ABSTRACT #1
EVALUATION OF FOUR DNA EXTRACTION METHODS FOR THE DETECTION OF *TRITRICHOMONAS FOETUS* IN FELINE STOOL SPECIMENS BY POLYMERASE CHAIN REACTION. SH Stauffer, AJ Birkenheuer, MG Levy, H Marr, JL Gookin. College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Feces are increasingly recognized as practical samples for molecular diagnosis of infectious disease. Extraction of PCR-quality DNA from feces can be challenging due to co-extraction of PCR inhibitors. Accordingly, we examined the effect of four commercially-available DNA extraction methods on sensitivity of PCR for detection of *Tritrichomonas foetus* (TF) in naturally-infected and TF-spiked feline stool.

Kits evaluated included ExtractMaster Fecal DNA Extraction Kit, Epicentre Biotechnologies (Kit A); QIAamp DNA Stool Mini Kit, Qiagen (Kit B); UltraClean Fecal DNA Kit, MoBio (Kit C); and ZR Fecal DNA Kit, Zymo Research (Kit D). In accordance with manufacturer instructions, DNA was extracted from 180 mg (A,B), 50 mg (C), 100 & 150 mg (D) aliquots of feline feces to which was added 20 µl volumes containing 0–10,000 cultured feline TF. Each kit was also used to extract DNA from the feces of each of 10 naturally infected and 10 uninfected cats. DNA was eluted in 300 µl (A), 200 µl (B), 50 µl (C), or 100 µl (D) of respective elution buffer. Endogenous PCR inhibitors in extracted DNA was examined by PCR amplification of an 876 bp gene fragment of bacterial 16S rRNA. DNA was then tested by single tube nested PCR for amplification of partial ITS1, 5.8S and ITS2 rRNA genes of TF.

Kit D provided the most sensitive detection of TF DNA as expressed by both organisms per DNA extraction and organisms per PCR reaction. To account for differences in DNA concentrations between kits (i.e. fecal sample size and elution volumes), the limit of detection for each kit as expressed by the number of TF per PCR reaction was as follows: Kit B = 250, Kit A = 167, Kit C = 100, Kit D (150 mg fecal sample) = 5, and Kit D (100 mg fecal sample) = 0.5. PCR performed on DNA extracted from cultured TF (no feces) or TF-spiked feces (100 mg) using Kit D was positive with as few as 10 TF per extraction. Further, DNA extraction using Kit D could be completed in the shortest time of all kits tested.

These studies identify the ZR Fecal DNA Kit as superior to the other kits tested for extraction of PCR-quality DNA from feline feces.

ABSTRACT #2
INVESTIGATION OF *ENTEROBACTER CLOACAE* INFECTIONS AT A SMALL ANIMAL VETERINARY TEACHING HOSPITAL. JS Weese. University of Guelph, Guelph, Ontario.

A wide range of pathogens can cause hospital-associated (HA) infections in small animal hospitals. Among these is *Enterobacter cloacae*, which is one of the most clinically relevant *Enterobacter* spp and a common cause of HA infection in humans. Recently, multidrug resistance has become a concern, particularly with emergence of extended-spectrum beta-lactamase and extended spectrum cephalosporinase producing strains. An infection control investigation was initiated at the Ontario Veterinary College Teaching Hospital (OVCTH) in the fall of 2007 in response to anecdotal concerns about *Enterobacter cloacae* infections in hospitalized animals.

Enterobacter cloacae was isolated from 45/36719 animals from January 1, 2005 to October 31, 2007, for an overall incidence of 1.2/1000 admissions. The monthly incidence rate ranged from 0 to 4.3/1000 admissions. Twenty-one (47%) cases were classified as community-associated, while 17 (38%) were hospital associated. Seven (15%) were community-onset but hospital associated, with three of these associated with other veterinary hospitals. There was no increase in the incidence of overall or hospital-associated infections during the study period.

