three cytokines increased by 10-10000 fold or more, peaking at 7-11 dpi. Thereafter, the RCNs decreased by 10-1000 fold and remained at approximately 10-100 fold higher than prior to infection. IL-5, IL-12p35 and IL-18 were frequently detected in most cats prior to infection, and rapidly returned to similar RCNs after peaking approximately 10-1000 fold higher a 4-7dpi.

This study demonstrates that it is possible to detect mRNA encoding feline cytokines from ocular swabs from cats using real-time RT-PCR. General trends in cytokine responses following *C.felis* infection were observed.

ABSTRACT #253

PHARMACODYNAMIC DATA OF FIVE FLUORINATED QUINOLONES TOWARD CANINE AND FELINE PATHOGENS FROM JAN 1999 THROUGH JUNE 2001. Boothe DM, Simpson BR. Auburn University, Auburn AL (Boothe) and Texas A&M University, College Station, TX.

This study prospectively compared *in vitro* resistance and potential efficacy of five fluorinated quinolones (FQ) toward aerobic canine or feline isolates (n=246) submitted to diagnostic laboratories. Microtube dilution was implemented at concentrations ranging from 0.03125 to 32 μg/ml. Drugs studied were (dose mg/kg) ciprofloxacin (CIP; 5-20), enrofloxacin (ENR; 5-20), difloxacin (DIF; 5-10), marbofloxacin (MAR; 2.5-5) and orbifloxacin (ORB; 2.5-7.5). Percent resistance of each organism to each FQ and pharmacodynamic indices were determined. Potency was measured as MIC_{50} or MIC_{90} and susceptibility as inhibitory quotient (IQ), defined as peak plasma drug concentration at the low or high dose of each FQ to MIC_{mean} (mean is the geometric mean). The organisms most commonly cultured and % resistant to all FQ were, for Gram negative (GN; n = 180; 20±3%), *Eschericia coli* (n=61; 39±5%; at least 55% from the urine), *Pseudomonas aeruginosa* (n=58; 16±3%), and Gram positive (GP; n=66; 17±3%,), *Staphylococcus sp* (n=17; 2± 1%) including *Staphylococcus intermedius* (n=19; 6± 5%) and *Streptococcocus* (n=20; 17±10%). Percent resistance did not differ among the FQ for any organism. For GN, the MIC_{mean} and $MIC_{50,}$ but not MIC_{90}, were lower than the breakpoint (MIC_{BP}) of each drug for all organisms. In general, potency for GN was ranked as CIP ≥ ENR ≥ MAR ≥ DIF ≥ ORB. However, the susceptibility (magnitude of IQ) was ENR ≥ CIP ≥ MAR ≥ ORB ≥ DIF. An IQ of 10 was achieved at the low dose only for ENR and MAR and only against *Proteus.* At the high dose, an IQ of 10 was achieved by ENR for all drugs and by DIF and ORB for none. For GP, potency was CIP ≥ ENR=MAR ≥ DIF ≥ ORB. However, the magnitude of IQ were ENR ≥ CIP ≥ MAR >ORB> DIF. Only ENR was able to achieve an IQ of 10 for GP organisms although CIP could for *S.intermedius.* This study of a limited number of organisms demonstrates ENR is the FQ most able to achieve targeted IQ for concentration dependent drugs and that the high dose of any FQ may be the more prudent dose for susceptible organisms. The use of generic CIP may not meet judicious antimicrobial use guidelines.

ABSTRACT #254

ATTEMPTED TRANSMISSION OF *CANDIDATUS MYCOPLASMA HAEMOMINUTUM* BY INGESTION OF *CANDIDATUS M. HAEMOMINUTUM*-INFECTED FLEAS. JE Woods, MM Brewer, MR Lappin, N Wisnewski. Department of Clinical Sciences, Colorado State University, Ft Collins, CO (Woods, Brewer, Lappin), Heska Corporation, Ft Collins, CO (Wisnewski).

Feline hemoplasmosis is caused by two Mycoplasma species; *Mycoplasma haemofelis* (Mhf) and *Candidatus M. haemominutum* (Mhm). In previous experiments, we showed that Ctenocephalides felis, can transfer Mhf but not Mhm between cats during hematophagous activity. In those studies, the cats were unable to groom and ingest the fleas or flea by-products. In a separate experiment, we failed to transmit Mhf between cats by feeding infected fleas or flea by-products. The goal of this study was to determine whether Mhm infection could be initiated by feeding Mhm-infected fleas and flea by-products.

Three, young-adult, mixed-sexed cats were used. One cat was a carrier of Mhm. The other two cats were negative for hemoplasmosis by a PCR assay that amplifies the DNA of both Mycoplasma species. One flea chamber containing 200 C. felis of equal male-to-female ratio was attached to the chronic carrier cat for five days during which time the fleas could feed. At chamber removal, a random sample of flea feces and eggs was positive for Mhm DNA by PCR assay. Chamber contents were then fed to each of the two Mhm-naïve cats as follows: one cat was fed 93 viable fleas (43 female, 50 male) and 0.274 gram of flea by-products, including feces, larvae and eggs. The other cat was fed 90 viable fleas (41 female, 49 male) and 0.354 gram of flea by-products. These items were mixed into 71 grams of a commercially available chicken-based human baby food to facilitate feeding.

A CBC and PCR assay performed weekly on both cats failed to document presence of Mhm organisms or DNA during the first eight weeks post-oral exposure. Results of this study suggest that ingestion of Mhm-infected fleas or flea by-products is not 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 #255

DETECTION OF *MYCOPLASMA HAEMOFELIS* AND *CANDIDATUS* MYCOPLASMA HAEMOMINUTUM DNA IN FELINE SERUM SAMPLES. ED Kennedy, J Hawley, M Brewer, MR Lappin. Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Prior to the development of PCR assays for the amplification of DNA of *M. haemofelis* (Mhf) or *Candidatus* Mycoplasma haemominutum (Mhm) from blood, prevalence studies were based on cytology. Since PCR assays are generally more sensitive than

cytology, it cannot be determined whether prevalence rates of Mhf or Mhm have changed over time. While our laboratory has thousands of stored feline serum samples, prior to PCR assay development, blood was generally not saved. Since Mhf and Mhm can disassociate from the surface of red blood cells, we hypothesized in this study that organismal DNA could be amplified from serum of infected cats by use of a currently available PCR assay.

Between 1998 and 2004, our laboratory completed a variety of feline infectious disease studies that required the submission of blood in EDTA and serum samples from the same cat. Cases selected for evaluation in this study included those for which adequate volumes of serum and blood were available for further study (n = 34). The samples had been saved at -20ºC until they were thawed on three separate days and stored at 4ºC until processed for PCR assay. For each of the sample sets, 200 µl of whole blood and 200 µl of serum were pipetted into separate tubes for DNA extraction using the blood and body fluid spin protocol of a commercially available kit (Qiagen QIAamp DNA Blood Mini Kit, Qiagen, Inc., Valencia, CA). An additional 300 µl of serum was pipetted into a separate 1.5 ml centrifuge tube and ultracentrifuged at 10,000 rpm for 5 minutes. The supernatant was removed**,** the pellet was resuspended in 200 µl of 0.01M PBS, and the DNA was extracted as for the blood and whole serum. A multiplex PCR assay for amplification of Mhf, Mhm, *Ehrlichia* spp., and *Anaplasma phagocytophilum* DNA was performed and interpreted as previously described.

DNA of Mhf or Mhm was detected in blood of six of 34 samples (17.6%) and 18 of 34 samples (52.9%), respectively. When serum results were compared to blood results, the sensitivities for Mhf and Mhm were 16.7% and 5.6% respectively. When serum pellet results were compared to blood results, the sensitivities for Mhf and Mhm,were 0% and 16.7% respectively. When serum results were compared to blood results, the specificities for Mhf and Mhm were both 100%. When serum pellet results were compared to blood results, the specificities for Mhf and Mhm were both 100%.

These results suggest that neither serum nor pellets derived from serum should be used for detection of haemotropic *Mycoplasma* spp when blood samples are available and that performance of PCR assays on serum is an inadequate technique for determination of the prevalence of haemoplasmosis.

ABSTRACT #256

CANDIDATUS MYCOPLASMA HAEMOMINUTUM INFECTIONS IN CLIENT-OWNED CATS. CA Reynolds, MR Lappin. Colorado State University, Fort Collins, CO.

Mycoplasma haemofelis and *Candidatus* Mycoplasma haemominutum are parasites of feline erythrocytes. In experimental inoculation studies performed to date, most cats inoculated IV with *M. haemofelis* isolates developed transient fever and severe hemolytic anemia, whereas cats inoculated IV with *Candidatus* M. haemominutum isolates remained clinically normal or developed mild anemia. Because only small number of isolates have been studied to date and naturally-infected cats are not likely to be inoculated IV, the pathogenic potential of different *Candidatus* M. haemominutum isolates is largely unknown. The purpose of this study is to report the clinical findings of a group of cats naturally infected with *Candidatus* M. haemominutum.

The laboratory records section of the Infectious Diseases Diagnostic Laboratory was searched for all feline cases from which samples had been submitted for *Mycoplasma* spp. PCR between January 2001 and June 2004. The cats were stratified into the following groups based on the results of a PCR assay capable of amplifying and differentiating DNA of the two *Mycoplasma* spp.: *M. haemofelis* alone; *Candidatus* M. haemominutum alone; both *Mycoplasma* spp.; and neither *Mycoplasma* spp.. The referring veterinarian of all cats that were PCR assay positive for DNA of *Candidatus* M. haemominutum was contacted and the complete medical record requested. The records were reviewed to determine clinical abnormalities, laboratory abnormalities, evidence of concurrent disease, treatment, and response to treatment.

Of 332 cats tested, 42 cats (12.7%) were PCR positive for DNA of *Candidatus* M. haemominutum alone. Complete medical records were available for 21 of the clinically ill cats. Fever, anorexia, lethargy, weight loss, and anemia were among the most common presenting abnormalities recorded by the referring veterinarians. Housing history was known for 17 cats; 16 were allowed outdoors. Anemia was a laboratory abnormality in 12 cats. Of these cats, six had evidence of other diseases that could have been the primary cause of anemia or could have activated haemoplasmosis. Those diseases included feline leukemia virus (two cats), immune-mediated myeloid and erythroid maturation arrest, lymphosarcoma, erythroid myelosis, and an unknown cause of polyarthritis. For six cats, '*Candidatus* M. haemominutum' was the only recognizable cause for the anemia. Of these cats, five were treated with doxycycline or enrofloxacin with or without a glucocorticoid and the anemia and other abnormalities resolved.

The combination of anemia, detection of '*Candidatus* Mycoplasma haemominutum' DNA in blood, exclusion of other causes of anemia or immune deficiency, and apparent response to therapeutic protocols that included drugs with known anti-hemoplasma activity suggests that some '*Candidatus* M. haemominutum' isolates cause anemia in naturally-infected cats.

ABSTRACT #257

INFECTIOUS DISEASES OF DOGS AND CATS ON ISLA ISABELA, GALAPAGOS. Julie Levy, Cynda Crawford, Sylvia Tucker, Rick Alleman, University of Florida, Gainesville, FL; Michael Lappin, Colorado State University, Fort Collins, CO; Edward Dubovi, Cornell University, Ithaca, NY; Michael Levy, North Carolina State University, Raleigh, NC.

Isabela is the largest island in the Galapagos archipelago and has the greatest diversity of endemic animal species. In addition to a human population of 1,500, the island is colonized by invasive domestic species, including cats and dogs, which are believed to threaten the native wildlife via predation, competition, and infectious diseases. Dogs and cats may not be transported between the islands, and vaccines are prohibited. Thus, Isabela represents a uniquely isolated population of dogs and cats. In May 2004, a project was undertaken to control the dog and cat population via sterilization. Blood samples were collected to determine the presence of infectious diseases that might threaten the domestic species, the wildlife, and the human residents of Isabela.

Blood samples were collected from 95 dogs and 52 cats, representing 30% of the dog population and 35% of the cat population. The ages ranged from approximately six weeks to adult for cats, and from six months to adult for dogs. Both sexes were included in the sample population. Samples were tested by serology, PCR assays, and light microscopy to assess for infection by selected viral, bacterial, rickettsial, protozoal, and parasitic infectious agents.

Dogs were commonly seropositive for antibodies against distemper virus (23%), parvovirus (100%), adenovirus types 1 and 2 (66%), parainfluenza virus (100%) but not canine coronavirus (0%). All dogs were negative for antibodies against *Ehrlichia canis, Borrelia burgdorferi*, *Trypanosoma cruzi* and 6 serovars of *Leptospira interrogans*. Antibodies to *Leishmania infantum* were detected in 4% of dogs and *Dirofilaria immitis* antigen was detected in 33% of the dogs.

Of the cats tested, none were infected with FeLV, FIV, or coronavirus. Antibodies against calicivirus (44%), feline herpesvirus 1 (10%), and panleukopenia virus (67%), were common. *Bartonella henselae* or *B. clarridgeaie* DNA was amplified from 44% of cats and 63% of the cats had *Toxoplasma gondii* IgM or IgG in serum. *Mycoplasma haemofelis* DNA was amplified from blood of one cat,

but all cats were negative for *Ehrilichia sp*. DNA in blood. One cat had *D. immitus* antibodies, but all cats were negative for heartworm antigen. No blood parasites were evident on stained blood films.

Distemper virus, parvovirus, adenovirus, parainfluenza virus, panleukopenia virus, herpesvirus, calicivirus, and *D. immitis* are likely endemic in the local dog and cat population. None of the viruses infect humans, but distemper virus and dirofilariasis are transmissible from infected dogs to sea lions. Evidence of *Leishmania* exposure in the dogs suggests that the human population is also at risk for infection with this organism. Cats carried two zoonoses, *B. henselae* and *T. gondii* that are found worldwide. The cats and dogs of Isabela had many of the infections found on the mainland of Ecuador. The use of routine parasite control and sterilization to prevent the birth of susceptible offspring would be expected to control a majority of the infections.

ABSTRACT #258

THE SEARCH FOR INTACT *BORRELIA BURGDOFERI* BACTERIA IN KIDNEYS FROM DOGS SUSPECTED OF SUFFERING FROM "LYME NEPHRITIS". TA Shanies, RE Goldstein, BL Njaa, DZ Atwater, YF Chang and KW Simpson. College of Veterinary Medicine, Cornell University, Ithaca, NY.

A unique renal syndrome commonly known as "Lyme nephritis" is a poorly characterized condition associated with proteinuria and often fatal renal failure, in dogs in Lyme endemic areas. The renal pathology characteristic of this syndrome is thought to include membranoproliferative glomerulonephritis as well as tubular necrosis and interstitial inflammation. An association with *Borrelia burgdorferi*, the causative agent of Lyme disease, has been suggested in a small number of studies. The aim of this study was to employ advanced molecular techniques in the search for intact *B. burgdorferi* in the kidneys of serologically Lyme positive dogs that exhibited appropriate clinical and histopathological features of Lyme nephritis.

Cases were obtained from the pathology tissue bank at Cornell University or sent in by veterinarians. Inclusion in the study group required: serologic evidence of natural exposure to *B. burgdorferi* (Western blot and/or C6 antibody), availability of renal tissue (frozen or paraffin embedded) exhibiting pathology consistent with Lyme nephritis. For polymerase chain reaction (PCR), DNA was purified from the renal tissue. PCR amplifications were performed on all samples using: a eubacterial primer set designed to amplify 16s rRNA, a *B. burgdorferi* primer set designed to amplify a segment of the OspA gene, and a primer set designed to amplify a canine genomic DNA housekeeper gene. DNA purified from *B. burgdorferi* bacteria grown in culture served as a positive control, and various blank solutions served as negative controls. Fluorescent in-situ hybridization (FISH) was performed on tissue sections by hybridizing a 5'-cy3-eubacterial probe for 16s rRNA. Immunohistochemistry (IHC) using a polyclonal antibody against *B. burgdorferi* and Modified Steiner silver staining (MS) were performed in some cases.

Ten frozen and seven paraffin-embedded renal tissue samples underwent PCR evaluation. Ten of those samples were included in preliminary FISH analysis. Of the 17 samples, seven had been evaluated with MS stain, and 10 with IHC. Two *B. burgdorferi* PCR reactions were positive, and five eubacterial PCR reactions were positive (including the two positive for *B. burgdorferi*). None of the samples evaluated by FISH showed conclusive evidence of intact bacteria (*B. burgdorferi* or other) within the renal tissue. MS stain evaluation was negative in all cases assessed. Two cases evaluated with IHC were judged as likely positive for the presence of small numbers of *B. burgdorferi*; 1 of these cases was also PCR positive, neither were FISH positive.

Using sensitive molecular techniques (PCR and FISH), we found limited evidence of the presence of intact *B. burgdorferi* or any other bacteria in the renal tissue of dogs with suspected Lyme nephritis. The identification of the organism in isolated samples of renal tissue may suggest a causal relationship. However, the inconsistent presence of the organism (in this preliminary study) may make immune complex disease a more likely pathogenic mechanism.

ABSTRACT #259

PREVALENCE OF WEST NILE VIRUS IN FERAL CATS. Cynda Crawford, David Hoch, Maureen Long, Julie Levy, University of Florida, Gainesville, FL; Amy Glaser, Cornell University, Ithaca, NY.

Since its introduction in 1999, West Nile virus (WNV) has become endemic in the U.S. This mosquito-borne virus causes serious, and often fatal, disease in several animal species, including humans. The prevalence of WNV and risk for exposure is monitored by detection of infection in sentinel animals. Prior to availability of a vaccine, horses served as sensitive sentinels for WNV prevalence because of high risk of mosquito exposure, susceptibility to virus infection, easy serological detection of infection, and close proximity to humans. Widespread vaccination has confounded serological identification of infected horses, so other species that live in close proximity to humans and other at-risk populations should be evaluated as sentinels. Since unowned free-roaming (feral) cats have a high risk of vector exposure and live in close proximity to humans and their companion animals, they may be a suitable sentinel species for WNV surveillance and prediction of exposure risks. The purpose of this study was to determine the prevalence of WNV in feral cats in Alachua County in north central Florida, and to identify risk factors that predispose feral cats to WNV infection.

Blood samples were collected from adult feral cats in Alachua County presented for sterilization to a monthly trap-neuter-return clinic. The samples were collected from approximately 50 cats per month from June 2003 to May 2004 to encompass seasons of high and low mosquito activity. Serum was screened for WNV antibodies using a virus neutralization assay, and for FeLV antigen and FIV antibodies using ELISAs. The Chi-square test was used to compare seroprevalence with gender, location, and FeLV or FIV coinfection

A total of 605 cats were tested, including 295 males and 310 females. In this population, 31% (187 cats) had antibodies to WNV. Only 3% of the cats were positive for either FeLV (20 cats) or FIV (21 cats) infection. There was no association of WNV antibody status with gender or FeLV and FIV coinfection. Seropositive cats lived in colonies in rural areas as well as suburban areas. The lowest prevalence (<20%) occurred during the spring months of April to May. The prevalence increased to approximately 50% during the fall/winter months of October to February. The peak month was November, when 65% of the tested cats were seropositive. The higher seroprevalence in the late fall and winter likely reflects greater exposure to infected mosquitoes during the summer months when mosquitoes are most active in north central Florida.

The prevalence of WNV infection in feral cats was similar to that reported for horses prior to vaccination, suggesting that feral cats are a suitable population for surveillance of WNV activity and prediction of exposure risks to humans in the same locale. The high prevalence of WNV infection in feral cats also suggests that pet cats exposed to mosquitoes in the same locale may be at significant risk for WNV infection.

ABSTRACT #260

THE ASSOCIATION OF *BARTONELLA* SPP. INFECTION WITH CHRONIC STOMATITIS IN CATS. KL Dowers, MR Lappin. Colorado State University, Fort Collins, CO.

Stomatitis is a debilitating disease in cats that leads to oral pain, anorexia, weight loss, and occasionally euthanasia in intractable cases. The syndrome is thought to have multiple causes; recent work suggests that *Bartonella* spp. may play a role in some cases. Establishing a causative link between bartonellosis and stomatitis

would justify routine testing of stomatitis cases and might suggest use of alternative antibiotic therapies, such as azithromycin. The objective of this clinical study was to determine the prevalence of *Bartonella* spp. DNA in blood and *B. henselae* serum antibodies in client-owned cats with histopathologically documented stomatitis as well as age- and geographically-matched healthy control cats.

Blood and serum samples from 34 affected cats and 34 age-matched healthy control cats were submitted by veterinarians from around the United States. DNA of *Bartonella* spp. was amplified from blood via a previously validated polymerase chain reaction (PCR) assay and serum antibody titers against *B. henselae* were determined by ELISA. All cats were tested for FeLV antigen and FIV antibodies. For cases where oral biopsy samples were obtained at the time of blood sampling, the PCR assay was also performed on tissue samples. Survey information regarding housing status (multiple or single cat households), previous FeLV and FIV testing, flea exposure, vaccination history and history of upper respiratory infections (URI) were collected for both affected and control cats, and lesion details and treatment trials were collected for affected cats.

No significant differences in the prevalence rates for PCR-positive cats between affected (8.89%) and control cats (8.89%) or for antibody-positive cats between the affected group (67.6%) and the control group (58.8%) were found. The only survey factor with significant correlation with stomatitis was history of URI [$p<0.05$]. Of the 18 oral tissue samples submitted, only 1 was PCR-positive.

This study underscores the difficulty of correlating *Bartonella* spp. test results with clinical disease in individual cats because of the high prevalence rates of antibody-positive animals within the healthy population, as reported in this and other studies. Treatment with anti-*Bartonella* spp. antibiotics may still be appropriate in refractory stomatitis cases, but a larger scale epidemiologic study should be conducted to further assess the usefulness of *Bartonella* spp. antibody and PCR testing of cats with chronic stomatitis.

ABSTRACT #261

DETECTION OF SELECT INFECTIOUS AGENTS IN THE BLOOD AND AQUEOUS HUMOR OF CATS WITH NATURALLY OCCURRING ENDOGENOUS UVEITIS. C.C. Powell and M.R. Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Chronic uveitis is a common cause of blindness in cats. Previous studies have used PCR assays to amplify DNA of *Toxoplasma gondii*, feline herpesvirus-1 (FHV-1), and *Bartonella* spp. from the aqueous humor of cats with active uveitis. Identification of these agents suggested a causal relationship with development of uveitis, however sample sizes were small and positive test results also occasionally occur in normal cats. The purpose of this study was perform PCR assays that amplify the DNA of *T. gondii*, FHV-1, and *Bartonella* spp. on aqueous humor from a larger group of cats identified by veterinary ophthalmologists as having endogenous uveitis.