The urinary tract was the most common site of infection (n=11, 24%). Wound infections (excluding surgical site infections) accounted for 8 (18%) of infections, with superficial and deep surgical site infections accounting for 7 (16%) and organ/space surgical site infections accounting for another 2 cases. Urinary tract infections were most common among animals with CA infection, accounting for 8/21 (38%) cases with wound infections accounting for 4 (19%) cases.

Of the 24 cases associated with the OVCTH, 17 (71%) had surgery, 15 (63%) were hospitalized in the intensive care unit, 10 (42%) had indwelling urinary catheters placed, and 20 (83%) had received antimicrobials prior to onset of infection. Risk factors for *E. cloacae* infection could not be determined because a noninfected control group was not evaluated. Surgical site infections accounted for 9 (38%) HA cases. Overall, only 2/11 (18%) urinary tract infections were associated with prior placement of a urinary catheter. Nine (20%) animals died or were euthanized and *E. cloacae* was implicated as a causative or contributing factor in 5 (56%) of those cases.

Two main antimicrobial phenotype patterns were identified. One (n=25) was characterized by susceptibility to fluoroquinolones, tetracycline, and trimethoprim with variable susceptibility to cefoxitin while the other (n=14) was characterized by resistance to these antimicrobials. Prior administration of antimicrobials was associated with presence of the more resistant phenotype (P=0.044) but there was no association between this phenotype and origin of infection (P=0.74) and no increase in the prevalence of this phenotype from 2005 to 2007 (P=0.97). Infections with this phenotype were not associated with nonsurvival (P=0.74).

There was no evidence of a, HA outbreak or increase in prevalence, yet identification of multidrug resistant *E. cloacae* in both CA and HA infections is concerning and requires ongoing surveillance.

ABSTRACT #3
***STAPHYLOCOCCUS PSEUDINTERMEDIUS:* A NEWLY RECOGNIZED PATHOGEN IN DOGS AND CATS.** MC Faires[1], D Slavic[2], JS Weese[1]. [1]Ontario Veterinary College, [2]Animal Health Laboratory, University of Guelph, Guelph, Ontario.

Staphylococcus intermedius has typically been regarded as the predominant pathogenic *Staphylococcus* spp in dogs and cats, and a leading cause of skin and soft tissue infections. In 2005, a novel *Staphylococcus* species, *Staphylococcus pseudintermedius*, was identified. This organism is closely related to, but distinct from, *S. intermedius*. Gene-sequence based methods are required to differentiate these two species; however, these techniques are rarely performed in clinical laboratories, and as a result the prevalence and characteristics of *S. pseudintermedius* are poorly understood. Recent evidence suggests that *S. pseudintermedius* may actually be the predominant *Staphylococcus* spp in dogs and cats but misidentified as *S. intermedius* by diagnostic laboratories. The objective of this study was to use sequence based methods to identify putative *S. intermedius* isolates from dogs and cats and to evaluate antimicrobial resistance and virulence factors among *S. pseudintermedius* isolates.

Isolates from dogs and cats identified as *S. intermedius* by conventional laboratory methods were obtained from the University of Guelph Animal Health Laboratory. Isolates were collected in a serial manner without selection. DNA was extracted, sequencing of the *sodA* gene was performed, and isolates were identified via sequence alignment with reference staphylococcal strains through GenBank (www.ncbi.nlm.nih.gov/blast/BLAST.cgi). Antimicrobial susceptibility testing was performed and PCR was used to identify various virulence factors and antimicrobial genes.