Previously validated PCR assays that amplify the DNA of *T. gondii*, *Ehrlichia* spp., *A. phagocytophilum*, FHV-1, and *Bartonella* spp. were performed on aqueous humor (AH) from 116 cats with naturally occurring endogenous uveitis. All assays other than FHV-1 were also performed on blood. Serum antibodies titers against *T. gondii* and *B. henselae* were also determined. The duration of ocular disease ranged from one day to several years. Overall, DNA of an infectious agent was amplified from the blood or AH of 19 cats (16.4%). DNA of *T. gondii* (four cases), and FHV-1 (one case) were amplified from the AH of some cats. DNA of *T. gondii* (four cases), *Ehrlichia* spp. (one case), *B. henselae* (seven cases), and *B. clarridgeaie* (2 cases) were amplified from the blood of some cats. One cat was positive in both blood and AH for DNA of *T. gondii*. For 27 cats, antibiotics had been administered prior to collection of blood and aqueous humor which may have lowered the numbers of positive *T. gondii*, *Ehrlichia* spp., *A. phagocytophilum*, and *Bartonella* spp. test results. For six of the seven *T. gondii* DNA positive cases and one of the nine *Bartonella* spp. DNA positive cases, serum antibody tests were concurrently negative for the respective organism. In these cases, the PCR result was the only evidence of exposure to the infectious agent.

PCR assay results documented current infection with an infectious agent in over 16% of the cats with endogenous uveitis in this study and several PCR positive cases were seronegative at the time of testing. These results suggest that in some cases, performance of PCR assays on blood or AH may add in ranking differentials for cats with endogenous uveitis. However, positive PCR assay results do not necessarily document clinical illness induced by the infectious agent.

ABSTRACT #262

INVESTIGATION OF THE ASSOCIATION OF VACCINATION WITH ENDOGENOUS UVEITIS IN CATS. AB Clode, CC Powell, SV Radecki, MR Lappin. Colorado State University, Fort Collins, CO (Clode, Powell, Lappin).

Uveitis, defined as inflammation of the iris, ciliary body, and choroid, is a clinical ophthalmologic condition noted commonly in the cat. Feline uveitis has many causes; a clinical diagnosis of feline idiopathic uveitis is generally made if there is no systemic or ophthalmic explanation for the ocular inflammation. In these cases, lymphocytic-plasmacytic inflammatory cell infiltrates are usually detected histopathologically, suggesting an immune-mediated process. In humans, vaccinations have been linked to several suspected immune-mediated conditions including thrombocytopenia, hemolytic anemia, and uveitis. In dogs, vaccinations have been associated with immune-mediated hemolytic anemia, thrombocytopenia, anti-thyroglobulin antibodies, and corneal edema. We hypothesize that vaccinations may be associated with the development of uveitis in some cats. The objective of this study was to compare the temporal associations of vaccinations between groups of cats presenting with and without uveitis.

Vaccination records were available for 52 cats with endogenous uveitis as determined by veterinary ophthalmologists (Group 1); these were divided into those with any positive infectious disease test (35 cats; Group 2) and idiopathic (17 cats; Group 3). The medical record database at the Veterinary Teaching Hospital was then searched for all new clinically ill cat accessions during a similar time period. A case was included if there was no evidence of ophthalmic disease, no evidence of diseases with a potential immune-mediated mechanism, and vaccination records were available (103 cats; Group 4). The age, breed, sex, and time between the last vaccination and onset of current illness for each cat with uveitis and clinically ill control cat without uveitis were recorded. To determine whether there was a temporal association of vaccination with development of uveitis, Wilcoxon's rank sum test was used to evaluate the difference in age between the cat groups, as well as to evaluate the difference in time interval between vaccination and presentation for uveitis or other clinical disease. The time intervals selected as indicating an association between vaccination and clinical presentation were four and eight weeks, based on previous criteria for vaccinations associated with immune-mediated disease in dogs and humans. The difference in the proportions of males and females in the four groups was evaluated using Fisher's exact test.

The numbers of cats vaccinated within four weeks were seven of 52 (13.5%), 6 of 35 (17.1%), one of 17 (5.9%), and 15 of 103 (14.6%) for groups 1-4, respectively. The numbers of cats vaccinated within 8 weeks were 14 of 52 (26.9%), 9 of 35 (25.7%), 5 of 17 (29.4%) and 30 of 103 (29.1%) for groups 1-4, respectively. There were no significant differences detected between groups for any parameter. Results of this study fail to link recent vaccination to development of uveitis in cats.

ABSTRACT #263

INFLUENZA VIRUS INFECTION IN RACING GREYHOUNDS. Cynda Crawford, Paul Gibbs, William Castleman, Richard Hill, University of Florida, Gainesville, FL; Ed Dubovi, Cornell University, Ithaca, NY; Ruben Donis, Iain Stephenson, Cathy Smith, Jackie Katz, Centers for Disease Control and Prevention, Atlanta, GA.

Recurrent outbreaks of severe respiratory disease characterized by coughing and fever have occurred in greyhounds at racing kennels in the U.S. in recent years. In January 2004, a typical outbreak occurred in 22 racing greyhounds in Jacksonville, Florida. Most of these dogs had fevers and cough, but eight died from hemorrhagic pneumonia. In June 2004, a similar outbreak occurred in thousands of greyhounds at race tracks in 6 states. There are no reports documenting the cause of these acute respiratory disease outbreaks in racing greyhounds. The objective of this work was to identify the etiological agent(s) involved in the January and June 2004 outbreaks.

Paired acute and convalescent nasal swabs and serum samples were collected from dogs with clinical signs and from asymptomatic dogs housed in contact with sick dogs. Bacterial cultures were performed with the nasal swabs. Virus neutralization and hemagglutination assays were performed on serum. Postmortem examinations were conducted on dogs that died during the outbreaks. Bacterial cultures, virus isolation studies, and immunohistochemistry assays for viral antigens were performed with respiratory tract tissues.

None of the common canine respiratory pathogens, including *Bordetella bronchiseptica*, distemper virus, adenovirus type 2, and parainfluenza virus, were identified as the etiological agent(s). Pathologic findings included: 1) severe pulmonary and pleural hemorrhage; 2) acute to subacute erosive to hyperplastic tracheitis, bronchitis, and bronchiolitis; and 3) bronchopneumonia. The epithelial lining and airway lumens in these tissues were infiltrated by neutrophils and macrophages. Influenza A subtype H3N8 virus was isolated from the lungs of a Florida dog that died in January and a Texas dog that died in June. Influenza H3N8 virus was recovered from archived lung tissue from a Florida greyhound that died during a respiratory disease outbreak in 2003. Genetic sequence analyses and phylogenetic comparisons determined that all three canine isolates were closely related and have evolved from contemporary strains of equine influenza H3N8. Immunohistochemistry demonstrated influenza antigen in bronchial gland epithelial cells, bronchial and bronchiolar epithelial cells, and in alveolar macrophages. Seroconversion to the canine influenza virus was demonstrated by hemagglutination inhibition and microneutralization assays.

Based on virus isolation from lungs, viral antigens in lung tissues, and seroconversion data, we conclude that the novel canine influenza virus was the etiological agent responsible for the January and June 2004 respiratory disease outbreaks in racing greyhounds. This represents the first report of influenza virus associated with respiratory disease in dogs.

ABSTRACT #264

INFLUENCE OF BODY SIZE ON COLONIC PERMEABILITY IN HEALTHY DOGS. D. Hernot[1], H. Dumon[1], V. Biourge[2], L. Martin[1] and P. Nguyen[1]. [1]Nantes National Veterinary School, Nantes, France; [2]Royal Canin Research Center, Aimargues, France.

Fed the same dry diet, large dogs show poorer fecal quality than small ones. A high colonic permeability could explain a low water and electrolyte net balance leading to a high fecal water content. The objective of the study was to evaluate the effect of body size on colonic permeability using the ratio of urinary lactulose to sucralose (L:S) and to determine whether colonic permeability is related to fecal sodium concentration and fecal quality.

Adult dogs of four breeds, varying from four to 55 kg body weight, were studied including six Miniature Poodles (MP), six Standard Schnauzers (SS), six Giant Schnauzers (GS) and six Great Danes (GD). A solution containing 150 mg/ml lactulose and 100 mg/ml sucralose was administered orally to each dog according to their body weight. All urine was collected 24 hours later and urinary L:S was calculated after separation of sugars by gas chromatography. Fecal sodium concentration was measured by flame photometry. Fecal moisture and scoring were recorded during the same period.

Fecal quality was significantly lower in large breeds (lower consistency and higher water content) and there were strong correlations between body size and fecal variables ($r=0.79$; $p<0.0001$ and $r=0.63$; $p=0.0025$, respectively for fecal scores and moisture). The urinary L:S ratio was significantly lower in GD, indicating a higher colonic permeability, than in MP, SS or GS (0.35 ± 0.12 for GD and 0.51 ± 0.05 for MP). Higher fecal sodium concentrations were found in GD compared to the three other breeds and we observed a good correlation between body size and fecal Na concentration ($r=0.63$; $p=0.0012$). Finally, we observed good relationships between urinary L:S ratio and fecal humidity or sodium content ($r=-0.60$; $p=0.0026$ and $r=-0.67$; $p=0.0005$, respectively).

Our results suggest that 1) body size would affect colonic permeability, like small intestinal permeability as it was reported in a previous study, 2) the higher fecal sodium concentration observed in large dogs could be explained by the higher colonic permeability and 3) both these variables could be important explanations for higher fecal moisture in large dogs.

ABSTRACT #265

EVALUATION OF SEVEN EXONS OF THE LIPOPROTEIN LIPASE GENE IN MINIATURE SCHNAUZERS WITH HYPERTRIGLYCERIDEMIA AND PANCREATITIS. R Schickel[1], JM Steiner[1], ML Cox[2], and DA Williams[1]. [1]Gastrointestinal Laboratory and [2]Canine Genetics Laboratory, Texas A&M University, College Station, TX.

A high prevalence of hypertriglyceridemia 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 105 mutations of the LPL gene have been described. Some of these mutations result in hyperlipidemia and in some cases pancreatitis. This goal of this study is to evaluate exons 2, 3, 4, 6, 7, 8, and 9 of the LPL gene in the Miniature Schnauzer for possible mutations.

Six Miniature Schnauzers were selected on the basis of a clinical history of pancreatitis, a serum triglyceride concentration above the upper limit of the reference range, and a serum cPLI concentration >200 µg/L. In addition, six healthy Miniature Schnauzers with serum triglyceride and cPLI concentrations within the reference range were selected for comparison. DNA was extracted from white blood cells or mucosal cells using a commercially available kit (Gentra Systems, PUREGENE). Primers were designed based on published sequences (Canine Genome Project (NCBI)) using Netprimer® software (Premiere Biosoft). All primers were designed to be situated at least 20 bp within the intronic regions, allowing for evaluation of exon-intron boundaries. Amplification conditions were optimized for all seven exons of the canine LPL gene investigated. PCR was performed and amplicons were resolved by electrophoresis through 2% agarose and visualized after staining with ethidium bromide. Sequencing reactions were carried out with the dye termination method and resolved on automated sequencing machines.

All six Miniature Schnauzers with a clinical history of pancreatitis, hyperlipidemia, and a serum cPLI concentration > 200 µg/L showed the same nucleic acid sequence in the evaluated regions of the LPL gene as the six healthy control Miniature Schnauzers.

We conclude that neither hypertriglyceridemia nor pancreatitis in the Miniature Schnauzer is associated with mutations of exons 2, 3, 4, 6, 7, 8, and 9 of the canine lipoprotein lipase gene. Whether any

potential mutation of the remaining expressed exons of the canine LPL gene, exons 1 and 5, could be the cause of hypertriglyceridemia and/or pancreatitis in the Miniature Schnauzer remains to be determined.

ABSTRACT #266

MOLECULAR IDENTIFICATION OF INTESTINAL BACTERIA IN HEALTHY DOGS. JS Suchodolski, CG Ruaux, JM Steiner, and DA Williams. Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX.

The normal 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 intestinal content. Recently, it has been recognized that the majority of microbial species cannot be identified using standard culture techniques. Reasons for this inability to culture many bacterial species include non-viable or stressed microorganisms, obligate requirements for coexisting flora or host-derived products, bias due to selectivity of culture media, and a lack of knowledge regarding essential nutrients for some bacterial species. Thus, a culture-dependent approach may underestimate the bacterial diversity of complex microbial communities such as those found in the intestinal tract. The aim of this study was to describe the intestinal microflora in healthy dogs by direct sequence analysis of the 16S ribosomal DNA (16S rDNA; gene encoding 16S ribosomal RNA).

Six healthy dogs, euthanatized for an unrelated project, were used for this study. Immediately after euthanasia intestinal content was collected from the duodenum, jejunum, ileum, and colon. Bacterial DNA was purified by phenol:chloroform:iso-amylalcohol extraction, and the 16S rDNA was amplified with universal bacterial primers at low PCR cycle numbers. Amplicons were ligated into linearized cloning vectors and chemically competent *Escherichia coli* were organisms transformed. Colonies were randomly selected, the plasmid DNA purified, and the 16S rDNA insert identified by bidirectional automated cycle sequencing. All non-redundant sequences were tested for possible chimeric structures, putative chimeras were excluded from further analysis. The cloned sequences were compared to existing 16S rDNA sequences in GenBank and in the Ribosomal Database Project (RDP).

From a total of 688 clones analyzed, 102 non-redundant bacterial 16S rDNA sequences were identified, with coverage of 85.2%. Forty (39.2%) of these sequences showed less than 97% sequence similarity to existing 16S rDNA sequences in the GenBank and RDP databases, and may represent as yet uncharacterized bacterial species. Seven major phylogenetic lineages were identified, with the majority (58.9%) of 16S rDNA sequences belonging to the *Clostridium*, *Bacteroides, Lactobacillus,* and *Fusobacterium* groups.

These data indicate that the canine intestinal microflora is very complex, and that the molecular approach described in this study can facilitate identification from the canine intestine of bacterial species that have not previously been characterized. The clinical significance of the diverse intestinal microflora in dogs, and alterations in bacterial diversity that may be present with gastrointestinal disease, need to be further investigated.

ABSTRACT #267

DYNAMICS OF THE JEJUNAL MICROFLORA IN RESPONSE TO FEEDING AND OVER TIME. JS Suchodolski[1], JA Harmoinen[2], CG Ruaux[1], JM Steiner[1], E Westermarck[2], and DA Williams[1]. [1]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, Texas A&M University, College Station, TX; [2]Department of Clinical Veterinary Sciences, Helsinki University, Helsinki, Finland.

The composition of the small intestinal microflora has a significant impact on the health status of an animal. While the canine fecal flora has been reported to be stable over time, limited data are available about the dynamics of the small intestinal microflora. Traditional bacterial culture techniques are laborious and not well suited to studying microbial dynamics. Amplification of bacterial 16S rDNA with subsequent separation by denaturing gradient gel electrophoresis (DGGE), allows rapid assessment of bacterial diversity in biological samples. The aim of this study was to evaluate the dynamics of the jejunal microflora after feeding and over time using these techniques.

Nine healthy dogs, each with a pre-existing chronically placed jejunal fistula inserted at one of two different locations, were used in this study. The location of the fistula was approx. 60 cm distal to the pylorus in 5 dogs and approx. 170 cm distal to the pylorus in 4 dogs. For evaluation of postprandial changes, jejunal juice samples were collected from all dogs before and 1, 2, 3, 4, 5, 6, and 8 hours after feeding a standard canine maintenance diet. Fasting jejunal juice samples were also collected by repeated sampling at eight different time points over a period of 15 days. Bacterial DNA was purified, the variable V6-V8 region of 16S rDNA amplified using universal bacterial primers, and PCR amplicons were subsequently separated by DGGE. Variation in the jejunal microflora over time or after feeding was evaluated by comparing similarity indices (Dice coefficient; 100% represents complete identity) of DGGE profiles using gel analysis software. Effect of time and fistula location on microflora was determined by repeated measures 2-way ANOVA. Dendrograms, showing clustering according to the similarity of banding patterns of individual samples, were constructed by the unweighted pair group method using arithmetic averages.

Feeding led to slight and transient changes in DGGE profiles with consecutive appearance or disappearance of unique bands. Mean ± SD similarity index of DGGE profiles was 74.4 ± 4.9% after feeding and 75.8 ± 5.4 over time. There was no significant change in band numbers or similarity indices either postprandially or over time ($p=0.49$). Location of the fistula had no significant effect on DGGE profiles. Constructed dendrograms revealed that DGGE profiles from individual dogs tended to cluster together regardless of feeding or changes over time.

These DGGE profiles indicate that dogs have a diverse and relatively stable jejunal microflora with marked differences between individual dogs. Feeding leads to slight and transient changes in bacterial diversity in some dogs, these changes do not appear to have a significant impact on the longer term diversity of the jejunal microflora.

ABSTRACT #268

PREVALENCE OF IgA DEFICIENCY IN GERMAN SHEPHERD DOGS BASED ON MEASUREMENT OF FECAL IgA CONCENTRATIONS. U Tress, JM Steiner, CG Ruaux, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

German Shepherd dogs (GSD) commonly develop gastrointestinal disorders, showing clinical signs such as diarrhea, vomiting, weight loss, and poor body condition. In humans, IgA deficiency is the most common primary immunodeficiency and is frequently associated with chronic gastrointestinal disease. Human IgA deficiency has been shown to be inherited. In dogs a condition comparable to human IgA deficiency has been described and breed predispositions have been reported, including the GSD. The goal of this study was to investigate the prevalence of IgA deficiency in GSD, with or without clinical signs of gastrointestinal disease, on the basis of measurement of fecal IgA concentrations using an in-house ELISA.

A total of 56 pure-bred GSD were investigated. Four one gram fecal samples (one sample taken on each of two consecutive days at an interval of four weeks) were collected from each dog. IgA was extracted and assayed using an in-house ELISA (JVIM 2004 18; 427). The reference range for the mean of four fecal IgA samples as determined by this ELISA has been reported as 0.22-3.24 mg/g feces.

The mean IgA concentration of all four fecal samples was calculated and IgA deficiency was defined as a mean value less than 0.22 mg/g feces. The owners of the GSD were asked about the current health status of their dogs placing emphasis on questions regarding gastrointestinal health.

Eight of 56 (14.2%) GSD were IgA deficient. Four IgA deficient GSD showed signs of gastrointestinal disease, such as vomiting and diarrhea, at the time of sample collection. Four IgA deficient GSD showed no signs of gastrointestinal disease. Two other GSD were not IgA deficient but showed signs of gastrointestinal disease (diarrhea and vomiting). The remaining 46 GSD were not IgA deficient, and no gastrointestinal signs were recorded during sample collection. IgA deficient GSD were significantly more likely to show gastrointestinal signs during the four weeks of sample collection (Fischer's exact test, p=0.0025).

The results of this study demonstrate that IgA deficiency, diagnosed on the basis of fecal IgA concentrations, occurs in 14.2% of our sample population. Occurrence of gastrointestinal signs is significantly more likely in GSD with IgA deficiency than in non-deficient GSD. Whether IgA deficiency may contribute to, or be a consequence of gastrointestinal disease remains to be determined.

ABSTRACT #269

CHARACTERIZATION OF SERUM BILE ACIDS IN DOGS WITH EXOCRINE PANCREATIC INSUFFICIENCY. DA Williams, CG Ruaux, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX

Increased fasting bile acid concentrations have previously been documented in dogs with exocrine pancreatic insufficiency (EPI) using colorimetric methods for determination of total bile acid concentrations. The objective of this study was to characterize the circulating bile acid pool in dogs with spontaneous EPI by specific identification of bile acid species using gas chromatography/mass spectrometry (GC/MS).

Serum samples (n=6), were randomly selected from dogs with spontaneous EPI diagnosed by the Gastrointestinal Laboratory at Texas A&M University. All EPI sera had concentrations of canine tryspin-like immunoreactivity (TLI) $\leq$ 2.0 µg/L as measured with a radioimmunoassay (Diagnostic Products Corporation). Control sera (n=6) were randomly selected from a set of banked serum samples from healthy dogs. Serum bile acids were characterized by GC/MS following specific separation of amino acid-conjugated, sulfated, and unconjugated bile acids via lipophilic anion exchange chromatography. The amino acid-conjugated and sulfated bile acids were deconjugated by overnight reaction with cholyl-glycine hydrolase (amino acid-conjugated) or cleaved with trifluoroacetic acid in dioxane and tetrahydrofuran (sulfated). Bile acids were then re-extracted and derivatized before GC/MS analysis. The total serum bile acid concentration (TBA) was calculated by addition of the conjugated, sulfated, and unconjugated bile acid concentrations. TBA, concentrations of unconjugated and sulfated bile acids, and proportion of TBA present as unconjugated and sulfated bile acids were compared between control dog sera and EPI dog sera using a statistical software package (GraphPad Prism 4.0). As TBA concentrations from the EPI dogs were not normally distributed, non-parametric tests were used. Statistical significance was accepted for values of $p<0.05$.

TBA concentrations in the sera of the dogs with EPI (7.25 to 90.84, median 14.43 µmol/L) were significantly higher than in the control dogs (0.34 to 3.07, median 0.95 µmol/L, p=0.002). Amino acid-conjugated bile acids predominated in all control dog sera, while in three EPI dogs unconjugated bile acids predominated. There was no significant difference in the percentage ofTBA present as sulfate-conjugates between EPI dogs and the control dogs (medians 6.26% vs. 8.12% respectively, p=0.937). Sulfated bile acid concentrations were significantly higher in EPI dog sera than in the control sera (medians 84.1 vs. 2299.0 nmol/L, p=0.002), the concentrations of sulfated bile acids and TBA showed a strong positive correlation (Spearman r=0.895, p=0.0002).

These data suggest that increased TBA concentrations may be common in dogs with spontaneous exocrine pancreatic insufficiency. These increased TBA concentrations are associated with increased serum concentrations of both unconjugated and sulfated bile acid species, possibly resulting from altered bacterial metabolism of bile acids during enterohepatic circulation.

ABSTRACT #270

BACTERIAL ISOLATION FROM HEPATIC TISSUE IN DOGS WITH LIVER DISEASE. D Bianco[1], ME Kerl[1], LA Cohn[1], WH Fales[2], SE Turnquist. [1]Department of Veterinary Medicine and Surgery, [2]Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, MO.

Along with food antigens and endotoxin, gut-associated bacteria may be delivered to the liver via portal circulation. While bacteria can be found in the liver of healthy dogs, bacteria may represent an additional factor contributing to the establishment and progression of disease in the presence of a compromised mononuclear phagocytic system. Hepatic bacterial infection is a significant source of mortality for people with decompensated hepatopathy. This retrospective study was undertaken to describe bacterial growth from hepatic tissue in dogs with clinicopathologic and/or histologic findings of liver disease.

Twenty-six dogs with clinicopathologic and/or histologic findings suggestive of liver disease, and which had bacterial cultures of hepatic tissue obtained during laparotomy or laparoscopy, were identified. Of these, eight had received antibiotics prior to culture. Samples were described according to the type and nature of histopathologic findings (neutrophilic inflammation, fibrosis, necrosis, biliary hyperplasia), and presence of sinusoidal neutrophilia, capsulitis, thrombosis, and nodular hyperplasia. Standard aerobic and anaerobic bacterial culture methods were used and isolates were tested for susceptibility to selected antimicrobial agents.