A total of 25 isolates were obtained from 21 dogs and 2 cats. Medical records were not available for 2 of the isolates. 25/25 (100%) were identified as *S. pseudintermedius* Severity of infection ranged from superficial dermatitis to rapidly fatal necrotizing fasciitis with the majority of isolates from otitis externa 9/23 (39.1%) and urinary tract infections 6/23 (26.1%). Antimicrobial susceptibility was as follows: amoxicillin/clavulanate 23/23 (100%), ampicillin 7/23 (30.4%), cephalothin 23/23 (100%), clindamycin 18/23 (78.3%), gentamicin 23/23 (100%), tetracycline 18/23 (78.3%), and trimethoprim/sulfa 19/23 (82.6%). Not all antimicrobials were tested for all isolates, based on laboratory protocols regarding antimicrobial panel and site of infection. Inducible resistance to clindamycin was detected by D-test in 1 isolate reported as clindamycin-susceptible (5.6%). Detection of virulence factors and antimicrobial resistance genes is ongoing.

This study identified *S. pseudintermedius* as an important pathogen in dogs and cats, and suggests that *S. intermedius* may not be a major concern in these species. Further studies are required to evaluate clinically relevant virulence factors to assist in understanding the pathogenesis of disease caused by *S. pseudintermedius*.

ABSTRACT #4

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AND MRSA CARRIAGE IN THREE POPULATIONS. S Kottler[1], JR Middleton[1], JS Weese[2], LA Cohn[1]. [1]University of Missouri College of Veterinary Medicine, Columbia, MO. [2]Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Considered primarily a human pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize animals. Prior studies have demonstrated a higher prevalence of MRSA colonization in healthcare workers (human and veterinary) than in the people with non-medically related occupations. We hypothesized that the prevalence of MRSA colonization would be higher in people and pets who reside in households with veterinary or human healthcare workers than in households without healthcare workers. One healthy volunteer (nasal swab) and a pet from the same household (nasal and rectal swabs) were sampled. Swabs were placed in enrichment broth and coagulase positive staphylococci (CPS) were identified by standard methods. CPS were classified as *S. aureus* using a latex agglutination test (LAT) and polymyxin B susceptibility. *Staphylococcus aureus* isolates were screened for methicillin resistance via PBP2a LAT and *mec*A PCR. Proportional data were compared using the Chi square test ($P < 0.05$).

Of the 601 sample sets (1,803 samples) obtained, 202 sets were from households with human healthcare workers (group 1), 223 from veterinary healthcare workers (group 2), and 176 from non-healthcare workers (group 3). And 103/601 (17.1%) pets sampled were cats and 498/601 (82.9%) were dogs. Overall, *S. aureus* was isolated from 166/601 (27.6%) of humans and 79/601 (13.1%) of pet nasal and rectal samples. For 26/245 (10.6%) *S. aureus* isolates, both a human and animal in the same household were colonized. Shared colonization was equally likely in all groups (10 from group 1, 10 group 2, and 6 from group 3).

Of 245 *S. aureus* isolates, 49 were MRSA (20%). Among *S. aureus* isolates, 31 of 166 human isolates (18.7%) were MRSA while 18/79 (22.8%) pet isolates were MRSA (Table). MRSA was present in 31/601 (5.2%) of humans sampled and 18/601 (3.0%) pets. There were no differences in prevalence of MRSA between groups of humans (9/202 group 1, 13/223 group 2, 9/176 group 3) or pets (6/202 group 1, 4/223 group 2, and 8/176 group 3). For 6/49 (12.2%) of the MRSA isolates, both an animal and human in the same household were colonized representing two households per group.

	Group 1	Group 2	Group 3	Total
MRSA among human SA isolates	9/60 (15%)	13/60 (22%)	9/46 (20%)	31/166 (19%)
MRSA among pet SA isolates	6/22 (27%)	4/34 (12%)	8/23 (35%)	18/79 (23%)

We were unable to demonstrate that colonization with *S. aureus* or MRSA was more common in households with healthcare workers. However, we found a greater prevalence of both *S. aureus* and MRSA in pet animals than has been previously demonstrated, and demonstrated that MRSA is found as commonly among *S. aureus* isolates from pets as from human isolates.