The final diagnoses were hepatitis/cholangiohepatitis (group A, n=11), vascular anomaly (group B, n=6), hepatic vacuolar degeneration (group C, n=6), and neoplasia (group D, n=3). A single bacterial species was isolated from 23 dogs, while three dogs had two types of bacteria. Direct growth from swab samples was found in nine, while 17 cultures produced growth only from enrichment broth. The most commonly identified bacterial organism was Staphylococcus epidermis, which was found in dogs in each of groups A (n=3), B (n=4), and C (n=4). Staphylococcus intermedius was cultured in group A (n=1) and group C (n=1). E. coli was identified from dogs in group A (n=2) and C (n=1). Enterococcus spp. were isolated from group A dogs (n=2). Pseudomonas spp. was isolated in one dog each from groups B and D, as was Serratia marcescens for one dog from groups A and D. Streptococcus spp. was isolated from 2 dogs in group C. Other bacterial species were isolated from a single dog each; Clostridium (A), Acinetobacter baumannii (A), Propionibacterium spp (A), Acinetobacter calcoaceticus Iwoffi (D).

Growth of bacteria was uniform from these diseased livers, but healthy dogs may also harbor a diverse hepatic bacterial population.[1] In our study, the most commonly isolated organisms were Staph. species. While studies of healthy dogs often describe Clostridium species as the most common hepatic organism, Staph have been isolated from healthy hepatic tissue and from dogs with induced hepatopathy and portosystemic shunting.[1-3] There was no pattern detected in regards to organism isolated and type or character of hepatic pathology.

1. Niza et al., J Sm Anim Pract. 45:401, 2004. 2. LM Cobb et al. J Comp Path 72:92, 1962. 3.LM Howe e al. AJVR 60:181, 1999.

ABSTRACT #271

INCREASED FECAL α_1-PROTEINASE INHIBITOR CONCENTRATIONS IN CATS WITH GASTROINTESTINAL DISEASE. K Fetz, JM Steiner, CG Ruaux, JS Suchodolski, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Gastrointestinal protein loss associated with gastrointestinal disease in cats has not been well described. In dogs and humans gastrointestinal protein loss can be assessed by the measurement of fecal α_1-proteinase inhibitor (α_1-PI) concentrations. The aim of this study was to evaluate fecal α_1-PI concentrations in a group of adult cats with chronic gastrointestinal disease.

Nine client-owned adult cats (seven male neutered, one female spayed, and one intact female; 1.5 to 16 years of age; three DSH, one DMH, one DLH, one Siamese, one Maine Coon, one Persian, and one Himalayan) were recruited from accessions to the Gastrointestinal Laboratory at Texas A&M University. Criteria for entry included a serum cobalamin concentration below the lower limit of the reference range (<290 ng/L), history and clinical signs consistent with chronic gastrointestinal disease, and the absence of an abnormally low serum feline trypsin-like immunoreactivity (fTLI) concentration. Fecal α_1-PI concentrations were determined using an in-house species specific ELISA in samples collected from three differing spontaneous bowel movements over three consecutive days. The upper limit of the reference range for mean fecal α_1-PI concentrations in healthy cats is 1.6 µg/g (wet weight). Serum from each cat was obtained on the last day that feces were collected and a routine serum biochemical profile was performed. Serum concentrations of cobalamin and folate were determined using specific competitive binding assays.

Six of nine cats had serum cobalamin concentrations below the lower limit of detection (<100 ng/L) and three cats had cobalamin concentrations ranging from 113 to 147 ng/L. Mean fecal α_1-PI concentrations were increased in eight of nine cats, ranging from 2.2 to 180.7 µg/g. The remaining cat had normal fecal α_1-PI concentrations in each of the three samples examined. Serum folate concentrations were above the upper limit of the reference range in four of nine cats and below the lower limit of the reference range in one of nine cats. Serum albumin concentration was below the lower limit of the reference range (<3.2 g/dL) in six of nine cats, ranging from 1.7 to 3.0 g/dL. Five of the six cats with low serum albumin concentrations also had decreased total protein concentrations, ranging from 5.5 to 6.3 g/dL (reference range: 6.5-8.9 g/dL), while the remaining cat had slightly increased serum globulin concentrations.

In conclusion, this study shows that increased fecal α_1-PI concentrations can occur in cats with chronic gastrointestinal disease associated with decreased serum cobalamin concentrations. Furthermore, two thirds of the cats investigated had hypoalbuminemia. These data would suggest that, in contrast to common clinical impressions, chronic gastrointestinal disease can be associated with gastrointestinal protein loss in the cat.

ABSTRACT #272

SERUM COBALAMIN AND FOLATE CONCENTRATIONS IN CATS WITH HYPERTHYROIDISM. JM Steiner[1], MA Peterson[2], CG Ruaux[1], S Ryburn[1], and DA Williams[1]. Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]The Animal Medical Center, Manhattan, NY.

Cats with hyperthyroidism commonly show vomiting and/or diarrhea, which are believed to be clinical signs due to direct effects of thyroid hormones on the gastrointestinal tract. However, signs of gastrointestinal disease do not resolve in some cats after ^{131}I treatment. The goal of this study was to objectively assess intestinal function in cats with hyperthyroidism by measuring serum concentrations of cobalamin and folate.

Serum samples were collected from 13 cats with hyperthyroidism. The diagnosis of hyperthyroidism was based on clinical signs compatible with hyperthyroidism, a palpable thyroid nodule on physical examination, and an increased serum total T4 concentration (mean ±SD: 17.9 ±10.6 µg/dL) using a radioimmunoassay (Diagnostic Products Corporation, reference range: 0.8-4.0 µg/dL). Serum cobalamin and folate concentrations were measured by competitive binding chemiluminescence assays (Diagnostic Products Corporation).

Serum cobalamin concentrations were below the lower limit of the reference range (290 ng/L) in three of the 13 hyperthyroid cats (23.1%). Serum cobalamin concentrations were in the low end of the reference range in 4 additional cats (serum cobalamin concentrations: 302, 326, 426, and 448 ng/L). Serum cobalamin concentrations were well within the reference range in the remaining 6 cats. Serum folate concentrations were below the lower limit of the reference range (9.7 µg/L) in 5 of the 13 hyperthyroid cats (38.5%). Serum folate concentrations were in the low end of the reference range in an additional 3 cats (serum folate concentrations: 10.1, 10.5, and 10.8 µg/L). Serum folate concentrations were well within the reference range in the remaining 5 cats. A total of 6 of 13 cats (46.2%) had either a decreased serum cobalamin concentration, a decreased serum folate concentration, or both. Eleven of the 13 cats (84.6%) with hyperthyroidism had a decreased or low normal serum cobalamin concentration and/or a decreased or low normal serum folate concentration.

Serum cobalamin concentrations were either decreased or in the low end of the reference range in 53.8% of hyperthyroid cats evaluated in this study. Also, serum folate concentrations were either decreased or in the low end of the reference range in 61.5% of hyperthyroid cats evaluated in this study. These findings suggest the presence of significant malabsorption of both cobalamin and folate in cats with hyperthyroidism, although hypermetabolsim may lead to increased vitamin requirements. Hyperthyroidism may directly or indirectly lead to vitamin malabsorption, or some hyperthyroid cats may have concurrent small intestinal disease. Whatever the mechanism, concurrent intestinal dysfunction and vitamin deficiencies appear to be common in hyperthyroid cats.

ABSTRACT #273

LASER CAPTURE MICRODISSECTION AND IN VITRO AMPLIFICATION OF RNA FROM PRIMARY MICRO-CULTURED CELLS: GROUND-BREAKING MOLECULAR TOOLS FOR GENE EXPRESSION ANALYSIS OF CANINE ENTEROCYTES. Shannon Walsh, Annika Linde, Maria Teresa Ortega, Sara Reppert, Frank Blecha & Tonatiuh Melgarejo. Kansas State University, Human Ecology & Veterinary Medicine, Manhattan, KS.

The P.A.L.M.® MicroBeam System (P.A.L.M. Microlaser Technologies AG, Germany) is a valuable tool that enables the identification and collection of specific individual cells from heterogeneous tissues or cultured cells with sub-micrometer precision through the process of laser microdissection. Although the laser microdissection is a powerful and advanced technique, the precision of cutting and collecting micro-sized areas from tissue or cultured cells yields only a small amount of total RNA (pico- to nanogram) that is generally not sufficient for analysis of gene expression (e.g. Northern Blot, Microarrays). The focus of research in our laboratory is the innate immune defense mechanisms of intestinal epithelial cells. A major barrier of canine enterocyte gene expression analysis has, however, traditionally been the significant challenge in culturing these cells. The goal of this study was twofold: 1) Develop a rapid and precise method to collect a target group of cells from a specific tissue of interest, and 2) Expand minuscule amounts of RNA isolated

from these cells to obtain enough material (i.e. micrograms) for gene expression analysis. A primary cell micro-culture of canine jejunal enterocytes was used for the microdissection technique. Canine enterocytes from adult beagles were seeded onto a P.A.L.M. LPC-membrane slide located at the bottom of a sterile Petri dish filled with supplemented culture media and allowed to attach for three hours at 37°C, 5% CO_2. After 24 hours the cells were switched to 34°C, 5% CO_2 to delay differentiation. Once clusters of attached cells covered the membrane, the slide was removed from the Petri dish and immediately fixed in 100% ethanol for three minutes at -20°C and then transferred for laser micro-dissection. The slide was then transferred to the P.A.L.M. ® MicroBeam System for micro-dissection and pressure catapulting spending less than 15 minutes on this step of the procedure to reduce RNA degradation. Groups of enterocytes were microdissected using a 20X objective and catapulted into a cap (positioned directly above the collection site) containing 35µl of lysis buffer to protect the RNA from degradation. The cap was removed and placed on ice for total RNA isolation using the RNeasy® Micro Kit (QIAGEN) and manufacturer's protocol. An amount of 20ng total RNA was isolated from the microdissected enterocytes and then amplified via MessengerAmp II aRNA Kit (Ambion). This novel and crucial *in vitro* transcription technique allowed us to amplify the extracted RNA up to 2,000 fold, thus enabling complete analysis of canine enterocyte gene expression via Microarray and Northern Blot studies. Our findings suggest that the combination of the described newfangled molecular techniques has the potential of becoming a revolutionary tool in gene expression studies of not only canine enterocytes, but also of numerous other types of cell lines.

ABSTRACT #274

USE OF RADIOGRAPHIC MEASUREMENTS IN DISTINGUISHING ACQUIRED MYASTHENIA GRAVIS FROM OTHER CAUSES OF ACQUIRED MEGAOESOPHAGUS IN DOGS. J.D.WRAY; Centre for Small Animal Studies, Animal Health Trust, Newmarket, Suffolk.UK.

The purpose of the study was to confirm that dogs with megaoesophagus due to myasthenia gravis (MG) display less oesophageal dilatation radiographically than those with megaoesophagus due to other causes.

Lateral thoracic radiographs of 66 non-anaesthetised adult dogs with megaoesophagus, in which concurrent acetylcholine receptor antibody (ACHRA) titre was known, were analysed retrospectively. Radiographs were examined without knowledge of final diagnosis. Correlation of thoracic inlet size (in mm) with weight and body surface area was evaluated with Pearson's correlation coefficient and maximum oesophageal diameter (in mm) was transformed to a 'relative oesophageal diameter' (ROD) by use of a ratio with thoracic inlet size. The dogs were divided into two groups according to 'MG' or 'non-MG' status and non-parametric tests (Mann-Whitney, Kruskal Wallis, Spearman rank correlation coefficient) were used to compare median ROD values between groups and to evaluate the relationship between ROD and age, weight and sex. A P value of <0.05 was considered significant. Breed-specific risk of MG was calculated using an Odds Ratio from the study population where there were a minimum of five dogs from one breed. A receiver Operating Characteristic (ROC) plot was used to evaluate a suitable ROD cut-off value which maximised sensitivity and specificity in distinguishing dogs with megaoesophagus due to MG from other causes.

Twenty dogs had MG and 46 were diagnosed with megaoesophagus due to other reasons. Thoracic inlet size correlated with body weight and body surface area. Median values of ROD for the MG and non-MG groups were 0.58 and 0.66 respectively and a statistically significant difference between the two groups was shown (P=0.029). There was no association between ROD and sex, age or weight. An increased odds ratio for MG existed in Golden retrievers and German Shepherd Dogs. ROC calculations showed an optimum cut-off value for ROD of 0.652 (sensitivity 80%, specificity 52%) in distinguishing MG from non-MG dogs with megaoesophagus.

Dogs with megaoesophagus due to MG in this study had a significantly lower relative oesophageal diameter than those dogs with megaoesophagus due to other causes. A single radiographic measurement ratio between maximum oesophageal diameter and thoracic inlet size may help distinguish dogs with megaoesophagus due to MG from those with other diseases.

ABSTRACT #275

COMBINED BLOOD TEST FOR GASTRIC EMPTYING AND ORO-CECAL TRANSIT TIME IN HEALTHY DOGS BEFORE AND AFTER TYLOSIN ADMINISTRATION. JM Steiner, JS Suchodolski, CG Ruaux, N Berghoff, K Fetz, U Tress, A Stoll, FH Rodriguez, P Xenoulis, and DA Williams. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Over the last decade many motility disorders have been described in human patients. However, little is known about motility disorders in dogs. This may be due, in part, to a lack of simple diagnostic modalities for assessment of gastrointestinal motility. Recently, use of a ^{13}C-octanoic acid breath test for estimation of gastric emptying (strictly duodenal filling), in dogs and horses has been reported. Also, a ^{13}C-lactose ureide breath test has been used to estimate oro-cecal transit time in humans. The goal of this study was to study a combined ^{13}C-octanoic acid and ^{13}C-lactose ureide blood test in healthy dogs before and after 4 weeks of tylosin (TL) administration.

Ten healthy research dogs belonging to an unrelated project were enrolled into the study. Food was withheld for at least 12 hours before the test. On the morning of the test an intravenous catheter was placed into the jugular vein of the dogs, a baseline blood sample of 1 ml was collected, and a dose of 2.5 mg/kg ^{13}C-octanoic acid and 10 mg/kg ^{13}C-lactose ureide was administered orally baked into a test meal of one egg yolk, mixed with five table spoons of canned maintenance food. Follow up blood samples of 1 ml each were taken at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, 210, 240, 270, 300, 360, 420, 480, 540, 600, 660, 720, 780, and 840 minutes after administration of the test meal. The same protocol was repeated in the same dogs after four weeks of TL therapy. Blood samples were immediately added into a 10 ml evacuated red-top tube containing 2ml 6N HCl for CO_2 extraction. Dose over base-line (DOB) was measured by use of an automated breath carbon analyzer (ABCA, Europa Scientific).

The time point for the first peak of DOB before TL administration was highly variable, ranging from 45 to 720 min after test meal administration (median 90.0 min; %CV: 124.0%). A clear second peak was detectable in seven dogs, ranging from 240 to 780 min after test meal administration (median 540.0 min). The time point for the first peak of DOB four weeks after TL administration was less variable ranging from 90 to 165 min after test meal administration (median 142.5 min; %CV: 20.1%). However, a significant difference of the median time point of the peak before or after TL administration could not be identified (p=0.4316). Also, a clear second peak was only detectable in two dogs after TL administration (660 and 780 min after test meal administration).

In conclusion, determination of a first peak after administration of ^{13}C-octanoic acid and ^{13}C-lactose ureide, putatively reflecting gastric emptying, is feasible. Administration of TL for four weeks led to less variability of the time of the first peak. A second peak, putatively reflecting oro-cecal transit time, could not be determined consistently within the first 840 minutes of the test, especially after four weeks of TL therapy. These findings would suggest that a ^{13}C-lactose ureide test would not be feasible for estimation of oro-cecal transit time in dogs using the current protocol.

ABSTRACT #276

PERCUTANEOUS ULTRASOUND-GUIDED TRANS-SPLENIC PORTAL VEIN CATHETERIZATION IN DOGS SUSPECTED FOR CONGENITAL PORTOSYSTEMIC SHUNT. M. Schneider, M. Plassmann, S. Scheidt; Small Animal Clinic (Internal Medicine), Justus-Liebig-University, Giessen, Germany.

Interventional therapy of congenital portosystemic shunts is of increasing interest. In the diagnostic and therapeutic procedure portal vein catheterization is needed. The aim of the prospective study was to describe the technique and results of percutaneous trans-splenic portal vein catheterization in dogs suspected for a congenital portosystemic shunt.

Between April 1999 and March 2003 we performed 40 percutaneous portal vein catheterizations in 31 dogs suspected for a congenital portosystemic shunt. Indications for the procedure were diagnosis (n=14), therapy (n=9) or reexamination after treatment (n=17). Dogs with a body weight less than 3.0 kg body weight were not included. Anesthesia was induced with acepromazin and levomethadon and maintained with inhalation of isoflurane. The patients were placed in dorsal recumbency and a biopsy-adapter was connected to a 7 MHz sector probe. Under ultrasound guidance a needle with mandarin (0.9 mm diameter and 10 cm length) was introduced into a splenic vein. After removing the mandarin and aspiration of blood a 0.018 inches soft tip guide-wire was placed through the splenic vein into the portal vein under fluoroscopic guidance. Either a 3F catheter lonely or a 4F catheter in combination with a 4F introducer sheet was used for portal vein catheterization. After the diagnostic or therapeutic intervention a bandage was wrapped around the abdominal cavity. After 12 hours an abdominal ultrasound examination was done to detect potential complications.

The median body weight was 9.0 kg (range 3.3 – 60.0). The access to the splenic vein was unsuccessful in four dogs. In four additionally dogs the catheter could only be placed into the splenic vein but not into the portal vein. The 3F catheter was used in 19 cases for angiography. The 4F catheter was used in 17 cases for coil-implantation and/or angiography. One dog developed an abdominal hemorrhage which needed a blood transfusion and recovered completely. Three other dogs showed a mild abdominal effusion. One dog developed a subcapsular splenic hematoma without clinical symptoms. Splenic vein thrombosis without clinically signs was found as a temporary or permanent complication in one case each.

Percutaneous ultrasound-guided portal catheterization is possible in most dogs with a congenital portosystemic shunt. Diagnostic and therapeutic intervention in the portal vein system can be done by this approach.

ABSTRACT #277

EFFECTS OF FEEDING FREQUENCY ON WATER INTAKE IN CATS. Kirschvink N.[1], Lhoest E.[2], Leemans J.[1], Delvaux F.[1], Istasse L.[2], Gustin P.[1], Diez M.[2] [1]Department for Functional Sciences, [2]Unit of Small Animal Nutrition, Department of Animal Production, Faculty of Veterinary Medicine, University of Liège, Belgium.

The objective was to determine the effects of variations in energy intakes and frequency of meals on spontaneous water intakes in healthy cats.

A colony of 24 adult cats (10 neutered males and 14 neutered females) aged between one and three years, body score ranging from 2 to 4, mean body weight (BW) 4.0 ± 0.6 kg, was given ad libitum access to water. All cats were sequentially fed the diet (% dry matter [DM], crude protein 35, crude fat 12, crude fiber 3, ash 9, Na 0.7, 4890 kcal metabolizable energy/kg DM) during three weeks and drinking water was measured during the last week. The diet was given at two energy (E) levels (Low E : 71 and High E : 91 kcal/kg BW/day), the total daily energy being provided by 1, 2 or 3 meals. Water intake was determined twice daily.

Table 1. Results.

	Daily energy intake kcal/kg BW	Na intake mg/kg BW	Water intake ml/cat/day	Water intake ml/g DM intake
Diets				
Low E- 1 meal	71	103	72 ± 10^{a}	1.23 ± 0.19^{a}
Low E – 2 meals	71	103	89 ± 4^{b}	1.54 ± 0.05^{b}
Low E – 3 meals	71	103	95 ± 6^{c}	1.67 ± 0.10^{c}
High E – 1 meal	91	128	$91 \pm 11^{a*}$	1.22 ± 0.15^{a}
High E – 2 meals	91	128	$109 \pm 11^{b*}$	1.44 ± 0.15^{b}

a, b, c, significantly different for within column and energy levels comparisons, * significantly different from respective Low E ($P < 0.05$).

For a given energy level, the water intake (ml/g DM) significantly increased by increasing meal frequency. With increasing energy intakes, absolute water intake (ml/day/cat) increased without influencing water intake expressed by g DM.

One dietary modification for the prevention and treatment of feline lower urinary tract disease might be the division of the daily diet in at least two or three meals, which seems efficient to increase drinking water consumption in healthy cats.

ABSTRACT #278

CREATINE DISPOSITION IN HEALTHY DOGS. ADJ Watson, E Jeunesse, V Laroute, G Costes, JP Braun, HP Lefebvre. Physiopathologie et Toxicologie Expérimentales INRA-ENVT, National Veterinary School of Toulouse, France.

Creatine is a popular dietary supplement used to increase exercise performance and fat-free mass in humans. This compound was recently shown to have beneficial effects in various human clinical conditions. Creatine is mainly found in skeletal muscle. It is obtained through the diet and is also synthetized in the liver, kidney and pancreas. There have been very few investigations of the pharmacokinetics of creatine in humans and animals. The aim of the present study was to investigate the pharmacokinetics of creatine in dogs after single iv and oral administration.

Five clinically healthy adult Beagle dogs were used. Animals were fasted overnight before dosing. Creatine was dissolved in sterile distilled water to obtain a solution at a final concentration of 12.5 µg/mL. The dose rate was 20 mg/kg. Blood was sampled just before administration of creatine. After bolus iv administration, blood was sampled at 2, 5, 10, 20 and 30 min, 1, 2, 4, 6, 8, 10 and 24 hours. After oral administration, bood was sampled at 15, 30, and 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours. Blood was collected in heparinized tubes and immediately centrifuged. Plasma creatine was frozen at –20°C until assayed by HPLC, the limit of quantitation being 5 µg/mL. Pharmacokinetic analysis was performed by a noncompartmental approach using WinNonLin software. Data are expressed as mean±SD.

Basal levels of plasma creatine (from 5.6 to 16.1 µg/mL) was above LOQ in 3 dogs. Following single iv administration, plasma clearance, steady state volume of distribution, mean residence time, and elimination half-life were 5.1±0.4 mL/kg/min, 207±24 mL/kg, 41±6 min and 32±5 min. Following single oral administration, peak plasma concentration and time to peak plasma concentration were 33±7 µg/mL and 33±7 min. The oral bioavailability was 86±10%.

These results indicate that the clearance of creatine is relatively close to the value of glomerular filtration rate in dogs, indicated that renal elimination of creatine may approximate GFR. The steady state volume of distribution corresponds to the extracellular fluid volume. Following oral dosing, which is the usual route of administration, the absorption occurs rapidly and the bioavailability is good. Further studies are however needed to assess pharmacokinetic variables

following repeated oral administrations and in individuals with renal failure.

ABSTRACT #279

COMPUTER ANALYSIS OF NUTRIENT SUFFICIENCY OF PUBLISHED HOME-COOKED DIETS FOR DOGS AND CATS. SD Lauten, TM Smith,CA Kirk, JW Bartges, and AM Adams. Department of Small Animal Clinical Sciences, University of Tennessee College of Veterinary Medicine, Knoxville, TN.

Veterinarians frequently rely on published home-cooked diets when commercial foods are inappropriate or rejected by their clientele. Forty-nine maintenance and 36 growth diets were collected from six books[a]. The diets were analyzed using a human software package[b] that utilizes several reputable ingredient databases including USDA, on food composition. All efforts were made to review individual ingredients to assure completeness of nutrient analyses. Diet analyses were compared against American Association of Feed Control Officials (AAFCO) recommendations, and the 1985/1986 National Research Council (NRC) Nutrient Requirements of Dogs and Cats, respectively. To allow for ingredient analytical variance, diets that were within 10% of recommended nutrient allowances were considered adequate.

Compared to AAFCO recommendations, 55% of the diets contained inadequate amounts of protein, with 77% of those being deficient only in taurine. Sixty-two percent were inadequate in vitamins, with 77% of those deficient only in choline. Eighty-six percent of the diets were inadequate in various minerals and 8% were inadequate in essential amino acids. Compared to NRC minimum nutrient requirements, 34% contained deficiencies in amino acids, but all were deficient in taurine only. Forty-five percent of the diets were deficient in vitamins; however, only one was deficient in multiple vitamins with the balance deficient only in choline. Twenty-one percent of the diets were deficient in minerals while 8% were deficient in essential amino acids. Many of the ingredients in the databases are not analyzed for taurine and choline, so diets determined as inadequate or deficient may be adequate.

Many of the published diets were nutritionally adequate; however, nutrients can vary significantly based on ingredient selection (i.e. size of eggs, or percent fat in meat sources). Additionally, diets may need to be revised or reformulated to specifically address key nutrients to improve nutrient profiles.

[a]Home-Prepared Dog and Cat Diets, Strombeck, DR. Manual of Companion Animal Nutrition & Feeding, Kelly and Wills, Eds. Manual of Veterinary Dietetics, Buffington, T, Holloway, C, and Abood, S. Natural Health for Dogs & Cats, Pitcairn, RH and Pitcairn, SH. Small Animal Clinical Nutrition, Hand, MS, Thatcher, CD, Remillard, RL, and Roudebush, P, eds. The Waltham Book of Clinical Nutrition of the Dog & Cat, Wills, JM, and Simpson, KW, eds. [b]ESHA Research, Food Processor SQL, version 9.6, Salem OR

ABSTRACT #280

Abstract Withdrawn.

ABSTRACT #281

DAY-TO-DAY VARIABILITY IN URINARY ALBUMIN EXCRETION. HM Syme, S.Cariese, AL.Cauvin and J. Elliott, Royal Veterinary College, London, UK.

Urinary excretion of protein has been demonstrated to be predictive of survival time in populations of azotemic and non-azotemic cats. However, in individual patients the significance of mildly elevated protein excretion in a single urine sample is difficult to interpret, and results may not be repeatable. This has led to the suggestion that multiple tests be performed. The aim of the present study was to quantify the day-to-day variability in urine albumin to creatinine (UAC) ratios in a population of clinically healthy cats.

Cats included in the study were believed to be healthy; most belonged to staff and students of the Royal Veterinary College. Four, free-catch, urine samples were collected from each cat; the first sample was collected prior to a period of hospitalisation during which the second and third samples were collected, the fourth sample was collected after the cat returned home. Urine samples were stored at –20^{o}C until analysis. Albumin concentration was measured in each urine sample using a species specific ELISA method that has been validated previously. Albumin concentration was divided by the urine creatinine concentration to yield the UAC measurement. To measure the variability in UAC measurements between the four samples the Coefficient of Variation (CV) was calculated. In addition, UAC values were classified as normal (<30 mg/g), mildly elevated (>30 mg/g) or elevated (>83 mg/g), and the numbers of cats from which discrepant results were obtained from the different urine samples was calculated. To determine if there was any effect of hospitalisation on UAC data was analysed by a repeated measures ANOVA.

Data from 24 cats was included in the study. As might be expected in urine samples obtained from normal cats the average UAC was low (9.2 ± 4.4 mg/g). The mean CV of the UAC measurements from the individual cats was 41% (range 8—95%), but only 2/25 cats had UAC values that were classified as mildly elevated, and none had elevated values. Of the two cats with mildly elevated UAC measurements, this result was found in only two of the four samples tested. There was no significant difference in UAC measurements from the different sampling times (P=0.08), although UAC measurements from individual cats were highly significantly related ($P<0.001$, $R^2=0.58$).

UAC does appear to show significant day-to-day variability. However, this does not appear to often result in measurements that could be interpreted as abnormal. This study now needs to be repeated in cats with significant renal dysfunction, and higher UAC measurements.

ABSTRACT #282

CHARACTERISTICS OF PROGRAMS INVOLVING CANINE VISITATION OF HUMAN HOSPITAL PATIENTS IN ONTARIO. S. Lefebvre[1], J. S. Weese[2], D. Waltner-Toews[1], A. Peregrine[3], R. Reid-Smith[1]. Depts. of Population Medicine[1], Clinical Studies[2] and Pathobiology[3] , University of Guelph, Canada.

Animal visitation programs are a source of comfort, motivation and socialization to people in healthcare facilities. Despite this, few details are known about the backgrounds of these animals. Because institutionalized populations are often more vulnerable than others to infections, such information is crucial to understanding any potential health risks associated with these otherwise beneficial activities. The purpose of this study was to: 1) enumerate hospitals in Ontario that permit dogs to visit their patients and the source of these dogs and 2) obtain details about these visitation programs and the participating dogs.

All Ontario hospitals listed with the Ontario Hospitals Association were surveyed by mail to request the information specified in 1) above. Of the 231 surveyed, eight failed to reply. Ninety percent of the 223 respondents indicated that dogs were permitted in their facilities. Five hospitals owned their own dogs; national therapy dog programs such as St. John Ambulance or Therapeutic Paws provided 35% of the dogs.

The sources of all dogs reported in the hospital surveys were invited to participate. All members that volunteered were included if their dogs actively visited hospitals, for a total of 102 dogs and 90 owners. Owners were interviewed using a standardized questionnaire. Ten dogs (10%) were associated with community-based groups (including kennel clubs), 73 (73%) with national agencies, 14 (14%) with hospital volunteer programs, and 5 (5%) with a humane society.

The screening protocols that dogs were required to pass in order to participate in their respective visitation programs were highly variable. The most common requirement was "core" vaccinations (defined as distemper, hepatitis, parainfluenza and parvo-viruses, plus rabies) – none of which protect against zoonoses with the exception of rabies. Only 16 of the 90 owners (18%) reported that annual fecal flotation was compulsory. Less than 50% of dogs (47) needed to pass a structured temperament test to qualify. The same number required annual veterinary health certification. Preparations for hospital visits also ranged widely, with the most common practice being grooming (53%). Eighteen owners (20%) said they were unaware of any preparatory requirements, and did not practice any. Sixty-six owners (73%) allowed their dogs on patients' beds, and 71 (79%) let their dogs lick the patients. Eighty (89%) had advised their veterinarians that their dogs visited hospitals, but only 13 (14%) reported that their veterinarians had discussed zoonoses with them. Thirty-six owners (40%) were unable to name one disease that people can catch from dogs.

Visitation of hospitalized patients by dogs has become the norm rather than the exception. These programs are highly variable in their screening requirements and infection control practices, leaving room for potential problems. Hospitals, visitation groups, and veterinarians need to work together to reach some common understanding for the protection of both people and pets.

ABSTRACT #283

A RETROSPECTIVE STUDY OF 94 CASES WITH HYPERCALCEMIA PRESENTED FROM APRIL, 2002 TO SEPTEMBER, 2004. T. H. Cho, B. T. Kang,, C. Park, D. I. Jung, E. H. Park, H. J. Kim, J. W. Kim, C. Y. Lim, and H. M. Park. School of Veterinary Medicine, Konkuk University, South Korea.

Hypercalcemia is the presence of abnormally high levels of calcium in the blood. When the serum calcium level rises above reference range, multisystemic manifestations become apparent. According to previous studies, non-parathyroid malignancy, most notably lymphosarcoma, is the most common cause of hypercalcemia in dogs. Other hemolymphatic malignant tumors (i. e., lymphocytic leukemia, multiple myeloma, myelo-proliferative diseases), anal sac apocrine gland carcinoma, and soft tissue tumors metastasizing to bone may also cause hypercalcemia. Less frequent causes include primary hyperparathyroidism, chronic or acute renal failure, hypoadrenocorticism, and hypervitaminosis D. Common causes of hypercalcemia in dogs have not been investigated in South Korea. The purpose of this study is to identify diseases that cause hypercalcemia of dogs.

In case group, total 94 dogs with hypercalcemia were evaluated and most of them were referred to the Veterinary Medical Teaching Hospital (VMTH) of Konkuk University from April of 2002 to September of 2004 were investigated. Because dogs less than 24 weeks of age have high values in calcium concentration, they are excluded in this study. Age, sex, adjusted total serum calcium concentration, and breeds of hypercalcemic patients were compared with those from control group of 94 eucalcemic dogs admitted to the department of internal medicine during the same period. In addition, diseases induced hypercalcemia in case group and those induced eucalcemia in control group were investigated and compared. The corrected total serum calcium concentration was calculated by this formula; Corrected calcium = measured calcium (□/□) – albumin (g/□) + 3.5. If the corrected total serum calcium concentration exceed 12.0 □/□, it is considered to be hypercalcemia. When it is between 9.0 and 12.0 □/□, it was considered to be eucalcemia.

During the study period, hypercalcemia was found in 94 dogs and 19 breeds were evaluated in case group. Control group was made up of 94 dogs and 18 breeds without hypercalcemia were examined for the same period. In case group, Shih-tzu (17.02 %) was most common breeds. In control group, it was highest in Yorkshire terrier (26.60 %). The most common causes induced hypercalcemia was renal related diseases (chronic renal failure (18.09 %), acute renal failure (14.89 %) and renal calculi (6.38 %)). Secondly, malignant neoplasia (lymphoma, hemangiosarcoma, chronic lymphocytic leukemia, mammary gland tumor, and multiple myeloma) was involved in hypercalcemia (8.5 %). Thirdly, diseases induced hormonal imbalance (hyperadrenocorticism, hyperthyroidism, hypo-adrenocorticism, and hypothyroidism) associated with hypercalcemia (6.4 %). Although this result is not similar to previous studies, this study could help veterinarians in South Korea to differentiate diseases induced hyperclacemia.

ABSTRACT #284

OCCURRENCE OF ANTIMICROBIAL RESISTANT BACTERIA IN HEALTHY DOGS AND CATS PRESENTED TO PRIVATE VETERINARY HOSPITALS IN SOUTHERN ONTARIO.

Colleen Murphy[1], Richard Reid-Smith[1,2], John Prescott[3], Brenda Bonnett[1], Cornelius Poppe[2], Patrick Boerlin[3], Scott Weese[4], Nicol Janecko[1] and Scott McEwen[1; 1]Department of Population Medicine, Ontario Veterinary College University of Guelph, Guelph Ontario, [2]Laboratory for Foodborne Zoonoses, Health Canada, Guelph Ontario, [3]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph Ontario, [4]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph Ontario, Canada.

The study of antimicrobial resistance of *Escherichia coli* (*E. coli*) isolates from companion animals has been limited to data reported from diagnostic laboratories and there are no published data on the prevalence of community carriage of *Salmonella* spp, methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin- resistant *Staphylococcus intermedius* (MRSI) and extended spectrum β-lactamase (ESBL) *E. coli* in companion animals. This was a community based, cross-sectional study whose objective was to establish the examined the prevalence and patterns of antimicrobial susceptibility of commensal *E. coli*, *Salmonella*, ESBL-*E. coli*, MRSA and MRSI in healthy dogs and cats.

Healthy dogs and healthy cats from private veterinary hospitals in Southern Ontario without a history of recent exposure to antimicrobials were enrolled into the study. Fecal samples were collected from using a rectal swab. The medical record was examined and a questionnaire administered to the pet owners. Standard isolation methods were used and susceptibility testing was performed in accordance to the NCCLS guidelines.

Samples were collected from 188 dogs and 39 cats. No animals were identified as carriers of *Salmonella*, ESBL-*E.coli*, MRSA or MRSI and five isolates of commensal *E.coli* were recovered from each animal. The prevalence of antimicrobial resistance in the commensal *E. coli* isolates recovered was lower than what has been previously reported in companion animals. The prevalence of resistance was highest in streptomycin, ampicillin, cephalothin and tetracycline in both dogs and cats. Multiple-drug resistance was observed with the most commonly observed pattern of resistance being streptomycin/tetracycline/trimethoprim-sulfamethoxazole in dogs and tetracycline/trimethoprim-sulfamethoxazole/nalidixic acid in cats. In addition, two dogs were identified as carriers of cephmycinase (CMY)-2 *E.coli.*

The data obtained from this study provide a baseline prevalence of resistance and patterns of resistance in companion animals. The pets in this study represent a community reservoir of antimicrobial resistant *E. coli* and resistance genes, including the CMY-2 gene, which could pose a risk to animal and human health. The results provide a starting point for investigating the impact of antimicrobial resistance in companion animals and contribute information to the global understanding of the epidemiology of antimicrobial resistance.

ABSTRACT #285

ORAL ABSORPTION OF FENBENDAZOLE (FBZ) AND OXFENDAZOLE (OXF) BY ALPACAS AFTER FEEDING 1.8% FENBENDAZOLE IMPREGNATED MINI-PELLETS: A COMPARISON WITH 10% ORAL SUSPENSION. J Lakritz, D. Linden, D. Anderson, T. Specht, C Barnum, K. Newman. Dept. of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

The purpose of this study was to determine whether camelids absorb sufficient active drug when 1.8% FBZ mini-pellets are provided in comparison with 10% oral suspension.

Nine healthy Alpacas were provided with either 2043 mg of FBZ (n = 5; 1.8% mini-pellets; 28.5 mg/kg), or 22 mg/kg 10% FBZ oral suspension (n =4). Blood samples were obtained from jugular catheters at time = 0, 0.5, 1, 2, 4, 8, 12, 24, 48 and 96 hours post-dosing. Plasma was harvested and analyzed by HPLC for FBZ and OXF simultaneously using a UV detector at 292nm. Blank plasma was spiked with known concentrations of both compounds to determine extraction efficiency, accuracy and precision and plasma concentrations of drugs in study samples.

After oral administration of either the 10% oral suspension (0.4 ± 0.14 µg/ml; range 0.28-0.61 µg/ml) or 1.8% FBZ impregnated mini-pellets (0.43 ± 0.16 µg/ml; range 0.29-0.7 µg/ml) resulted in peak plasma concentrations of FBZ that exceeded previously reported concentrations observed in other studies. Plasma concentrations of OXF observed exceeded those of FBZ (0.65 ± 0.27 µg/ml; range 0.46-0.85 µg/ml 10% oral suspension; 0.64 ± 0.08 µg/ml; range 0.58-0.75 µg/ml 1.8% mini-pellets). Plasma concentrations of FBZ exceeded the limit of quantitation for 48 h, and OXF exceeded the LOQ for 96 h post dosing. Estimated half-life of FBZ elimination was 26.5 ± 8 h after oral suspension, versus 38 ± 25 h for mini-pellets. The area under the concentration versus time curve was 22.8 ± 11 h●µg/ml for suspension versus 22 ± 7.9 h●µg/ml for mini-pellets. The mean residence time was 39 ± 13 h for suspension versus 40 ± 10 h for mini-pellets. The data obtained suggest that oral absorption of FBZ from the FBZ impregnated mini-pellets in comparison to the oral suspension is sufficient to warrant use clinically. The use of feed impregnated with drug should improve client compliance by alleviating the need for restraint and forcible administration to animals. Since FBZ is converted to OXF by oxidation, remains active against many nematodes (including *Trichuris* spp.) and plasma concentrations were similar or exceeded plasma concentrations of FBZ, total active anthelmintic concentrations in plasma were >1 µg/ml at 24 hours post-dosing.

ABSTRACT #286

CARDIAC TROPONIN-I PLASMA CONCENTRATION IN NORMAL HORSES AND IN HORSES WITH ARRHYTHMIAS AND TOXICITIES. JH Foreman, MA Oyama, BS Tennent-Brown, KK Seino, TE Goetz, DD Sisson. Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL.

Cardiac troponin-I (cTnI) is a highly sensitive and specific marker of myocardial injury and can be detected in plasma by immunoassay techniques. Elevations in cTnI in dogs are correlated with heart size and survival time, and can be used as a blood-based biomarker of cardiac disease (Oyama and Sisson 2004). Measurement of cTnI in horses has been reported previously in normal and post-exercising Thoroughbred racehorses (Phillips et al. 2003), horses with fumonisin toxicity (Smith et al. 2002), and one horse with ventricular tachycardia (Cornelisse et al. 2000). Our hypothesis in this study was that horses with clinically-apparent myocardial disease would have measurably-elevated plasma cTnI concentrations when compared to normal horses' plasma concentrations.

Inclusion criteria over a three-year period required a diagnosis of cardiac disease accompanied by ECG and cardiac ultrasound examinations (n=20 cardiac cases). A second group of normal horses and ponies (n=12) were also studied as a reference group. Jugular venous blood samples were collected in 8ml evacuated blood tubes containing 143 IU of sodium heparin. Samples were centrifuged within 30 minutes after collection and the plasma was separated and frozen at –70 C for batched cTnI analysis by use of the human Access AccuTnI assay. The lower limit of detection was 0.01 ng/ml and the upper limit was 100 ng/ml of plasma. Results from normal horses and ponies were used to establish a reference range to which were compared results from horses with cardiac diagnoses.

Normal equine plasma cTnI ranged from 0.01 to 0.03 ng/ml (n=12). Horses with benign atrial fibrillation (n=8) had cTnI ranges of <0.01-0.09 ng/ml, with 4 horses having cTnI falling outside the reference range (0.04, 0.05, 0.06, and 0.09). Horses with murmurs and no arrhythmias (n=3) included tricuspid insufficiency (0.05), mitral insufficiency (0.07), and aortic insufficiency (0.02). One foal with an atrial septal defect and signs of congestive heart failure had cTnI of 0.53 ng/ml. Horses with ventricular arrhythmias (VPCs or ventricular tachycardia) and documentable toxicities (n=5) had cTnI of 0.05, 0.21, 0.31, 15.18, and >100 ng/ml. Toxicities included Streptococcal myositis (n=3), white snakeroot poisoning (n=1) and red maple leaf exposure (n=1). Horses with ventricular arrhythmias but no documentation of myocardial toxicity (n=3) had cTnI of 0.34, 0.46, and 80.42 ng/ml.

It was concluded that horses with myocardial toxicities and ventricular arrhythmias often had severe elevations in plasma cTnI, with concentrations decreasing gradually as the cardiac arrhythmias diminished in frequency and character. Horses with more benign conditions such as uncomplicated valvular insufficiencies or atrial fibrillation also often had cTnI falling outside the normal range, but these were not nearly as elevated as in horses with ventricular arrhythmias.

ABSTRACT #287

EQUINE PARS INTERMEDIA PITUITARY ADENOMA (CUSHING'S DISEASE): STEROID HORMONE PROFILES IN HEALTHY HORSES UNDERGOING DEXAMETHASONE SUPPRESSION, THYROTROPIN RELEASING HORMONE (TRH), AND ADRENOCORTICOTROPIC HORMONE (ACTH) STIMULATION. Kellie A. Fecteau(1), John C. Haffner(2), Hugo Eiler(1), Frank M. Andrews(3), Jack W. Oliver(1). (1)Department of Comparative Medicine, (3)Department of Large Animal Clinical Sciences, The University of Tennessee, College of Veterinary Medicine, Knoxville, TN; (2)Horse Science Center, Middle Tennessee State University, Murfreesboro, TN.

Equine pars intermedia pituitary adenoma (PIPA) is often characterized by a paradox: high concentration of plasma ACTH (+10-fold) concurrent with normal concentrations of cortisol. Dogs, cats, and ferrets exhibiting clinical signs of Cushing's with normal cortisol concentrations and increased adrenal intermediate steroids are considered to have aberrant or atypical Cushing's syndrome. Atypical Cushing's syndrome has been reported in dogs, cats, and ferrets and opens the possibility that steroid intermediate imbalances may be present in horses with Cushing's. The objective of this work was to initiate a database of reference values for the adrenal steroids progesterone (P), 17-hydroxyprogesterone (17-OHP), androstenedione (ANDRO), cortisol (C), and aldosterone (ALD), and the sex steroids testosterone (T) and estradiol (E), during dexamethasone (Dx) suppression and after TRH and ACTH stimulation.

Four geldings and seven mares 4-19 years old and weighing 454-568 kg from the Horse Science Center were used. A Combined Dx suppression/TRH/ACTH stimulation test protocol was used. Blood samples were collected at 0-time; then, Dx (40 µg/kg, IM) was injected and blood collected at 3 h; then, TRH (2.0 mg/horse, IV) was injected and blood collected at 30 minutes and at 90 minutes; then,

ACTH (Cortrosyn™) (60 µg/horse, IV) was injected and blood collected 60 minutes later.

Mean concentrations of P, 17-OHP, ANDRO, T, E, ALD, and C in geldings were 0.025, 0.047, 1.4, 0.02, 0.07, 0.08, and 42.0 ng/ml, respectively, and in mares mean concentrations were 5.2, 0.64, 2.2, 0.018, 0.08, 0.05, and 51.8 ng/ml, respectively. Coefficients of variation for all hormones were between 12.8% and 41.1%. In both sexes, cortisol (96% in geldings, 85% in mares) was the predominant steroid followed in geldings by ANDRO (3.4%), and all other steroids were less than 0.17% each. In mares, P (9.9 %) was the second highest steroid, followed by ANDRO (3.6%), 17-OHP (1.0%), and remaining steroids were less than 0.13% each. Certain steroids were significantly higher ($P<0.05$) in mares compared to geldings; P (242-fold), 17-OHP (13.6-fold), and ANDRO (1.6-fold). Other steroids were not affected by sex.

Out of the seven steroids studied, Dx injection suppressed ($P<0.05$) C only. TRH did not stimulate secretion of any steroids; ACTH stimulated secretion of 17-OHP (1.5-fold), ANDRO (2.2-fold), and C (3.5-fold). This data provides for the first time comprehensive information on blood steroid levels in horses. The study of the effect of PIPA on adrenal steroid profiles is underway and intermediate steroid hormones are expected to have diagnostic value.