ABSTRACT #5

EFFECTS OF ZAFIRLUKAST, A CYSTEINYL-LEUKOTRIENE RECEPTOR ANTAGONIST, IN A LOW DOSE ENDOTOXIN INFUSION MODEL OF FELINE SEPSIS. Sharp CR, DeClue AE, Reinero CR. University of Missouri, College of Veterinary Medicine, Columbia, MO.

Cysteinyl–leukotrienes (cys-LT) are important inflammatory mediators contributing to systemic inflammation and hemodynamic instability during sepsis and there is experimental evidence that inhibiting the effects of leukotrienes ameliorates these sequelae. We hypothesized that the cys-LT receptor antagonist, zafirlukast, an inexpensive and readily available cys-LT receptor antagonist, would attenuate systemic inflammation and hemodynamic derangement in a low-dose endotoxin infusion model of feline sepsis.

This was a randomized, blinded, placebo-controlled crossover design, utilizing 6 adult cats. The cats were given a priming dose of lipopolysaccharide (LPS) (1 mcg/kg, IV) at time 0 and then either placebo (flour) or zafirlukast 10 mg orally at 6 and 12 hours. The cats were challenged at 14 hours with LPS (2 mcg/kg/h × 6 h, IV). There was a 30 day washout period between evaluations. Rectal temperature, heart rate (HR), systolic arterial blood pressure (BP), plasma interleukin-6 (IL-6) concentration, and tumor necrosis factor (TNF) activity were evaluated. Repeated measures ANOVA with post-hoc Tukey's test or paired t-test were used for statistical analysis with a p-value < 0.05 considered significant.

LPS administration induced significant systemic inflammation and hemodynamic derangement as indicated by increased mean±SD rectal temperature at 4, 5, and 6 hours (baseline, 39.1±0.5; 4 h, 39.9±0.4; 5 h, 40.0±0.3; 6 h, 39.8±0.4 °C; $p < 0.001$), increased IL-6 concentrations at 3 hours (baseline, 68.8±194.8; 3 h, 883.7±492.2 pg/mL; $p < 0.004$), increased TNF activity at 1.5 hours (baseline, ND; 1.5 h, 151±49 ng/ml; $p < 0.001$), and relative hypotension at 6 hours (baseline, 145.7±19.8; 6 h, 110±27.54 mmHg; $p = 0.03$), when compared to baseline regardless of treatment group. There was no significant difference between the placebo and zafirlukast treatment groups in regards to rectal temperature, HR, BP, IL-6 concentrations, or TNF activity at any time point.

These data indicate that antagonism of cys-LT with zafirlukast has neither beneficial nor deleterious effects in this model of feline sepsis.

ABSTRACT #6

FACTORS ASSOCIATED WITH ORGAN DYSFUNCTION AND DEATH IN DOGS WITH SEPSIS. JM Burkitt, K Hopper. University of California, Davis, School of Veterinary Medicine, Davis, CA.

Sepsis is the systemic response to infection, and is associated with a high mortality rate in dogs. Literature regarding the clinical course, distant organ dysfunction, and factors associated with mortality in dogs with naturally occurring sepsis is limited. The purpose of this investigation was to describe factors associated with distant organ dysfunction and death in a heterogeneous group of septic dogs admitted to an intensive care unit.

Post-hoc analysis was completed on prospectively collected data from septic dogs admitted to an intensive care unit from October 2004 to October 2005. Animals were included only if they fit criteria for the systemic inflammatory response syndrome due to a confirmed infectious etiology. Signalment, infected anatomic site(s), infectious agent(s), clinical course, and outcome were collected on a standardized data collection sheet. Categorical variables were analyzed using Pearson's chi-square test, and $P < 0.05$ was considered significant.