ABSTRACT #288

EQUINE CUSHING'S DISEASE: EFFECT OF DEXAMETHASONE SUPPRESSION ON CORTISOL RESPONSE TO ADRENOCORTICOTROPIC HORMONE (ACTH) STIMULATION IN HEALTHY HORSES. John C. Haffner(1), Kellie A. Fecteau(2), Hugo Eiler(2), Jack W. Oliver(2), Frank M. Andrews(3). (1)Horse Science Center, Middle Tennessee State University, Murfreesboro, TN; (2) Clinical Endocrinology Service, Department of Comparative Medicine, (3) Department of Large Animal Clinical Sciences, The University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

Aberrant or atypical Cushing's syndrome is a condition in which plasma concentration of cortisol is normal, however clinical signs of hypercortisolism persist. Cortisol biosynthesis intermediates are known to have intrinsic glucocorticoid activity and cause atypical Cushing's in dogs, cats, ferrets, and humans, when cortisol concentration is normal. Equine Cushing's disease or pars intermedia pituitary adenoma (PIPA) is characterized by a paradox: high concentration of plasma ACTH, normal level of plasma cortisol, and clinical signs of hypercortisolism. Often steroid biosynthesis imbalances are expressed under ACTH stimulation or dexamethasone (Dx) suppression, not in resting or baseline conditions. The objective of this research was to optimize the ACTH dose-response relationship in horses undergoing dexamethasone suppression for the purpose of further development of testing protocols for equine Cushing's disease based on intermediate steroid analysis. Twenty-four horses divided into eight groups of three each were used. A Combined Dx suppression/ACTH stimulation test protocol was used. Blood samples were collected at 0-time; then, Dx (40 µg/kg, IM) was injected and blood collected at three hours; then, ACTH (Cortrosyn™) (various dosages 0, 2, 5, 10, 20, 60, 125, 250 µg / horse, IV) was injected and blood collected at 0-time, 60, 120, 180, and 240 minutes. The experiment was repeated in nine of the horses with ACTH stimulation alone.

Dexamethasone suppressed plasma cortisol (for over 24 hours) to a mean of 14 ng/ml at 180 min from a baseline of 50 ng/ml at 0-time. There was a linear ($r = 0.87$) ACTH dose-response relationship until the 60 µg ACTH dose. Horses were highly sensitive to ACTH injection, since the smallest dose of 2.0 µg/horse caused a cortisol net gain ($P<0.05$) of 10 ng/ml at 60 minutes. A dose of 60 µg/horse maximally stimulated adrenal glands and caused a cortisol gain ($P<0.05$) of 85ng/ml at 60 min., then, cortisol concentrations declined linearly ($r= – 0.93$). Duration of ACTH effect was directly correlated ($r = 0.97$) to dose size. At 240 minutes, concentrations of cortisol were still 60%, 40%, 36%, 9% and 9% above baseline value (250 µg, 125 µg, 60 µg, 20 µg and 10 µg dose, respectively). ACTH response was similar ($P = 0.05$) in the group of nine horses, with and without Dx suppression. It was concluded maximal diagnostic stimulation of cortisol can be attained with 60 µg Cortrosyn ™/horse, which is significantly less than what is reported in the literature. The above provides a basis for exploring the usefulness of combined testing and steroidal panel analysis in the diagnosis of equine Cushing's disease.

ABSTRACT #289

DIAGNOSTIC VALUE OF A COMBINED DEXAMETHASONE SUPPRESSION/THYROID-RELEASING HORMONE STIMULATION TEST IN EQUINE CUSHING'S DISEASE. F.M. Andrews, N. Frank, C.S. Sommardahl, H. Eiler, B.W. Rohrbach, M.D. McCracken College of Veterinary Medicine, The University of Tennessee.

Equine Cushing's disease (ECD) results from hypertrophy, hyperplasia or a functional adenoma in the pars intermedia of the pituitary gland and is frequently recognized in older horses. Recently, a combined dexamethasone suppression/thyrotropin-releasing hormone stimulation (DEX/TRH) test was introduced to diagnose ECD, but this test has not been validated. The purpose of this study was to estimate the sensitivity and specificity of the test for the diagnosis of ECD by comparing test results with histopathologic findings.

Forty-nine horses (median: 13 years of age, range 2 to 33 years) that were donated to the University of Tennessee VTH were used in this study. Within two days of arrival, the DEX/TRH test was administered between 8 and 10am, as previously reported.[1] The DEX/TRH test was considered positive for ECD when plasma cortisol concentration increased to ≥66%, at 30 minutes after the TRH administration or if cortisol concentration was >1.0 µg, 24 hrs after DEX administration. After the test was administered, horses were humanely euthanatized with an overdose of barbiturate. At necropsy, the pituitary gland (PG) from each horse was harvested, fixed in 10% formalin and routinely prepared for histopathologic examination for the presence of a discrete mass or hyperplasia in pars intermedia of the PG. The pathologist was blinded to the test results. The results of the DEX/TRH test were then compared with the histopathologic changes. These data were then used to estimate the sensitivity and specificity of the test. The positive and negative predictive values were calculated using the sensitivity, specificity and pretest probability (prevalence of ECD) based on the number of positive and negative horses enrolled in the study.

Seventeen (eight mares, eight geldings, and one stallion) of the 49 horses (35%) had ECD on histopathologic examination. The median age of horses with ECD was 23 yrs (range, 7-33 yrs) and the median age of the horses that did not have ECD was 10 yrs (range 2-30 yrs). The sensitivity and specificity of the DEX/TRH test were 88% and 78%, respectively. Positive and negative predictive values, based on the prevalence of horses affected in this study were 68% and 93%, respectively. When the population tested was limited to horses ≥ 13 years of age, 14/25 (56%) were affected with ECD and positive and negative predictive values were 83% and 83%, respectively.

In conclusion, the DEX/TRH test, when combined with clinical assessment of the patient, is useful in the diagnosis of ECD. When the test was applied to horses ≥ 13 years of age, prevalence of disease changed from 36% to 56% and the positive predictive value improved to 83%.

[1]*Eiler H, Oliver JW, Andrews FM, et al J Am Vet Med Assoc 1997;211(1):79-81.*

ABSTRACT #290

USE OF SUBTRACTIVE HYBRIDIZATION TO IDENTIFY DIFFERENTIALLY EXPRESSED GENES IN ISCHEMIC EQUINE INTESTINAL MUCOSA. MV Crisman, J. Tschetter, L.Beex, S.Woody, Dianne Little, A. Blikslager. VA-MD Regional College of Veterinary Medicine. Molecular Diagnostics Lab. Blacksburg, VA. & Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Approximately 10% of horses suffer a bout of colic each year with 0.7% of the cases resulting in death, indicating colic is a serious disease syndrome in horses. Much of the mortality is associated with ischemic-injured intestine during strangulating obstruction, yet there is limited understanding of the molecular events associated with ischemic injury of the intestinal epithelium. Identification of differentially expressed genes during ischemic injury will expand our understanding of colic and may lead to novel targeted therapeutics in the future. The objective of this study was to isolate and identify differentially expressed genes in equine jejunum following a two-hour ischemic event compared to normally perfused jejunum. We utilized Suppressive subtractive hybridization (SSH-PCR) to clone genes that are differentially expressed between equine jejunum injured by two hours of complete ischemia as compared to time-matched control jejunal tissues. Our method is based on the PCR-select™ (Clonetech Lab, Inc.). Positive clones from each subtracted cDNA population were selected for sequencing and BLAST analysis. Expression of selected clones was further evaluated by northern blot analysis.

Results. Of the 384 clones that were selected, 157 were confirmed to possess cDNAs corresponding to differentially expressed genes by dot blot analysis. Two genes, fatty acid binding protein 2 (FABP2) and calcium activated chloride channel 4 (CLCA4) were further confirmed to be differentially expressed by northern blot analysis. FABP2 is classified as a transport binding protein to saturated long chain fatty acids and is implicated in long-chain fatty acid uptake, metabolism or transport. FABP2 was down-regulated in ischemic jejunum. Additionally, CLCA4 was down-regulated during ischemia. Proteins in the chloride channel family have recently been shown to be critical to early tight junction closure following ischemic injury. Further analysis of genes associated with tight junction integrity is currently underway.

Conclusions. Suppressive subtractive hybridization can be used to detect changes in expression of a broad array of genes, as confirmed by northern blot analysis of select genes. These initial results have identified a pool of equine intestinal epithelial genes that are differentially expressed following a two-hour ischemic event. In particular, genes indicative of deranged metabolic activity and genes potentially involved in early repair events were identified, and will be the focus of future studies on mechanisms of epithelial repair that may have relevance to enhancing recovery of equine colic patients.

ABSTRACT #291

PRELIMINARY EVALUATION OF THE ROLE OF CLOSTRIDIUM DIFFICILE IN DUODENITIS PROXIMAL JEJUNITIS IN HORSES. Luis G. Arroyo, Henry Staempfli; Joyce D. Rousseau; J. Scott Weese; Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Duodenitis proximal enteritis (DPJ) is an acute sporadic syndrome in horses characterized by inflammation and edema of the duodenum and jejunum. The cause of this disease remains unknown, however, clostridia, Salmonella spp and mycotoxins have been suggested as being the initiating cause. *Clostridium difficile* is an important cause of enterocolitis in horses and while colitis in the most commonly reported presentation, this organism is known to have an affinity for the small intestine and its role in DPJ requires further scrutiny.

This preliminary study evaluated the role of C. difficile in DPJ. Nasogastric reflux was collected at the time of hospitalization from five horses with clinically diagnosed DPJ and six horses with nasogastric reflux due to other small intestinal lesions. Selective enrichment culture was performed by inoculating 2ml of nasogastric reflux into a capped test tube with cycloserine-cefoxitin-fructose broth and incubated at 37°C for one week. Broth cultures were subcultured onto blood agar plates and incubated at 37°C in an anaerobic chamber for 48 hours.

Clostridium difficile was isolated from 5/5 horses with DPJ but 0/6 horses with nasogastric reflux of other etiologies. Toxins A and or B were detected in 4/5 isolates using a commercial immunoassay. Three horses with DPJ were euthanized due to the severity of the disease while the remaining two cases fully recovered. Histological changes in the small intestine of horses succumbing to DPJ included sloughing of the villus epithelium, submucosal edema, fibrinoid necrosis of vessels walls and thrombosis of blood vessels within the submucosa, muscularis and subserosa.

These changes, while somewhat non-specific, are consistent with lesions present in the small intestine of horses with *C. difficile enterocolitis*. Further investigation into the role of *C. difficile* in DPJ is indicated.

ABSTRACT #292

PHARMACOKINETICS AND PHARMACODYNAMICS OF PANTOPRAZOLE IN CLINICALLY NORMAL NEONATAL FOALS. Clare A. Ryan, L. Chris Sanchez, Steeve Giguère, Thomas Vickroy; University of Florida College of Veterinary Medicine, Gainesville, FL.

Proton pump inhibitors (PPIs) are a mainstay of treatment for acid-related ulceration in humans and horses. Currently, only an oral preparation of omeprazole is approved for use in horses. The purpose of the present study was to investigate the pharmacokinetics and pharmacodynamics of pantoprazole following IV or intragastric (IG) administration in healthy neonatal foals.

Seven healthy foals between six and 12 days of age at the start of the study were evaluated. Trials were conducted in a randomized block design such that each foal received each treatment with a 96-hour washout period between experiments. Treatments included 1) no drug administration, 2) intravenous pantoprazole (1.5 mg/kg), and 3) intragastric pantoprazole (1.5 mg/kg). Intragastric pH was recorded for 24 hours after drug administration for pharmacodynamic evaluation. Plasma pantoprazole concentrations were measured using HPLC. For each foal, the plasma concentration versus time data was analyzed based on noncompartmental pharmacokinetics. Pearson product moment correlations were used to determine the strength of association between the AUC of the plasma pantoprazole concentration versus time curve, and the AUC of the 24 h pH versus time curve. Mean hourly pH was calculated for each experiment. Time and treatment effects were examined with a repeated measures ANOVA followed by the Holm-Sidak method for multiple comparisons.

Plasma concentrations of pantoprazole were detectable within five minutes following IV or IG administration. There was no significant difference in elimination rate constant between IV and IG routes of administration. The time to peak plasma concentration and peak plasma concentration after pantoprazole administration were 0.25 hours and 4.08 µg/ml, respectively. Bioavailability of IG administered pantoprazole was 41%. Baseline mean hourly pH ranged from 1.5 to 6.1. Mean intragastric pH was not significantly different between the three groups for the first hour. There was a statistically significant increase in mean hourly pH relative to untreated foals during hours 2-24 after IV or IG pantoprazole administration.

Based on these data, intravenous administration of pantoprazole causes a significant increase in intragastric pH. Thus, the IV formulation of pantoprazole may provide a clinically useful alternative means of acid suppression in foals unable to tolerate

enteral administration of a proton pump inhibitor. However, further studies are required to investigate the use of this drug in critically ill patients. Intragastrically administered pantoprazole had poor bioavailability, but administration by this route also caused a significant, prolonged elevation of intragastric pH versus untreated foals.

ABSTRACT #293

SERUM OPSONIZATION CAPACITY, PHAGOCYTOSIS AND OXIDATIVE BURST ACTIVITY IN NEONATAL FOALS IN THE INTENSIVE CARE UNIT. RB Gardner, DV Nydam, JA Luna, M Bicalho, MB Matychak, MJBF Flaminio. Cornell University, Ithaca, NY.

Although the phagocytic capacity of healthy neonatal foals has been reported to be comparable or superior to that of adult horses, serum opsonization capacity has been shown to develop with age. Serum opsonization capacity may be further compromised as opsonins are consumed during sepsis. In human patients with sepsis, phagocytosis, oxidative burst activity and opsonization capacity may be decreased.

The purpose of this study was to identify and characterize phagocytosis, oxidative burst activity and serum opsonization capacity in sick foals admitted to the intensive care unit and to evaluate the temporal effects of plasma transfusion on serum opsonization capacity in sick foals when compared to healthy control foals with adequate colostrum consumption.

Blood samples were collected from 18 neonatal foals that were admitted to the intensive care unit at the Cornell University Hospital for Animals with suspected bacterial infections, and from 10 healthy control foals at four time points (at admission prior to administration of plasma or at birth prior to ingestion of colostrum, respectively; and at subsequent 24 hours, five and 10 days). Hospitalized foals were characterized as sick (sepsis score <11) or septic (sepsis score ≥ 11) and received between one and four liters of commercial fresh frozen plasma intravenously. Phagocytosis of propidium iodide labeled *Staphylococcus aureus* and oxidative burst activity indicated by the oxidation of dehydrorhodomine 123 were tested using flow cytometric analysis. Bacteria were opsonized with pooled serum from healthy adult horses. Autologous opsonization capacity was determined by testing individual foal sera and phagocytes from a healthy adult horse. Immunoglobulin G (IgG) and serum complement component 3 (C3) levels were determined using radial immunodiffusion. Data was analyzed using non-parametric techniques (e.g. Kruskal-Wallis, Wilcoxin Rank Sum) and alpha was set at p=0.05.

Phagocytosis and oxidative burst activities of hospitalized foals were inferior to that of control foals at 24 hours ($p<0.05$). Opsonization capacity of hospitalized foals was superior to that of control foals at birth/admission and on day 10 ($p<0.05$). IgG levels were similar between groups after colostrum ingestion or plasma administration and C3 levels were significantly higher in septic foals at the initial time point ($p<0.05$).

These results demonstrate that the phagocytic capacity and oxidative burst activity of sick or septic foal neutrophils is decreased, and that sick or septic foals that receive plasma transfusions have similar opsonization capacity, IgG levels and C3 concentrations, when compared to healthy foals following colostrum consumption.

ABSTRACT #294

PATTERN OF INFLAMMATORY MEDIATOR EXPRESSION AT THE DEVELOPMENTAL STAGE AND AT THE ONSET OF ACUTE LAMENESS IN THE BLACK WALNUT EXTRACT MODEL OF LAMINITIS. A. M. Cochran, Auburn Univ., Auburn, AL; S. J. Black, Univ. of Massachusetts, Amherst, MA; J. K. Belknap, The Ohio State Univ., Columbus, OH.

Due to many similarities between the equine case at risk of laminitis and that described for human sepsis patients, we were interested to determine the pattern of inflammatory mediator expression at two early stages of laminitis with reference to that described in clinical cases and models of sepsis.

In this study, we used real time quantitative PCR (RT-qPCR) techniques to assess the expression levels of numerous cytokines and two cyclooxygenase (COX) isoforms at a developmental stage and acute clinical stage of laminitis using the black walnut extract (BWE) model of equine laminitis. Duplicate RT-qPCR reactions were performed on individual cDNA samples obtained from mRNA purified from laminar tissue harvested from: 1) horses three hours after nasogastric (NG) administration of water (developmental control group, n=5), 2) horses 10 hours following NG administration of water (acute lameness control group), 3) horses at a well-described developmental stage following NG administration of BWE (onset of leucopenia, n=5), and 4) horses at the onset of clinical lameness following NG administration of BWE (n=5). Statistical analysis (ANOVA) was performed following log transformation of the data (results presented in Table 1).

	IL-1	IL-4	IL-6	IL-8	IL-10	IL-12	TNF-	COX-1	COX-2
Developmental Period	36* (+)	12* (+)	1034* (+)	21* (+)	3* (-)	3* (+)	1.5 (+)	1	11* (+)
Acute Lameness	10* (+)	2 (+)	668* (+)	7* (+)	1	2 (+)	1	2 (-)	12* (+)

Table 1: Fold increase (+) or decrease (-) in laminar mRNA levels of BWE vs. control horses.
*** indicates significant difference vs. control group (P<0.05)**

Although results indicate marked tissue inflammation as described in sepsis cases and models, the lack of TNF-α upregulation and the downregulation of the "anti-inflammatory cytokine" IL-10 are not consistent with the cytokine expression profile characteristic of sepsis. Additionally, when mRNA levels are compared at the two different time points, it appears that the peak changes in expression of proinflammatory cytokines occur early in the developmental stage of laminitis. These data demonstrate: 1) a unique pattern of inflammatory mediator expression in laminar tissue in early stages of BWE-induced laminitis, and 2) a need for aggressive therapeutic approaches towards inflammation in both the horse at risk and the horse in early clinical stages of laminitis.

ABSTRACT #295

EQUINE NEUTROPHIL ACTIVATION AND EXPRESSION OF CELL SURFACE DIFFERENTIATION ANTIGENS. J. Loftus, Univ. of Massachusetts, Amherst, MA; D. P. Lunn, Colorado State University, Ft. Collins, CO; J. K. Belknap, The Ohio State University, Columbus, OH; S. J. Black, Univ. of Massachusetts, Amherst, MA.

In this study we investigate equine blood neutrophils *in vitro* with a view toward developing a robust activation system and identifying markers of resting and activated cells. Resting neutrophils were isolated from ice-cold heparinized blood, at 4°C as follows: (i) by dextran (1.5% wt)-facilitated rouleaux of erythrocytes, (ii) recovery of leukocytes from plasma (250 x g, 10 min) (iii) selective pelleting of neutrophils and monocytes by centrifugation over Ficoll-Hypaque (400 x g, 20 min), (iv) selective banding of neutrophils by centrifugation of the resuspended pellet over 90% Percoll (60,000 x g, 40 min). The majority (>95%) of isolated cells had the characteristic nuclear morphology of polymorphonuclear leukocytes and no discernable intracellular reactive oxygen intermediates as

determined by staining with DCFH-DA dye (the fluorescent intensity of which is elevated in the presence of H_2O_2). Isolated resting neutrophils strongly expressed EqCD44 and EqMHC Class I, expressed EqCD11a/18 at an intermediate level, and weakly expressed EqCD13 as determined by staining with mAb. The resting neutrophils did not express EqMCH Class II or lymphocyte-associated differentiation antigens namely, EqCD4, EqCD5 or EqCD8. Resting equine blood monocytes/macrophages expressed EqMHC Class II as well as CD13, while resting equine blood lymphocytes did not express EqCD13. Purified equine blood neutrophils retained their resting phenotype when incubated for 2 hours at 37°C and at a density of $<10^6$ cells/ml medium in phenol red-free RPMI 1640 medium supplemented with 5 mg bovine serum albumin (BSA)/ml and in "Costar" culture-ware that was pre-coated with BSA. The neutrophils acquired an activated phenotype, defined below, when incubated at $<10^6$ cells/ml medium in culture-ware lacking the BSA coating, when incubated at 5×10^6 cells/ml medium in BSA-coated dishes, and when incubated at $<10^6$ cells/ml medium in BSA-coated dishes in medium supplemented with *E. coli* lipopolysaccharide serotype 026.B6 (LPS; 100ng/ml medium) and 5% equine serum. Equine serum activated the neutrophils only to a minor degree in the absence of LPS, whereas activation of equine neutrophils by LPS was significantly reduced in the absence of serum (consistent with a role for serum LPS-binding protein). Irrespective of the method of activation, activated equine neutrophils exhibited: 1) uniform, intense staining with DCFH-DA dye indicating that the cells had mounted a respiratory burst, and 2) an elevated level of the aminopeptidase EqCD13 in the cytoplasm and on the cell surface in approximately 60% of the cells resulting in clear biphasic staining of the population. The distribution of EqCD13 was punctate on resting and activated neutrophils, suggesting a raft-distribution; activation was associated with coalescing of the putative EqCD13+ rafts into larger aggregates.

We conclude that the level of expression of EqCD13, as well as staining with DCFH-DA can be used to evaluate equine neutrophil activation.

ABSTRACT #296

DIFFERENTIAL GENE EXPRESSION IN INNATE IMMUNE RESONSES TO *RHODOCOCCUS EQUI* INFECTION IN FOALS AND ADULT HORSES. J.L. Watson, K.A. Jackson, K.L. Moyer, W.D. Wilson , S.K. Hietala. Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA.

The objective of this study was to compare the innate immune response of foals to the intracellular pathogen *R. equi* with that of their adult Dams. Two in vitro cell culture systems were used. Primary alveolar macrophages and dendritic cells (DCs), differentiated from peripheral blood mononuclear progenitors, were infected with a virulent strain of *R. equi* (33701+) and its isogenic, avirulent plasmid-cured pair (33701-). Total RNA was reverse transcribed and real time RT-PCR (TaqMan®) was used to quantify mRNA transcripts for pro-inflammatory and immunoregulatory cytokines. Infection of alveolar macrophages with *R. equi* induced the up-regulation of IL-1β, IL-6, IL-8, IL12p40 and TNF-α. When compared to their adult dams, foals showed a significantly smaller magnitude up-regulation of IL-6 and IL-8 at six hours and a significantly larger magnitude up-regulation of IL-12p40 at 24 hours. The presence of bioactive TNF-α was confirmed by bioassay using WEHI-13VAR cells as early as six hours after infection. In contrast to the observed up-regulation of several cytokines, infection with *R. equi* resulted in a dramatic decrease in transcription of natural resistance-associated macrophage protein 1 (NRAMP1) in foals and adults. Infection of equine DCs induced variable upregulation of IL-1β, 1L-6, IL-8, IL-12p40 and TNF-α in foals and adults. Detection of bioactive TNF-α in DC culture supernatants six and 24 hours after infection confirmed secretion of this cytokine by *R. equi* infected DCs. Suppressive subtractive hybridization was performed using mRNA from *R. equi* infected and uninfected alveolar macrophages from foals and adults and 30 genes were found to be differentially expressed. Differentially expressed genes were from a number of gene families including chemokines, endosome/lysosome trafficking, SLAM family (activating receptors) and STATs (signal transducer and activator of transcription).