Forty-seven dogs were included in the investigation. Twenty dogs (42%) were discharged from the hospital alive. The most commonly infected anatomic sites were the peritoneal cavity (n = 14; 30%), the subcutaneous tissues (11; 23%), and the lung (9; 19%). Forty-five of the infections were bacterial (96%) and the remaining two were fungal. Septic shock occurred in 10/47 dogs (21%). Distant organ dysfunction occurred in 21/47 dogs (45%) and was associated with a surgical disease process ($P = 0.03$), hypoglycemia ($P = 0.011$), the presence of septic shock ($P = 0.002$), and death ($P = 0.019$). Fourteen of 21 dogs (67%) of dogs with distant organ dysfunction died or were euthanized for grave prognosis. Hypoglycemia ($P = 0.045$), total number of failing organs ($P = 0.003$), and presence of septic shock ($P < 0.001$) were each associated with death or euthanasia for severe illness. All dogs that developed septic shock died.

Distant organ dysfunction occurs frequently in dogs with sepsis and is associated with death. Distant organ dysfunction may be seen more commonly in dogs with hypoglycemia, a surgical disease process, or septic shock. Septic shock in the dog carries a particularly poor prognosis.

ABSTRACT #7

EFFECTS OF ALLOANTIGEN EXPOSURE AND CELL-ASSOCIATED MUCOSAL FIV CHALLENGE ON FELINE TOLL-LIKE RECEPTOR GENE EXPRESSION. K Cairns, S Kumar, S Leavell, BD Assogba, MJ Burkhard. Department of Veterinary Biosciences, College of Veterinary Medicine, Center for Retrovirus Research, The Ohio State University, Columbus, OH.

Toll-like receptors (TLRs) are important players in innate viral immunity. Endosomal TLRs (3,7,8,9) recognize viral nucleic acid while cell surface TLRs (2,4) sense viral protein. Many viruses activate TLRs, including cytomegalovirus, respiratory syncytial virus, and HIV. Although often protective, there are circumstances in

which TLR activation augments viral pathogenicity. Nine feline TLRs exist and in vitro FIV infection alters TLR expression. Alloantigen exposure also modulates anti-viral immunity. In cats, mucosal exposure to alloantigen induces cellular alloimmune responses that are associated with reduced viral burden after mucosal FIV challenge.

The goal of this study was to investigate TLR gene expression in feline peripheral blood mononuclear cells (PBMC), intra-epithelial (IEL) and lamina propria (LPL) lymphocytes and iliac (ILN), mesenteric (MLN) and popliteal (PLN) lymph nodes to determine if alloantigen exposure and cell-associated mucosal FIV challenge modulate TLR gene expression.

Specific pathogen free (SPF) cats were vaginally exposed weekly for 12 weeks to lymphocyte media (n = 7) or allogeneic PBMC in media (n = 7). Blood samples were obtained prior to exposure. Twelve weeks post-exposure, 3 cats per group were euthanized to collect blood and tissues. Remaining animals (n = 4 per group) were vaginally challenged with cell-associated FIV. Blood and tissue samples were obtained 12 weeks post-challenge at euthanasia. RNA was extracted and TLR expression was analyzed by real-time RT-PCR. Standard curves were generated from plasmids containing each TLR and GAPDH and TLR copy number was normalized to GAPDH.

Basal TLR expression was variable based upon tissue type. Alloantigen exposure did not alter TLR expression in PBMC, IEL or LPL. However, it did modulate TLR expression in lymph nodes, particularly the MLN where TLR 1,4,5,6 were decreased and TLR 7,9 were increased. TLR expression was also decreased in ILN (TLR9) and PLN (TLR 2,3,8). These findings suggest diffuse nodal alloantigen processing, which impacts innate immunity via TLR signaling pathways.

FIV challenge significantly increased TLR 7 expression, decreased TLR 2 expression, and had variable effects on TLR 6,8,9 expression in most tissues. This is notable given the endosomal location of TLR 7, 8 and 9 and the importance of these TLRs in antiviral immunity. FIV exposure had variable effects on the other TLRs. These viral effects on TLR expression were also modulated by prior exposure to alloantigen.

These data indicate a role for TLRs in both alloantigen processing and host response to FIV. Previous data show allogeneic immune responses increase the threshold for susceptibility to mucosal FIV infection. These data suggest this effect may occur via TLR pathways. Additional studies are indicated to further characterize the role of both TLRs and alloantigen exposure in FIV.