These data support the following conclusions:

1. Foal and adult alveolar macrophages respond to *R. equi* infection via enhanced transcription of both pro-inflammatory and immunoregulatory cytokines.
2. *R. equi* induces the down-regulation of NRAMP1 in both foal and adult alveolar macrophages. 3. Foal and adult DCs may respond to *R. equi* infection via enhanced transcription of both pro-inflammatory and immunoregulatory cytokines.
4. Numerous non-cytokine genes are involved in innate responses to *R. equi* infection.

ABSTRACT #297

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF PERIPHERAL BLOOD-DERIVED DENDRITIC CELLS FROM JUVENILE AND MATURE HORSES. BA Sponseller[1,2], DM Wong[1], SK Clark[2], DE Jones[3]. [1]Department of Veterinary Clinical Sciences, [2]Department of Veterinary Microbiology and Preventive Medicine, [3]Department of Veterinary Pathology, Iowa State University, Ames, IA.

Considerable gaps in knowledge regarding immune maturation in humans exist; however, it is well recognized that human childhood is characterized by increased susceptibility to infectious diseases, particularly those caused by intracellular pathogens. Recent studies suggest that antigen presenting cell function is an important factor that could, in part, explain juvenile vulnerability to disease.

Dendritic cells (DC) are a fundamental link between innate and adaptive immunity. They are present at the sites of antigen entry, migrate from peripheral sites to secondary lymphoid organs, and have a superior capacity to stimulate naïve T-cells. Subsequent to antigen encounter, DC are very effective at producing cytokines, especially Th1-trophic IL-12, a cytokine crucial for the initiation of cellular immune responses. Interestingly, age related differences in the capacity of IL-12 p70 synthesis have been observed where mononuclear cells from children show reduced ability to synthesize bioactive IL-12 p70.

We hypothesize that age-related differences in antigen presenting cell function may help explain foal susceptibility to respiratory pathogens in general, and the intracellular pathogen *Rhodococcus equi*, in particular. To test this hypothesis, we have initiated a comparative study of circulating mononuclear cells generally known to produce Il-12. We compared IL-12 p40 production of stimulated and non-stimulated peripheral blood mononuclear cells, monocyte-derived macrophages, and blood-derived DC obtained from juvenile and adult horses. In addition, we compared the phenotype of both macrophages and DC by flow cytometry and tested both cell subsets for their ability to promote the proliferation of heterologous lymphocytes in mixed lymphocyte reactions (MLR).

Our results confirm previously reported phenotypic differences in equine macrophages and DC. Moreover, differences in IL-12 synthetic capacity were observed among the different cell types. Most strikingly, lower levels of IL-12 p40 were detected in stimulated juvenile PBMC compared with adult PBMC. However, in vitro-derived juvenile DC were functionally capable of generating IL-12 p40 to the same level as adults. These results suggest foals may have a deficiency in the number and/or function of DC that predisposes them to development of disease caused by intracellular pathogens. Also, our data suggest that the in vivo functional deficit may be

overcome by propagating DC in vitro. More studies are needed to further characterize these differences.

ABSTRACT #298

EVALUATION OF FIVE COMMERCIALLY AVAILABLE ASSAYS FOR THE DIAGNOSIS OF FAILURE OF PASSIVE TRANSFER OF IMMUNITY IN FOALS. Steeve Giguère and Rachel Davis. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Sepsis is the leading cause of morbidity and mortality in newborn foals. Several studies have documented a positive correlation between failure of passive transfer of immunity (FPT) and bacterial sepsis in foals. FPT is typically defined as serum IgG concentrations of less than 400 mg/dl after 24 h of age whereas partial FPT is defined as serum IgG concentrations between 400 and 800 mg/dl. The prevalence of FPT in foals has ranged between 5 and 20%. The objective of this study was to assess the performance of five commercially available assays for the diagnosis of FPT and partial FPT in foals.

One hundred blood samples were collected at the time of admission from foals presented to the University of Florida Veterinary Medical Center (n=65) and at various times post-partum from clinically normal foals (n=35). Immunoglobulin G concentration in serum was assessed using zinc sulfate turbidity (Equi Z, VMRD), glutaraldehyde coagulation (Gammacheck E, Veterinary Dynamics), semiquantitative immunoassays (Snap, Idexx Laboratories; Midland 4 and 8 Quick Test Kits, Midland Bioproducts) and a quantitative immunoassay (DVM Stat, CAA). Two single radial immunodiffusion assays (VMRD and Triple J Farms) were used as gold standards. Sensitivity, specificity and accuracy were calculated and compared between assays.

The prevalence of FPT at serum concentration of IgG < 400 mg/dl and IgG < 800 mg/dl was 27% and 42%, respectively. For the detection of IgG < 400 mg/dl, sensitivity of the DVM Stat test (100%) was not significantly different from that of the Midland 4, Equi Z and Snap tests (89%). Specificity of the DVM Stat (97%) and Snap (93%) tests was significantly higher than that of the Equi Z (78%) and the Midland 4 (79%) tests. Similarly, accuracy of the DVM Stat (97%) and Snap (93%) tests was significantly higher than that of the Equi Z (82%) and the Midland 4 (81%) tests. For the detection of IgG < 800 mg/dl, sensitivity of the DVM Stat (98%), the Gammacheck E (93%), the Equi Z (81%), and the Snap (81%) tests were significantly higher than that of Midland 8 test (52%). Specificity of the Midland 8 (100%), the Snap (95%), and the DVM Stat (83%) tests was significantly higher than that of the Equi Z (57%) and Gammacheck E (59%) tests. Accuracy of the DVM Stat (89%) and Snap (89%) was significantly higher than that of the Equi Z (67%) and Gammacheck E (73%) tests.

The predictive value of a negative test is particularly important to ensure identification of most foals with FPT. At a prevalence of 15%, the predictive value of a negative test would be greater than 90% for each of the assays evaluated. For most assays, the predictive value of a positive test is much lower indicating the potential for false positives with these screening tests.

ABSTRACT #299

SEVERE ACUTE RHABDOMYOLYSIS IN FOUR HORSES ASSOCIATED WITH *STREPTOCOCCUS EQUI* SUBSPECIES *EQUI* INFECTION. BT Sponseller[1], SJ Valberg[2], BS Tennent-Brown[3], JH Foreman[3], P Kumar[4], JF Timoney[4]. [1]Department of Veterinary Clinical Sciences, Iowa State University, Ames, IA. [2]Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN. [3]College of Veterinary Medicine, University of Illinois, Urbana, IL. [4]Gluck Equine Research Center, University of Kentucky, Lexington, KY.

Streptococcus equi subsp. *equi* (*S. equi*) typically causes upper respiratory tract infection and purulent lymphadenitis in susceptible horses and may be accompanied by numerous complications. Recently, sequelae affecting the muscular system have been reported in horses infected with, exposed to or vaccinated with the organism, including an immune mediated myositis associated with malaise and lumbar and gluteal muscle atrophy, as well as an often fatal infarctive myopathy resembling Henoch-Schönlein purpura. In this report, we describe a further complication of *S. equi* infection characterized by acute severe rhabdomyolysis without clinical evidence of muscle atrophy or infarction.

Four Quarterhorses, one to seven years of age, with submandibular lymphadenopathy and guttural pouch empyema culturing positive for *S. equi* developed a stiff gait that proceeded to recumbency in three horses. Once recumbent, the three horses deteriorated rapidly despite aggressive antimicrobial and anti-inflammatory treatment, necessitating euthanasia within 24-48 hours. One of these horses had been vaccinated for strangles using an intranasal modified live vaccine (Pinnacle®) at four months of age. A fourth horse with unknown vaccination status remained standing and recovered completely after treatment with penicillin for 10 days followed by sulfamethoxazole-trimethoprim for 26 days. Common clinicopathologic findings included mature neutrophilia, hyperfibrinogenemia, marked elevations in creatine kinase (115,440-587,070 IU/l), elevations in aspartate aminotransferase (600 – 14,520 IU/l) and variable electrolyte derangements. Serum from three animals (including the survivor) was available for serologic testing. *S. equi* M protein titers were low except in the vaccinated horse. Serum antibody levels to a recently identified myosin binding protein (Se18.7) were high in all three horses.

Necropsy revealed large multifocal, pale, friable areas of muscle representing severe myonecrosis, most prominently in the gluteal, hamstring and epaxial muscles. Histopathologic evaluation of affected muscle revealed profound acute myonecrosis with histiocytic or lymphohistiocytic myositis. Periodic acid Schiff staining of unaffected muscle of two horses showed no abnormalities. *S. equi* was identified in affected muscle of the euthanized horses using immunofluorescent stains for both Lancefield group C carbohydrate and *S. equi* M protein. Rhabdomyolysis was attributed to either an inflammatory cascade resembling streptococcal toxic shock or potentially direct toxic effects of *S. equi* within muscle tissue.

ABSTRACT #300

WEST NILE VIRUS TITERS IN A SEMI-FERAL HERD OF PONIES IN PENNSYLVANIA. Sue M. McDonnell, Elkanah H. Grogan, Amy L. Glaser[1], and Pamela A. Wilkins. University of Pennsylvania School of Veterinary Medicine New Bolton Center, Kennett Square, PA and [1]Veterinary Diagnostic Laboratory, Cornell University, Ithaca, NY.

West Nile Virus (WNV) first appeared in horses in Pennsylvania in 2000. Natural exposure and antibody titer responses to WNV within a stable population of equids over time have not been previously reported. A herd of Shetland-type ponies (n=50-65), maintained continuously at pasture in Chester County Pennsylvania since 1994, with no known WNV disease and not vaccinated against WNV, was studied. Serum samples were obtained in Autumn of 2003 after mosquito season for this geographic region (A03), Spring of 2004 before mosquito season (S04) and Autumn of 2004 after mosquito season (A04). Microtiter serum neutralization testing (MSNT) was performed by the Veterinary Diagnostic Laboratory at Cornell University in S04 and A04. MSNT titers were considered positive at 1:16 or greater. ELISA was performed by the Pennsylvania Animal Diagnostic Laboratory System (PADLS) in A03, S04 and A04. ELISA titers were reported as either positive or negative at 1:400 only.

In A03, 23/61 (38%) ponies were ELISA positive, including 2/12 (17%) foals. In S04, 19/47 (40%) ponies were MSNT positive and 8/34 (24%) ELISA positive. Of yearlings, 3/12 (25%) were MSNT positive, including the 2 ELISA positive A03 foals. Of ponies $\geq$ 2 years old (mature), 16/33 (48%) were MSNT positive, including 14 ELISA positive in A03. Of the 11 mature A03 ELISA seropositive ponies available for retesting in S04, 7 (64%) remained ELISA positive. In A04, 40 of 63 (63%) ponies were MSNT positive while nine of 63 (14%) were ELISA positive. A04 MSNT positives included 5/14 (36%) foals, 6/13 (46%) yearlings and 29/36 (81%) mature ponies, while ELISA positives included 0/14 foals, 1/13 (8%) yearlings and 8/36 (22%) mature ponies. For 30 tested on all occasions and both assays, results for seven (23%) were consistently positive and for eight (27%) consistently negative. There were no instances of an MSNT seropositive pony becoming MSNT seronegative (6-month period); there were 11 instances of ELISA seropositive ponies that became ELISA seronegative (1-year period). For the A04 sample set, 31 ponies had discrepant MSNT and ELISA results, all with MSNT positive and ELISA negative.

In Pennsylvania, 2003 was the peak year so far for equine clinical cases of WNV, with > 500 equine cases reported. For 2004, Pennsylvania had only nine reported cases with none in Chester County. For this herd, MSNT positives increased from 40% before to 63% after the 2004 mosquito season. Of 43 yearlings and mature ponies tested both in S04 and A04, 12 of 20 (60%) that were MSNT negative in S04 were MSNT positive in A04. These data demonstrate persistence of MSNT positivity, and increased herd seropositivity due to natural exposure, over a 6-month period during mosquito season in a WNV endemic area. Animals with positive ELISA titers may become seronegative during periods of no mosquito activity and remain seronegative during the next mosquito season, possibly related to the large titer cut-off.

ABSTRACT #301

SUCCESSFUL TREATMENT OF *COCCIDIOIDES IMMITIS* PNEUMONIA WITH FLUCONAZOLE IN TWO HORSES**.** J.C. Higgins[1,2], G. S. Leith[2], D. Pappagianis[3], N. Pusterla[4]. [1]Loomis Basin Large Animal Services, Loomis, CA, [2]Arizona Equine Medical and Surgical Center, Gilbert, AZ, [3]Department of Medical Microbiology and Immunology, School of Medicine, [4]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis.

Coccidioidomycosis in horses has generally proven to be a devastating disease once the animal begins showing clinical signs. In the literature, equine cases that have presented with signs of disease have commonly had a fatal outcome despite attempted treatment with various antifungal agents. Only one successful treatment of coccidioidomycosis osteomyelitis with itraconazole in the horse has been reported. Oral fluconazole has been shown to be a highly bioavailable azole antifungal compound with a high volume of distribution and a long elimination half-life in the horse, but has not been reported for the treatment of equine coccidioidomycosis. Because of its long half-life, fluconazole may show a cumulative effect and therefore warrants therapeutic drug monitoring throughout the course of treatment to adjust the dose if necessary. The present report describes two cases of pulmonary coccidioidomycosis that were successfully treated with fluconazole and outlines the therapeutic drug monitoring procedure that was followed.

Two adult geldings, ages four and 20, were each diagnosed with pulmonary coccidioidomycosis based on positive serologic titers, clinical signs compatible with infection, radiographic evidence, hematological abnormalities consistent with ongoing inflammation, as well as culture of the organism from the transtracheal wash of one of the horses. Both horses received a loading dose of 14mg/kg fluconazole PO once, followed by 5mg/kg PO SID for five to six months. In both horses, clinical signs diminished within two weeks, but hematological abnormalities persisted for up to four months. Both horses showed a decreasing titer throughout treatment and have continued to decrease at one year past the end of treatment. Plasma levels of fluconazole were analyzed and found to be within the therapeutic range established for *C. immitis* in human beings.

In conclusion, the results of these two cases suggest that pulmonary coccidioidomycosis may be successfully treated with fluconazole.

ABSTRACT #302

COMPARATIVE ANALYSIS OF CYTOKINE SIGNATURES IN THE CEREBROSPINAL FLUID OF HEALTHY HORSES AND HORSES WITH SELECTED NEUROLOGICAL DISORDERS. N. Pusterla[1], C.M. Leutenegger[1], P.A. Conrad[2], B.C. Barr[3], W.D. Wilson[1]. [1]Department of Medicine and Epidemiology, [2]Department of Pathology, Microbiology and Immunology, [3]California Animal Healthy and Food Safety Laboratory System, School of Veterinary Medicine, University of California, Davis, CA.

The goal of this study was to determine the gene transcription of selected cytokines in the cerebrospinal fluid (CSF) of healthy horses and horses with cervical stenotic myelopathy (CSM), West Nile virus (WNV) encephalitis and spinal cord trauma using TaqMan PCR. The study material consisted of CSF collected at necropsy from 30 horses (12 healthy horses, eight horses with confirmed CSM, four horses with confirmed WNV encephalitis and 6 horses with confirmed spinal cord trauma). Total RNA was extracted from the spinal fluid nucleated cells, transcribed to complementary DNA and assayed for equine GAPDH, TNF-α, IFN-γ, IL-2, IL-6, Il-8, IL-10, iNOS and TGF-β by TaqMan PCR. Final quantitation of cytokine transcription was done using the comparative C_T method and was reported as relative transcription or the n-fold difference relative to a calibrator (weakest value across all target genes for normal CSF samples).

The housekeeping gene GAPDH was expressed in all samples, reflecting a successful RNA extraction and cDNA transcription. The cytokine profiles expressed by nucleated cells from the spinal fluid of healthy horses was a balance between pro-inflammatory (TNF-α), anti-inflammatory (IL-6, IL-10) and Th1 (IL-2, IFN-γ) cytokines and growth factor (TGF-β). Horses with CSM expressed elevated TNF-α, absent IL-6 and normal TGF-β, IL-10 and IL-2. The cytokine profile of horses with WNV was characterized by high IL-6, absent TNF-α. Horses with spinal trauma expressed elevated IL-6 and normal IFN-γ and TGF-β. Interleukin-8 was not detected in any CSF sample and iNOS was only expressed by one healthy horse.

Despite the small number of samples for each group, our preliminary results seem to suggest distinct gene signatures expressed by nucleated cells in the CSF of healthy horses and horses with different inflammatory neurological disorders. Cytokine profiles could in the future contribute to the differential diagnosis in situations where conventional laboratory parameters fail to provide diagnostic clues.

ABSTRACT #303

EFFECT OF EXERCISE AND ORAL (N-3) FATTY ACID-ANTIOXIDANT SUPPLEMENTATION ON BLOOD OXIDANT MARKERS AND ERYTHROCYTE MEMBRANE FLUIDITY IN HORSES. B. de Moffarts[1], K. Portier[2], N. Kirschvink[1], J Coudert[3], N. Fellmann[3], E. Van Erck[1], C. Motta[4], J. Pincemail[5], T. Art[1]and P. Lekeux[1]. [1]Department for Functional Sciences, Fac. Vet. Med., University of Liège, Belgium, ²MCU, Equine Department, National Vet. School of Lyon, [3]Laboratory of Sport Physiology and Biology, Fac. of Med., University of Auvergne, [4] CHU Pontchaillou, Laboratory of Biochemistry, Rennes, France, [5]Probiox S.A., Centre Hospitalier Universitaire, University of Liège, Belgium.

The aim of this study was to assess the effect of exercise and oral antioxidant supplementation enriched in (n-3) Fatty Acids on

« erythrocyte membrane fluidity » (EMF), (n-3)/(n-6) fatty acids ratio and« oxidant markers » in eventing horses.

Twelve healthy and regularly trained horses were randomly divided in two groups; group 1 received during four weeks an oral antioxidant cocktail enriched with (n-3) fatty acids , whereas group 2 was placebo-treated. At the end of the treatment period, all horses performed a standardized exercise test (SET) under field conditions. Venous blood was sampled before starting the treatment (T0), immediately before (R) as well as 15 minutes (E15') and 24 hours (E24h) after the SET. Beside assessment of the EMF determined by electron spin resonance using the relaxation-contraction time (Tc inversely proportional to EMF) and the determination at T0 and at R of the (n-3)/(n-6) fatty acids ratio in plasma and in erythrocyte membrane, the following oxidant markers were determined at all time points: glutathione peroxidase (GPx), superoxide dismutase (SOD), uric acid (UA), antioxidant capacity of water-soluble (ACW) and liposoluble (ACL) plasma components, lipid peroxides (Pool), oxidized proteins (oxProt), copper (Cu), zinc (Zn).

The SET induced a significant ($p<0.05$) increase of Tc (3.72±0.12 versus [vs] 5.05±0.45 ns) (R vs E24h) and a significant increase of UA (2±0.85 vs 6.9±1.2 $mg.L^{-1}$), ACW (52.5±13 vs 98.2±23 $nmoleqAA.mL^{-1}$) (R vs E15') and oxProt (3.79±0.4 vs 5.33±0.5 $10^{-2}nmol.mgprot^{-1}$) (R versus E24h) in both groups. In placebo-treated horses (group 2), GPx significantly decreased (242±13 vs 223±12 IU/gHb) (T0 versus R), whereas the Cu/Zn ratio (1.9±0.12 vs 2.28±0.13) (T0 versus R) as well as Tc (3.83±0.18 vs 4.56±0.45 ns) (R versus E15') significantly increased, which was not the case for horses receiving the antioxidant cocktail. The evolution of the (n-3)/(n-6) fatty acids ratio in plasma (delta R-T0: 5.7±1.3 vs –8.8±3.7 %) (group1 vs group2) and in erythrocyte membrane (delta R-T0: 6±0.4 vs 1.4±0.7 %) (group1 vs group2) was significant.

In conclusion, exercise induced an oxidant imbalance and a decrease of EMF in horses, which could both be modulated by an orally administrated cocktail composed by natural antioxidants and (n-3) fatty acids.

ABSTRACT #304

DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO CHANGES IN MAGNESIUM INTAKE IN THE HORSE: A MEASURE OF INTRACELLULAR MAGNESIUM? Maureen Wichtel[1], Stephanie Power[2], Gary Quamme[3], Lawrence Hale[2] and Jeffrey Wichtel[1]. [1]Atlantic Veterinary College and [2]Department of Biology, University of Prince Edward Island, Charlottetown, PEI, Canada; [3]Department of Medicine, University of British Columbia, Vancouver, BC, Canada.

We wished to test whether gene expression in equine mononuclear cells responds to changes in Mg intake, and to evaluate differential gene expression as a potential indicator of intracellular Mg. Six adult horses were maintained in tie stalls, fed low-Mg Timothy hay, and randomly assigned to one of two experimental treatments and three experimental periods of 14 days each, arranged in a crossover design. Treatments were: Low-Mg (sham treatment with molasses) and High-Mg (30g MgO PO SID in molasses). Blood and spot urine samples (via catheter) were obtained on days 8, 11 and 14 of each period. Transcripts of two genes, MRG1 and N33, were measured in mononuclear cells using real-time RT-PCR using equine β-actin for normalization.

Mean plasma Mg, urinary Mg clearance and MRG1 mRNA level differed in response to changes in Mg intake (Fig. 1).

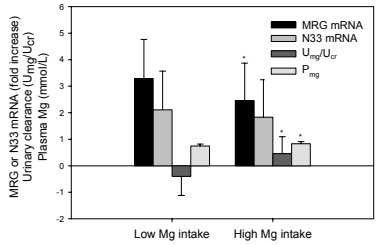

Figure 1. Mononuclear cell expression of MRG1 and N33 mRNA, urinary clearance of Mg (all log-transformed), and plasma Mg concentration, in response to Mg intake. * denotes significance ($P<0.05$).

Of these, clearance was most closely related to Mg intake ($R^2=0.33$). MRG1 mRNA increases in response to decreasing Mg intake in the horse, but individual variation in basal mRNA precludes the use of a single mononuclear cell sample as a clinical indicator of intracellular Mg status. We are investigating transcripts of other genes that decrease in response to decreasing Mg, for use as an alternative to, or in combination with, the MRG1 gene.

ABSTRACT #305

ROUTE OF CARBOHYDRATE ADMINISTRATION AFFECTS POSTEXERCISE MUSCLE GLYCOGEN STORAGE RATE IN HORSES. R. Geor, L. Larsen, L. Waterfall, L. Stewart-Hunt, S. Pratt and J. McCutcheon. Ontario Veterinary College, University of Guelph, Guelph, ON Canada.

When compared to humans, horses are slow to replenish muscle glycogen stores after glycogen-depleting exercise, with as much as 48-72 hours required for complete repletion even when high carbohydrate meals or supplements are provided. In the present study, we tested the hypothesis that glucose delivery from the gastrointestinal tract limits the rate of muscle glycogen storage in horses when carbohydrate is administered during the early postexercise period. Specifically, we predicted that muscle glycogen storage would be enhanced when glucose was administered IV but not when an equivalent glucose dose was given orally. In a randomized crossover design, seven mature horses completed a two-hour bout of treadmill exercise on three occasions to deplete muscle glycogen by ~50%. After exercise horses received: 1) an intravenous glucose infusion (IV; 0.5 g/kg bwt per hour for 6 hours), 2) oral glucose boluses (OR; 1 g/kg bwt at 0, 2 and 4 hours post-exercise), or 3) no glucose supplementation (CON). Blood samples for measurement of glucose and insulin concentrations were collected before exercise and during the 6-h treatment period. Muscle biopsies (middle gluteal m.) for measurement of muscle glycogen content and glycogen synthase (GS) activity were taken before and after exercise and at 3 and 6 hours of recovery. Statistical analysis was performed by two-way ANOVA with repeated measures ($P<0.05$). Data are presented as means ± SD.

The average glucose concentration during the 6-h treatment period in CON, OR and IV were, respectively, 4.4 ± 0.5, 6.5 ± 0.9 and 15.4 ± 3.0 mmol/L. Plasma glucose concentrations were significantly higher in IV and OR than in CON throughout treatment. However, the increase in plasma glucose during IV was more than twofold greater than during the OR treatment. The average serum immunoreactive insulin responses of the IV (75 ± 19 μU/ml) and OR (35.2 ± 10.1 μU/ml) treatments were also significantly greater than that of the CON (7.2 ± 2.1 μU/ml) treatment. Muscle glycogen concentrations after exercise were not different amongst the three treatments (IV, 254 ± 31; OR, 249 ± 26; CON, 230 ± 30 mmol/kg dry wt). However, glycogen storage rates were significantly higher in IV than in CON and OR during the first 3 h (IV, 14.6 ± 5.5; CON, -1.2 ± 2.1; OR, -4.0 ± 3.9 mmol/kg dw/h) and second 3 h (IV, 32.6 ± 9.9; CON, 7.9 ± 4.0; OR, 17.9 ± 6.2 mmol/kg dw/h) of recovery.

Therefore, muscle glycogen concentration was significantly higher in IV than in OR and CON at 6 hours of recovery (IV, 396 ± 29; O, 287 ± 23; CON, 250 ± 35 mmol/kg dw). GS activity was not affected by treatment. In conclusion, intravenous glucose (3 g/kg over 6 hours), but not the equivalent glucose dose administered *per os,* increased the rate of postexercise muscle glycogen storage in horses. These findings suggest that intestinal glucose uptake and/or hepatic metabolism of absorbed glucose may limit the rate of post-exercise muscle glycogen storage in horses when supplemental carbohydrate is provided via the oral route.

ABSTRACT #306

REPEATABILITY OF INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS FROM MINIMAL MODEL ANALYSIS OF THE FREQUENTLY-SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TEST IN HORSES. R. Geor, R. Li, L. Waterfall, L. Larsen, L. Stewart-Hunt, J. McCutcheon. Ontario Veterinary College, University of Guelph, Guelph, ON Canada.

Minimal model analysis for estimation of insulin sensitivity (Si), glucose effectiveness (Sg) and the acute (0-10 min) insulin response to glucose (AIRg) has recently been used in equine studies of glucose metabolism. However, to date the interday variance in measurements obtained from minimal model analysis has not been reported. Thus, the primary objective of this study was to determine the variance in minimal model estimates (Si, Sg, and AIRg) derived from an insulin-modified frequently-sampled intravenous glucose tolerance test (FSIGT) in horses. A second objective was to evaluate the variability of minimal model estimates from a reduced sample FSIGT protocol. Eight mature Standardbred horses underwent three FSIGT procedures over a seven-day period under conditions of fixed diet and limited physical activity. After collection of baseline blood samples, an IV bolus of glucose (0.3 g/kg bwt) was given, and insulin (30 μU/kg bwt IV) was injected at 20 minutes after glucose administration. Further blood samples for measurement of plasma glucose and insulin concentrations were collected after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 min. Minimal model analysis was applied to the full 30-sample data set and a reduced 12-sample data subset (0, 2, 4, 10, 19, 22, 30, 40, 50, 70, 90, 180 min). Intraclass correlation coefficients (ICC) and coefficients of variation (CV) were calculated to assess repeatability. Paired Student's *t* tests were performed to compare full and reduced sample protocols. Data are reported as mean ± SD.

Mean Si from the 30-sample data set was $1.78 \pm 0.78 \times 10^{-4}$ $min^{-1} \cdot (mU/L)^{-1}$ with an ICC of 0.91 and average interday CV of 13.4 ± 5.6% (range 6-26%). Mean Sg and AIRg were, respectively, 1.77 ± 0.42 x 10^{-2} min^{-1} and 234.9 ± 68.8 $mU \cdot min \cdot L^{-1}$. Corresponding interday CV and ICC were 17.8 ± 6.5% (range 5-24%) and 0.55 for Sg, and 16.1 ± 8.4% (range 6-28%) and 0.64 for AIRg. Mean Si calculated from the 12-sample data set ($1.18 \pm 0.69 \times 10^{-4}$ $min^{-1} \cdot (mU/L)^{-1}$) was not significantly different (P = 0.16) from the mean calculated from the full data set. However, the mean CV from the reduced data set was greater than that calculated from the full data set (36.8 ± 14.5% vs. 13.4 ± 5.6%) and the ICC was lower (0.43 vs. 0.91). Mean values and average interday CV for Sg and AIRg calculated from the 12-sample data set were similar to corresponding values derived from the complete data set. However, the ICC for repeat determinations of Sg was lower for the 12-sample (0.22) when compared to the 30-sample (0.55) data set. It is concluded that minimal model analysis of the insulin-modified FSIGT provides repeatable estimates of insulin sensitivity and glucose dynamics in healthy horses. However, the repeatability of Si estimates is greatly reduced when the FSIGT protocol is simplified by decreasing the number of samples to 12.

ABSTRACT #307

COMPARISON OF INSULIN SENSITIVITY IN HORSES DERIVED BY THE EUGLYCEMIC CLAMP, MINIMAL MODEL METHOD OR ORAL GLUCOSE TOLERANCE TEST. S. Pratt, R. Geor, J. McCutcheon and J. Cant. University of Guelph, Guelph, Ontario, Canada.

In human medicine, two methods are recommended for assessment of tissue sensitivity to insulin, the euglycemic clamp (EC) and minimal model analysis of an insulin-modified frequently-sampled intravenous glucose tolerance test (FSIGT). Both the EC (Rijnen et al. 2003) and minimal model (Hoffman et al. 2003) approaches have been applied in horses but the relationship between insulin sensitivity measures obtained with these procedures has not been reported. Thus, the primary objective of this study was to determine the relationship between values for insulin sensitivity measured with the EC and FSIGT in a cohort (n=16; seven geldings, nine mares) of clinically healthy horses. A second objective was to determine which measure(s) of glucose and insulin obtained at rest or during an oral glucose tolerance test (OGTT) is the strongest correlate of insulin sensitivity measured directly using the EC. The EC, FSIGT and OGTT were administered in all horses with a minimum of three days between procedures. The ratio of glucose infusion rate to mean serum insulin (M/I) was the measure of insulin sensitivity in the EC. For the FSIGT, insulin sensitivity (Si) was quantified by the variation in glucose disappearance rate due to unitary increases in insulin. Minimal model analysis also yielded AIRg, the acute (0-10 min) insulin response to glucose, and an estimate of glucose-mediated glucose disposal (Sg). Area under the glucose (AUCg) and insulin (AUCin) curves, the product of AUCg and AUCin, and the sum of the insulin to glucose ratios (Sum I/G) were calculated from the OGTT. From resting plasma glucose and insulin concentrations, the Quantitative Insulin Sensitivity Check Index (QUICKI; 1/[log glucose + log insulin]) and Homeostasis Model Assessment (HOMA = glucose x insulin/22.5) were calculated. Pearson correlation coefficients were calculated to describe the relationships between M/I and: a) Si, Sg and AIRg, b) OGTT-derived measures, and c) QUICKI and HOMA. The data are presented as means and 95% confidence interval (CI).

A technical error precluded determination of M/I in one horse. Accordingly, comparisons are based on data from 15 horses. Mean M/I and Si were, respectively, 2.03 (1.70, 2.37) x 10^{-2} $mg \cdot kg \cdot^{-1} min^{-1}$ per $\mu U \cdot ml^{-1}$ and 2.93 (2.26, 3.60) x 10^{-4} $min^{-1} \cdot (\mu U/ml)^{-1}$. There were strong correlations between Si and M/I (r = 0.87, 95% CI: 0.63, 0.95; p < 0.001) and between log transformed AIRg and M/I (r = -0.80, 95% CI: -0.93, -0.49; P < 0.001). However, Sg was not significantly correlated with M/I (r = -0.33, P = 0.22). Similarly, QUICKI (r = 0.08) and HOMA (r = 0.12) were poorly correlated with M/I. Of the OGTT-derived variables, AUCin (r = -0.57, P = 0.03) and Sum I/G (r = -0.55, P = 0.03) were most strongly correlated with M/I. Given the strong correlation between M/I and Si, both the EC and minimal model/FSIGT procedures appear useful for assessment of insulin sensitivity in horses. However, calculations based on resting or OGTT-derived plasma glucose and insulin may not be suitable for documentation of insulin sensitivity.

ABSTRACT #308

BLOOD PRESSURE MONITORING IN NEONATAL FOALS: ASSESSMENT OF TWO INDIRECT OSCILLOMETRIC MONITORS, EFFECT OF SITE OF CUFF PLACEMENT AND THE RELATIONSHIP OF PERIPHERAL ARTERIAL BLOOD PRESSURE TO CARDIAC OUTPUT. Steeve Giguère, Harvey Knowles, Alexander Valverde, Eric Bucki. College of Veterinary Medicine, University of Florida, Gainesville, FL.

Arterial blood pressure monitoring is routine practice in equine neonatal intensive care units, allowing recognition of some cardiovascular derangements and titration of therapy with

intravenous fluids, vasopressors, and inotropic agents. However, blood flow rather than blood pressure is the driving force for tissue perfusion and measurement of cardiac output is required for calculation of global oxygen delivery and consumption. Because measurement of cardiac output is currently considered impractical for routine use in foals, indirect arterial blood pressure is commonly used as an indication for potential blood flow. The objectives of this study were to assess the accuracies of two automated indirect oscillometric monitors for measurement of mean arterial pressure (MAP) in foals, to determine the optimal site of cuff placement for MAP monitoring, and to determine the relationship between arterial blood pressure and cardiac output in anesthetized foals. Ten neonatal foals were anesthetized and instrumented with a catheter in the metatarsal artery for direct MAP monitoring and measurement of cardiac output by lithium dilution. Concurrent MAP measurements were obtained with Cardell and Dinamap oscillometric monitors with cuffs placed at 3 different sites (coccygeal, metatarsal and median arteries). Blood pressure was manipulated by varying the depth of anesthesia and administration of dobutamine or phenylephrine.

There was a statistically significant ($P = 0.025$) interaction between the type of monitor and cuff placement site. With the Cardell monitor, placement of the cuff over the coccygeal artery resulted in a significantly lower bias than placement over the median or dorsal metatarsal artery ($P < 0.0001$ and $P = 0.0149$, respectively). There was no significant difference in bias with cuff placement site using the Dinamap monitor. There was no significant difference in bias between the Cardell and Dinamap monitor with cuff placement over the coccygeal or metatarsal artery. Cuff placement over the median artery resulted in a significantly lower bias with the Dinamap than with the Cardell monitor ($P = 0.0007$). At constant end-tidal isoflurane concentration, systemic vascular resistance was significantly ($P < 0.001$) higher in foals given phenylephrine (867 ± 49 dynes·s·cm^{-5}) than in the same foals during administration of dobutamine (418 ± 36 dynes·s·cm^{-5}). The correlation between cardiac index and MAP was significantly ($P = 0.025$) higher when data collected during phenylephrine administration was excluded ($r = 0.77$), compared to that obtained using the complete data set ($r = 0.47$). Indirect oscillometry using a cuff placed over the coccygeal artery or dorsal metatarsal artery is an acceptable method for measuring MAP in foals. Arterial blood pressure is a poor indicator of blood flow in anesthetized foals when vascular resistance is altered.

ABSTRACT #309

ROMIFIDINE-MORPHINE COMBINATION FOR SEDATION OF FOALS: CLINICAL ASSESSMENT OF TWO PROTOCOLS FOR ADMINISTRATION. C Robert, S Jacquet, A Bertin, JM Denoix, C Desbois, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.

The combined administration of romifidine and morphine has been shown to be a cheap and effective method for sedation-analgesia in horses. To prevent excitation, the morphine is usually administered after α2-agonist induced sedation. The purpose of the present study is to compare some behavioural and physiological effects of the intravenous administration of romifidine (Sedivet^{R}) and morphine injected either simultaneously or five minutes apart in foals.

As part of a large research program on osteochondrosis, 41 Anglo-Arabian foals were sedated for routine radiographic examination. Mean age was 170 ± 23 days (mean ± SD). The foals were divided into two groups balanced for age, weight and diet (weaned or not, low or high dietary level) and sedated blindly according to the following protocols:

group	t_o : 1st injection	+ 5 minutes : 2nd injection
1	romifidine 44 μg/kg + morphine HCl 0,1 mg/kg	NaCl 0,9%
2	romifidine 44 μg/kg + NaCl 0,9%	morphine HCl 0,1 mg/kg

The heart rate was recorded at 1-minute intervals using a heart rate monitor. Physiologic and behavioural observations including respiratory rate, presence of arrhythmia, inspiratory noises, sweating, muscle stiffness, ataxia and intestinal motility, were conducted before drug injection and six and 20 minutes after. Responses to visual, auditory and cutaneous stimulation were scored from 0 to three. Sedation was assessed subjectively and scored as unsatisfactory, moderate, good or excellent. All side effects observed were noted. Differences between the two protocols were evaluated using the Khi-2 test.

The two protocols resulted in a sedation good enough to realise all the radiographs (including the stifles). The foals demonstrated a significant decrease in mobility and in reactivity to external stimulation. Heart rate decreased in the 30 seconds following the first injection. The decrease seemed to be more important and quicker with romifidine alone, but the difference was not statistically significant. There was a marked decrease in intestinal motility that appeared earlier ($p<0.001$) in group 2. Usual undesirable side effects of α2-agonist - inspiratory noises, polypnea, ataxia, paradoxical reaction – were observed in both groups; they were statistically more frequent in group 2 ($p<0.01$). Conversely, foals in group 2 were easier to manipulate: the mean number of restraint techniques used (twitch, tail) and the manipulation score were significantly lower; the proportion of sedation judged excellent was higher ($p<0.005$).

This study demonstrates that romifidine and morphine could be administered simultaneously for sedation in foals. A unique intravenous injection is easier to realise and side effects associated to α2-agonists appear to be better controlled by morphine. But, the quality of sedation seems to be slightly better with two injections five minutes apart.

ABSTRACT #310

MEDICAL TREATMENT WITH MICONAZOLE IN FOUR CASES OF GUTTURAL POUCH MYCOSIS. A Giraudet, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France.

Guttural pouch mycosis is a well-known disease in horses that can lead to devastating consequences due to uncontrollable haemorrhage. Several surgical treatments have been described to treat successfully the disease. However, these treatments are sometimes expensive and may involve radiation exposure for the surgeons and for the clinical staff. Moreover, in the case of neurological damage, the neurologic deficits seldom fully recover after surgery. The purpose of this case series was to demonstrate the efficacy of a medical treatment for guttural pouch mycosis when surgery in not an option.

Four horses, three adult horses and one pony ranging in age from four to nine, were treated medically for guttural pouch mycosis. Two horses (Horses 1 and 2) were presented for mild unilateral bleeding. The other two horses (Horses 3 and 4) were presented only for dysphagia, but displayed other subtler neurological deficits. Horse 3 had a unilateral lesion, in Horse 4 both pouches were involved. None of these cases were surgical candidates, mainly for financial concerns. The owners were warned of the risk of life threatening haemorrhages, but elected for an attempt with a medical treatment. Treatment consisted in daily administration of miconazole in the affected pouch during one week, followed by every other day administration during the following two weeks. On Horse 3 and 4, twice a week administrations were performed for three more weeks. The administration were performed under endoscopic control, using a equine female urinary catheter, mild sedation was required. The pouches were endoscopicaly assessed prior treatment and after treatment except for Horse 1 that was not re-examined post treatment due resolution of clinical signs and lack of compliance of the owner. Horses 1 and 2 were treated with a diluted solution from the injectable form (70 mg of miconazole diluted with isotonic saline to reach a 10 ml volume for each treatment) the dosage was minimized due to the cost of the injectable form. Horses 3 and 4 were treated

with gynaecological preparation of miconazole (400 mg per treatment) administration of the ointment through the catheter was facilitated by warming the filled syringe in warm water. This molecule was chosen because of a good activity against *Candida* spp and *Aspergillus fumigatus*, the later being a very common cause of guttural pouch mycosis.

The mild bleeding (Horses 1 and 2) resumed during the first week of treatment and did not reoccur. The neurological signs (Horses 3 and 4) actually ceased eight and 12 weeks respectfully after the last treatment. All horses recovered, the shortest follow up on these cases being six months. This brief series demonstrate the possibility of a medical treatment for guttural pouch mycosis, particularly when surgery cannot be performed. More work should be done to evaluate the advantage of medical treatment when neurological lesions alone are present.

ABSTRACT #311

MEASUREMENT OF LAMINAR PERFUSION IN THE NORMAL HORSE USING DYNAMIC COMPUTED TOMOGRAPHY. Puchalski SM, Kruger EF, Wisner ER, Hornof WJ, Galuppo LD, University of California, Davis.

The purpose of this study was to evaluate a dynamic computed tomography technique to measure the perfusion and permeability of lamina in the normal horse.

Ten horses free from lameness were placed used in this study. A complete distal extremity radiographic examination, performed the day of the CT exam, which did not demonstrate any significant abnormalities, was available for each horse. Induction and anesthesia was maintained using a routine protocol. Each horse was then placed in lateral recumbency with the dependent limb in the CT gantry. An 18 gauge catheter was place in the median artery of this limb using ultrasonographic guidance. A CT image was obtained at the same location every other second for 90 seconds before, during and after intra-arterial iodinated contrast administration. Contrast material was administered into the artery for a total of 30 seconds (3mL/sec) beginning five seconds after image acquisition. After the images were acquired the horses were recovered in a routine fashion. Automated software was used to evaluate these images and develop a time density curve for an artery within the image, the dorsolateral, dorsal and dorsomedial lamina. Density measured in Hounsfeld units has a linear relationship with iodine concentration in tissue, therefore, the Patlak equation using the arterial input function can be used to calculate the perfusion and the vascular permeability in mL/min/mL for each of the specified areas of interest.

Table 1. Results.:

Location Lamina	Mean Perfusion, ±σ (ml/min/mL)	Range	Mean Perm. ±σ (ml/min/mL)	Range
Dorsal	0.56 ± 0.33	0.145 – 0.780	0.07 ± 0.04	0.045 – 0.150
Dorsomedial	0.33 ± 0.1	0.074 – 0.567	0.08 ± 0.03	0.070 – 0.330
Dorsolateral	0.33 ± 0.2	0.056 – 0.607	0.07 ± 0.05	0.042 – 0.450

This study showed that dynamic computed tomography is useful for the evaluation of blood flow within the foot of the horse. Alterations in blood supply to the foot, including arteriovenous anastomoses, regional perfusion deficits are implicated in different disease processes and may play a role in the development and propagation of laminitis in the horse. There is significant variability in the values obtained in this study that may be in part due to blood pressure changes under anesthesia and the normal vascular physiology of the foot. Further investigation of this technique in horses with laminitis may show a significant variation from the above stated normal values which may have both clinical and research applications.

ABSTRACT #312

ACYCLOVIR PHARMACOKINETICS IN THE HORSE. PA Wilkins, M Papich* and RW Sweeney. University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square PA and * North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Acyclovir is virustatic drug commonly employed in treatment of alpha-herpes virus infections in humans and some veterinary species. Equine herpes myelencephalopathy (EHM) results from infection of horses with equine herpesvirus type 1 (EHV-1), an alpha-herpes virus, and preliminary investigation of EHV-1 isolates from recent EHM outbreaks suggest that: (1) EHV-1 is sensitive to acyclovir and (2) acyclovir may be a rational therapy for the treatment of EHM. There are no descriptions in relevant literature of the pharmacokinetics of acyclovir in horses. We undertook an investigation of the pharamacokinetics of acyclovir in adult horses.

Six healthy adult females horses, mean weight 587 kg, were treated with either intravenous (IV) acyclovir infusion (10 mg/kg infused over one hour in 1 L crystalloid fluid) or oral (PO) acyclovir (20 mg/kg) administration in a randomized cross-over design. An approximate one-week washout period was allowed between trials. Blood was collected from catheters placed within a jugular vein at time 0, prior to administration, and at multiple intervals thereafter for 24 hours. Blood was immediately placed in heparin tubes, centrifuged, and the plasma frozen at -80°C. Plasma was assayed for acyclovir concentrations using an HPLC technique. Peak concentration (mean ± SD) for IV acyclovir was 13.7 ± 5.9 μg/ml at the completion of the one hour infusion. The half-life of the distribution phase (alpha) was 0.16 hr while the half-life of the elimination phase (beta) was 9.6 hr. The steady state volume of distribution was 3926 ± 1205 ml/kg. We were unable to measure pharmacokinetics after PO acyclovir as plasma concentrations were detected in only 2/6 horses and only between 20 minutes and three hours post-administration. Peak values obtained in these two horses were ~ 0.11 μg/ml (2 hr) and ~ 0.08μg/ml (3 hr) respectively.

These data suggest that, as in humans, the bioavailability of orally administered acyclovir is poor in horses. The pharmacokinetics of orally administered acyclovir deserves further study. Modification of the oral dosage form to enhance its absorption, or investigation of one of the pro-drugs of this class, is suggested. Because of the prolonged elimination phase in horses, multiple dosing of oral antiviral drugs from this class may allow sufficient plasma accumulation.

ABSTRACT #313

THE PHARMACOKINETICS OF ACYCLOVIR IN ADULT HORSES FOLLOWING SINGLE INTRAVENOUS AND INTRAGASTRIC DOSING. Bradford G. Bentz, Lara K. Maxwell, Cyril R. Clarke, Ronald S. Erkert, Christopher M. Royer, Michael S. Davis, Charles G. MacAllister; Oklahoma State University, College of Veterinary Medicine, Stillwater, OK.

The purpose of this investigation was to describe the bioavailability and pharmacokinetic parameters of acyclovir following intravenous (IV) and intragastric (IG) administration to healthy adult horses.

Six healthy adult horses (three geldings, three mares) were used in a randomized crossover study with a three-by-three Latin square design. These horses were randomly divided into three groups of two horses consisting of one mare and one gelding. The three treatments administered were: 1) 10 mg/kg of injectable acyclovir diluted in 1 liter of normal saline and delivered as an IV infusion over 15 minutes, 2) 10 mg/kg of acyclovir (crushed 800mg tablets suspended in 500 ml of water) delivered IG, and 3) 20 mg/kg of acyclovir delivered IG. A two-week washout period was provided between each treatment. Serum samples were obtained at times 0, 5, 10, 20, 30, 60, 90, 120, 180, 240, 480, 720 min and at 24 hours following oral dosing. Serum samples were also obtained at 0, 5, 10, 15, 20, 25, 35, 45, 75, 105, 120, 180, 240, 480, 720 min and at 24 hours

following IV dosing. Blood samples were permitted to clot and serum was separated and frozen at -20°C until analysis. Serum samples were assayed using reversed phase high performance liquid chromatography with fluorescence detection. Serum samples were deproteinated with perchloric acid, cooled and centrifuged. Twenty-five microliters of the supernatant were injected onto a C18 column and acyclovir was eluted under isocratic conditions of 4% acetonitrile in 15 mM potassium phosphate buffer at a pH of 2.1. The limit of quantitation of the assay was 0.04 µg/ml. The assay exhibited suitable accuracy (96%), precision (C.V. = 7%) and recovery (98%). The IV data were analyzed by a 3-compartment pharmacokinetic model and oral data were analyzed non-compartmentally.

Oral administration of acyclovir at both dose rates was associated with high variability in serum acyclovir-time profiles, low maximal serum concentrations (C_{max}), and poor bioavailability. For example, following an IG dose of 20 mg/kg, the mean C_{max} was 0.20 ± 0.10 (SD) µg/ml and the mean bioavailability was 2%. The mean terminal elimination rate associated with IV dosing of acyclovir was 13.4 hours and the mean total body clearance was 4.96 ml/min/kg. Adverse effects were noted in one horse administered IV acyclovir, characterized by sweating, colic and generalized muscle tremors.

Previously published *in vitro* data indicate that acyclovir concentrations of 0.30 to 0.45 µg/ml are necessary for inhibition of various strains of EVH I. Yet another reference cited a concentration of 7 µg/ml for inhibition of a Kentucky EHV I strain. Therefore, the results of this investigation are not supportive of a therapeutic benefit when acyclovir is orally administered to adult horses at dosages as high as 20 mg/kg. Additional pharmacokinetic and safety studies would also be necessary to support the use of intravenous acyclovir in adult and neonatal equidae.

ABSTRACT #314

CONCENTRATIONS OF DOXYCYCLINE IN PLASMA, INTERSTITIAL FLUID, POLYMORPHONUCLEAR LEUKOCYTES AND AQUEOUS HUMOR FOLLOWING ORAL ADMINISTRATION IN HORSES. Jennifer L. Davis, Jacklyn H. Salmon, Mark G. Papich. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

The purpose of this study was to determine the pharmacokinetics and safety of doxycycline in the horse following single and multiple doses of 20 mg/kg and to examine its penetration into the interstitial fluid (ISF), polymorphonuclear leukocytes (PMNL), and the aqueous humor.

Six healthy adult horses were used. A small pilot study was performed, prior to the actual experiment, to determine the effects of feeding on doxycycline absorption. Following this, each horse received a single dose of doxycycline hyclate at 20mg/kg by nasogastric tube and plasma samples were drawn. Following a minimum 10-day washout period, each horse received five doses of 20 mg/kg doxycycline hyclate in a similar manner at 12-hour intervals. Plasma, ISF, PMNL and aqueous humor samples were drawn. All samples were analyzed by high pressure liquid chromatography (HPLC) with UV detection. Plasma protein binding was determined using an *in vitro* microcentrifugation technique. Data were subjected to pharmacokinetic analysis. Horses were monitored for any adverse reactions throughout the study period.

Feeding decreased the plasma maximum concentration (C_{max}) and area under the concentration-time curve (AUC), and increased the time to maximum concentration (T_{max}). Based on these results, horses were fasted eight hours prior to and two hours after drug administration for the rest of the study. Doxycycline showed favorable kinetics in the plasma following oral dosing and exhibited a T_{max} of 1.63 ± 1.36 hr, a C_{max} of 1.74 ± 0.3 µg/mL, and an elimination half-life of 12.07 ± 3.17 hr. Plasma protein binding was 81.76 ± 2.43%. $C_{max\ isf}$:$C_{max\ plasma}$ and AUC_{isf}:AUC_{plasma} ratios were 17.88 ± 3.49% and 24.84 ± 7.54%, respectively. This correlated with the calculated % protein unbound drug in the plasma (18.24%). Doxycycline showed significant accumulation within PMNLs. The C_{max} and AUC of doxycycline in the equine neutrophil were 17.27 ± 8.98 and 14.58 ± 6.64 times higher when compared to plasma C_{max} and AUC, respectively. Aqueous humor concentrations were between 7.5% and 10% of plasma concentrations at the times measured. One horse developed fever, leukopenia, diarrhea and laminitis 52 hours following administration of the last dose and was euthanized. Culture of tissues and feces for *Salmonella* sp., and *C. perfringens* enterotoxin tests were negative.

Based on the results of this study, we recommend doxycycline at a dose of 20 mg/kg PO q24h for susceptible intracellular bacteria and for bacteria with an MIC ≤ 0.25 µg/mL. For less susceptible bacteria with an MIC of 0.5-1.0 µg/mL, we recommend 20 mg/kg PO q12h, although this may carry an increased risk of gastrointestinal side effects. Feeding schedules should be adjusted accordingly.

ABSTRACT #315

THE PHARMACOKINETICS AND TISSUE DISTRIBUTION OF CEPHALEXIN IN THE HORSE. Jennifer L. Davis, Jacklyn H. Salmon, Mark G. Papich. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Cephalexin is an oral, first-generation cephalosporin antibiotic with activity against most gram-positive organisms, and some highly susceptible gram-negative organisms. To date, the pharmacokinetics of cephalexin in the horse has not been reported. The purpose of this study was to determine the plasma pharmacokinetics of cephalexin in the adult horse following oral and intravenous administration. To characterize tissue distribution, we also examined the disposition of cephalexin into the interstitial fluid (ISF), and aqueous humor, as well as the *in vitro* plasma protein binding. Dosing recommendations were made based on established pharmacokinetic and pharmacodynamic parameters used to predict efficacy for cephalosporin antibiotics which target a plasma concentration greater than or equal to the MIC for 50% of the dosing interval.

Six healthy adult horses were used for this study. Cephalexin hydrate was dissolved in sterile saline to a final concentration of 10 mg/mL and administered to horses as a slow IV bolus at a dose of 10 mg/kg. Plasma samples were collected at predetermined times. Following a two-week washout period, cephalexin capsules were dissolved in water and administered via a nasogastric tube at a dose of 30 mg/kg. Plasma, interstitial fluid, and aqueous humor samples were collected. All samples were analyzed by high pressure liquid chromatography (HPLC) with ultraviolet detection. Horses were monitored for adverse reactions throughout the study.

Following intravenous administration, cephalexin was rapidly excreted with a clearance of 3.23 ± 0.7 mL/min/kg, and a plasma half-life ($t_{1/2}$) of 2.2 ± 0.75 hr. The volume of distribution at steady was 0.23 ± 0.04 L/kg. Following oral administration, cephalexin was rapidly absorbed and was detected in 5 out of 6 horses at 10 minutes after dosing. The average maximum plasma concentration was 3.47 ± 1.21 µg/mL and the time to maximum plasma concentration was 0.97 ± 0.31 hr. The plasma $t_{1/2}$ was 1.77 ± 0.56 hr. Bioavailability was low at 5.0 ± 2.8 %. Plasma protein binding was 22.93 ± 7.94%. Cephalexin was detected in all but one of the ISF samples analyzed. The AUC_{isf}:AUC_{plasma} ratio was 80.55 ± 11.42% which corresponded to the predicted concentration based on % protein unbound drug in the plasma (77.07%). The $t_{1/2}$ in the ISF was similar to that of plasma at 2.49 ± 1.35 hr. Cephalexin was not detected in any of the aqueous humor samples analyzed, which agrees with predictions based on blood-ocular barrier penetrability. No adverse events were noted during this study.

Despite the low oral bioavailability of cephalexin, adequate concentrations were achieved in the plasma and interstitial fluid for the treatment of susceptible gram-positive bacteria (MIC ≤ 0.5 µg/mL) at a dose of 30 mg/kg PO administered every eight hours.

Multiple dose studies are necessary to establish the safety of cephalexin for long-term use in horses.

ABSTRACT #316

EFFECT OF DETOMIDINE ON SOMATIC AND VISCERAL NOCICEPTION AND DUODENAL MOTILITY IN CONSCIOUS HORSES. L. Chris Sanchez, Johanna Elfenbein, Jodi Mock, Sheilah Robertson, and Cynthia Kollias-Baker. University of Florida College of Veterinary Medicine, Gainesville, FL.

Alpha-2 antagonists such as detomidine are commonly used in horses. The purpose of the study reported here was to evaluate the effects of detomidine on visceral and somatic nociception as well as effects on heart and respiratory rates, sedation, and duodenal motility.

Visceral nociception was evaluated using two methods of threshold detection, colorectal distention (CRD) and duodenal distention (DD). Each employed the use of a Mylar® balloon and a computer-controlled barostat for distention. The duodenal balloon was placed via a permanent gastric cannula with endoscopic guidance. This balloon and barostat were also used for assessment of duodenal motility between threshold testing protocols. Somatic nociception was assessed via thermal threshold (TT) determination. A probe containing a heater element and adjacent temperature sensor placed on the withers was used for thermal stimulation. Nose-to-ground height was used to assess sedation. Heart and respiratory rates were determined by manual count over a period of 30 seconds. Detomidine was administered by bolus injection into a jugular vein at dosages of 10 or 20 µg/kg. Five horses were used for the study, and each horse received each treatment. All data were analyzed by means of a three-factor ANOVA with the fixed factors of group and time and the random factor of horse (SAS Proc Mixed). When a significant time*group interaction was detected, the difference between groups for each time point was compared with a simple t test, whereas all later time points were compared to time 0 for each group by a Bonferroni t test for multiple comparisons.

Detomidine administration produced a significant, dose-dependent decrease in nose-to-ground height, heart rate, and skin temperature. A significant decrease in respiratory rate was also noted in response to detomidine administration, but this was not dose-dependent. Detomidine administration caused a significant increase in CRD threshold at the 20 µg/kg dose for at least 165 minutes post-treatment, but this effect was only significant at the 15 minute time point following the 10 µg/kg dose. A significant difference from time 0 was not detected for TT at any time point following detomidine administration at either dose. A marked, immediate decrease in amplitude of duodenal contractions was also noted following detomidine administration at either dose.

Comparatively, detomidine caused a longer period of visceral analgesia as determined by CRD but a shorter period of analgesia as determined by DD than has been previously reported in models using cecal distention at similar doses. The lack of somatic analgesia as determined by TT testing may be related to a marked decrease in skin temperature, likely caused by peripheral vasoconstriction.

ABSTRACT #317

ENDOTHELIN AND NITRIC OXIDE PRODUCTION BY EQUINE BRONCHIAL EPITHELIAL CELLS CULTURED UNDER AIR-LIQUID INTERFACE CONDITIONS. L.R.R. Costa, K. O'Reilly, R. Truax, T. Foster, J. R. Johnson, R. M. Moore; Equine Health Studies Program, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA.

Several mediators have been implicated in the pathogenesis of equine airway diseases. Amongst them, nitric oxide (NO) derived from inducible nitric oxide synthase and endothelin (ET) were shown to be increased in airway epithelium of asthmatic and horses affected with summer pasture-associated obstructive pulmonary disease (SPAOPD). Moreover, a number of stimuli, especially cytokines, have been incriminated in the induction of ET and NO synthesis. The overall goal of this study was to stimulate cultures of differentiated equine bronchial epithelial cells with LPS, TNF-alpha and IL-4 and measure the synthesis of ET and NO.

Fresh post-mortem specimens of lung tissue were obtained from two adult horses affected with SPAOPD while horses were in clinical remission (i.e., without signs of respiratory disease, intrapleural pressure difference less than 10cm of water, and neutrophil in bronchoalveolar lavage less than 15%). The bronchial epithelium was dissected, subjected to cold trypsinization and cultured on Transwells with Dulbecco's modified Eagle's medium:Ham's F12 (1:1 v/v) containing fetal bovine serum and epithelial growth factor (EGF) as previously described. Once cultures were established, they were placed in air-liquid interface (ALI) and maintained in a serum-free media containing low concentration of EGF. After nine days, the cells were stimulated basolaterally with LPS (10 ng/ml), human recombinant TNF-alpha (5 and 20 ng/ml) or equine recombinant IL-4 (1%, 10% and 50% v/v). Cell-free supernatants from the bottom of the wells were harvested at 24, 48 and 72 hours and stored at –70C until assayed for ET and NO. ET concentrations were determined using a commercially available sandwich enzyme-linked immunosorbent assay (Biomedica). NO determination was performed using an electrochemical detection system, ISO-NO Mark II. Morphologic differentiation of the cell cultures after 14 to 28 days in ALI was evaluated using light microscopy (thin-sections were stained with Toluene Blue), confocal microscopy (stained for cytokeratin and actin) and transmission electron microscopy.

Stimulation with hrTNF-alpha, LPS or eqrIL-4 for 24 and 48 hours, and eqrIL-4 for 72 hours resulted in increased production of ET (ranging from 1.5 to 4 fold). Stimulation with hrTNF-alpha, LPS and eqrIL-4 for 48 hours and eqrIL-4 for 72 hours induced 1.5 to 2.5 fold increases in NO production by primary bronchial epithelial cell culture. Our results suggest that bronchial epithelial cells represent a potentially important source of ET and NO in response to cytokine (TNF-alpha and IL-4) stimulation. The interactions of these mediators may play a role in the pathogenesis of SPAOPD.

ABSTRACT #318

FLUID MOVEMENT FROM PULMONARY VASCULATURE IN EXERCISING HORSES AFTER ACUTE AND CHRONIC INHIBITION OF CARBONIC ANHYDRASE. M. Vengust[1], H. Stämpfli[2], L. Viel[2], A. Nunez de Moraes[3], G. Heigenhauser[4]. [1]University of Ljubljana, Veterinary Faculty, Slovenia; [2]University of Guelph, Ontario Veterinary College, Guelph, Ontario, Canada; [3]UDESC Centro de Ciências Agroveterinárias Lages, SC, Brasil; [4]McMaster University Medical Centre Hamilton, Ontario, Canada.

In athletic horses, the kinetics of fluid across the lung at rest and during exercise remains poorly understood. Specific airway diseases in horses, especially non-septic inflammatory airway disease and EIPH, are associated with abnormal pulmonary hemodynamics and abnormal pulmonary interstitial fluid equilibrium. This study was designed to determine fluid movement across the alveolar-capillary barrier during variable degrees of CO_2 retention, which was achieved with carbonic anhydrase (CA) inhibition.

Six Standardbred horses (five to six years old) were exercised until fatigue on a high speed treadmill (Säto Sweden) at 80% VO_2 peak a) without CA inhibition (control), b) with acute CA inhibition, and c) chronic CA inhibition. CO_2 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 three 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_2 elimination and O_2 uptake were sampled simultaneously five minutes apart. During exercise, the sampling was performed in 60 sec intervals until fatigue. Changes in blood volume (ΔBV %) were calculated from changes in hemoglobin, hematocrit, and plasma

protein values in venous and arterial blood. Cardiac output (Qp L/min) was calculated using Fick principle. Fluid movement (FM L/min) across the lung was then quantified based on Qp and ΔBV (FM = (Qp x ΔBV)). Variables were analyzed using two-way repeated-measures ANOVA ($P<0.05$). A significant F ratio was further analyzed using Tukey post-hoc analysis. Data is expressed as mean ± SE.

CA inhibition had a significant effect on fluid movement across the pulmonary vasculature ($P=0.04$). At rest there was no fluid movement in control (0.3±0.8 L/min) and AcIn (0.2±1 L/min); in ChIn 1.3±0.5 L/min of fluid moved from the pulmonary interstitium into the pulmonary circulation (different from control and AcIn: $P<0.05$). During exercise in control experiment fluid moved from the pulmonary circulation into the pulmonary interstitium at a rate of 9.4±2.4 L/min; in AcIn 1.6±2.4 L/min and ChIn remained relatively stable at 1.8±2.4 L/min. Mean exercise ChIn was significantly different from control and AcIn ($P\leq0.04$).

Fluid readily moves between lung compartments during exercise in normal horses. However, altered CO_2 homeostasis along with changes in acid-base status seems to affect fluid movement in lungs. Based on findings of this study it can be concluded that the kinetics of the pulmonary interstitial and vascular fluid movement adapt rapidly and efficiently to physiological and possible pathological changes during strenuous exercise as it occurs in racing horses.

ABSTRACT #319

EFFECTS OF ORALLY ADMINISTRATED DOSES OF A 5-HT4 RECEPTOR AGONIST, MOSAPRIDE, ON ELECTROINTESTINOGRAPHY IN HORSES. N. Sasaki[1] , K. Okamura[2], Y. Ujimasa[2], H. Yamada[1]. [1]Department of Veterinary Surgery, Obihiro University of Agriculture and Veterinary Medicine, Japan; [2]Animal Science, Dainippon Pharmaceutical CO., LTD., Japan.

A high incidence of digestive disorders has been reported in horses with abnormal function of the digestive tract, and the regulation of the intestinal motility was important to treatment. 5-hydroxytryptamine$_4$ receptor agonist (5-HT$_4$ receptor agonist) is a gastroprokinetic agent that acts on the cholinergic postganglionic fiber ends in the intramural plexus of the digestive tract, and acetylcholine release is stimulated. Mosapride is a benzamide derivative with a morpholine ring, and is a gastroprokinetic agent whose mechanism is a selective 5-HT$_4$ receptor agonist. Mosapride has been used in humans for the treatment of the gastrointestinal motility dysfunctions of reflux esophagitis, chronic gastritis, and postoperative ileus. In the horse, electrical activity of the Electrointestinography (EIG) in the small intestine was increased by oral administration of mosapride at 2mg/kg, suggesting its potential for prokinetic action on small intestine motility in equines. On the other hand, neither the optimal dose of mosapride nor the effects on other digestive tracts are examined. In this study, the effects of a serotonin receptor agonist mosapride on the small intestine of the horse after oral administration were examined by using EIG.

Six adult healthy thoroughbreds were used. EIG measurements were performed using a Electrogastrography system (Nipro EG, Japan). EIG surface electrodes were installed at three sites: the front edge of the tuber coxae (using another EIG mini-amplifier), the intersection of the horizontal line extending from tuber coxae and the rear edge of the last rib (using an uninductive electrode), and the apex of an inverted regular triangle formed by placing the other two electrodes on the other apexes (using an EIG mini-amplifier). At a sampling rate of 1 Hz, the frequency of electrical activity was measured within the range of 1.7 to 12 cycles per minute (cpm). EIG analysis was analyzed with fast Fourier transform (FFT) analysis. 0, 0.5, 1.0, 1.5, and 2.0 mg/kg mosapride (mosapride citrate, Dainippon Pharmaceutical CO., LTD., Japan) were dissolved in 200ml water, respectively, and administered through a nasogastric tube. The % mean amplitude for a 30-min period at three hours after administration of drugs is expressed as a percentage of that for the 30-min period before administration.

The % mean amplitude of EIG of orally administered mosapride on the small intestine were 127.0±12.5, 137.7±22.2, and 151.1±24.0, respectively, at 1.0 ,1.5, and 2.0 mg/kg, all of which were significantly greater than that at 0 mg/kg. The % mean amplitude of EIG of orally administered mosapride on cecum were 130.1±34.5 and 151.6±45.2 at 1.5 and 2.0 mg/kg, respectively, and were significantly greater than that in 0 mg/kg.

It was clear that mosapride promoted the prokinetic action of gastrointestinal motility at 1.0, 1.5, and 2.0 mg/kg in the small intestine, at 1.5 and 2.0 mg/kg in the cecum. Therefore, the 5-HT$_4$ agonist mosapride may be useful for treatment of gastrointestinal dysfunction in the horse.

ABSTRACT #320

MEASUREMENT OF INTRA-ABDOMINAL PRESSURE IN NORMAL HORSES. Louise L. Southwood and Pamela A. Wilkins. New Bolton Center, University Of Pennsylvania, Kennet Square, PA.

An increase in intra-abdominal pressure (IAP) has been recognized in human critically ill patients to cause serious pathophysiological alterations to the respiratory and cardiovascular systems as well as to abdominal organ function. Intra-abdominal hypertension (IAH) is defined as an IAP in excess of 20 to 25 mmHg and abdominal compartment syndrome (ACS) occurs when IAH is associated with organ dysfunction. Some of the changes that are associated with IAH in human patients have been observed in horses with abdominal disease. Prior to evaluation and clinical application of IAP measurements in critically ill horses, normal values need to be described. The purpose of this study was to obtain normal values for IAP in horses.

Measurements of IAP were performed in horses under standing sedation and under general anesthesia in dorsal and lateral recumbency. IAP was measured using bladder catheterization and a water manometer. The penis and penile urethra or vulva was aseptically prepared for catheterization. A stallion or Foley catheter was routinely placed into the bladder via the urethra. The bladder was emptied. Connecting tubing and a water manometer were attached to the stallion/Foley catheter. Air bubbles were removed. Approximately 100 mL of sterile saline were infused into the bladder. In the standing horse, the tuber ischii was used as the landmark and measurements were made 0 to 11 inches (in.) ventral to the tuber ischii. The tuber ischii was also used as the landmark for horses in dorsal recumbency, and measurements were also made 0 to 11 in. ventral to the tuber ischii. The ventral midline was used as the landmark for horses in dorsal recumbency and measurements were made 0 to 10 in. above the ventral midline. Multiple (> 3) measurements were made at each location. Values during inspiration (max.) and expiration (min.) were recorded. The maximum IAP that should be considered normal for each position was determined.

There was variability in IAP measurements between horses; however, the repeatability was good. In standing horses, the IAP should not exceed 7 cmH_2O at the level of the tuber ischii and many measurements in this location were negative. The median difference between the max. and min. IAP was 2 cmH_2O. In dorsally recumbent horses, IAP should not exceed 10 cmH_20 during expiration when the manometer is more than seven inches ventral to the tuber ischii and in laterally recumbent horses, the IAP should not exceed 10 cmH_2O during expiration when the manometer is greater than 6 inches above the ventral midline. The median difference between the max. and min. values were 1.75 and 3 cmH_20 in dorsal and lateral recumbency, respectively.

While there is some variability in IAP measurements between horses, this technique should be useful for assessing IAH in horses with abdominal disease. Variability may be due to the position of the horse and detrusor muscle tone. Further studies in clinical cases using this technique are needed.