ABSTRACT #8

DETECTION OF FELINE CALICIVIRUS RNA, FELINE HERPESVIRUS-1 DNA, AND *BARTONELLA* SPP. DNA IN TISSUES OF CATS WITH AND WITHOUT GINGIVOSTOMATITIS. K Dowers, N Wilkerson, JR Hawley, M Brewer, MR Lappin. Department of Clinical Sciences, Colorado State University, Ft. Collins, CO.

Gingivostomatitis (GS) is a common syndrome in cats; feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), and *Bartonella* spp. are common differential diagnoses. Currently, there is minimal information available concerning the presence of nucleic acids of these microbes in the oral tissues of cats with or without GS. The objective of this study was to report the prevalence rates of FCV RNA, FHV-1 DNA, and *Bartonella* spp. DNA in gingival tissues of cats.

Fresh tissue biopsies from affected areas of cats with GS (n = 42) were obtained and submitted on ice by practicing veterinarians throughout the United States. A 6 mm skin biopsy punch was used to collect a full thickness biopsy from the right palatoglossal arch of normal cats housed in a Humane Society (n = 19) in Colorado after euthanasia for reasons unrelated to the study. The normal cats had lived in the Humane Society for 1 to 33 days (median = 7 days) and 14 of 19 had been administered an intranasal, modified live FCV, FHV-1, and panleukopenia on admission. The majority of cats with GS had been vaccinated but it is unknown when the last inoculation was given. DNA and RNA were extracted from the fresh tissues by use of a commercially available kit and previously described PCR assays (FHV-1 and *Bartonella* spp.) or reverse transcriptase PCR assay (FCV) was used to amplify the target DNA or RNA.

FCV RNA was amplified from a statistically greater number of cats with GS than control cats (Fischer's exact test; p = 0.001). *Bartonella* spp. DNA was amplified only from cats with GS and all were *B. clarridgeiae* but the significance of these findings is unknown as *Bartonella* spp. are regionally defined by flea risk and the control cats came from a low flea risk state.

	Organisms		
Cat group	FCV	FHV-1	*Bartonella* spp.
GS	17/42 (40.5%)	2/42 (4.8%)	3/34 (8.8%)
Control	0/19 (0%)	3/19 (15.8%)	0/19 (0%)

The results suggest that FCV was associated with stomatitis in some of the cats with GS. In addition, administration of an intranasal modified live FCV containing vaccine approximately 1–33 days prior to oral tissue biopsy is unlikely to result in a positive result in the reverse transcriptase PCR assay for FCV used here.

ABSTRACT #9

PREVALENCE OF HEMOTROPIC MYCOPLASMA INFECTION IN THE FELINE SHELTER AND CLIENT-OWNED POPULATIONS OF SASKATOON AND EVALUATION OF THE PRECISION OF TWO FELINE HEMOTROPIC MYCOPLASMA QUALITATIVE PCR. BD Nibblett[1], E Snead[1], C Waldner[2], S Taylor[1], M Jackson[3]. [1]Department of Small Animal Clinical Sciences, [2]Department of Large Animal Clinical Sciences, [3]Department of Pathology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, CANADA.

The objective of part A of this study was to evaluate the prevalence of subclinical hemotropic Mycoplasma (HM) infection in two distinct feline populations: a local shelter population (SPCA) and a client-owned population. The objective of Part B of this study was to evaluate the inter- and intratest variability of two independent qualitative PCR assays used for the diagnosis of feline HM infections.

For part A, an EDTA and serum blood sample was collected from 58 SPCA and 57 client-owned cats determined to be healthy based on physical examination. The packed cell volume, total protein, and retroviral status were determined for each cat. In addition, each cat was screened for a subclinical HM infection using a) a qualitative polymerase chain reaction (PCR) assay for the 16S rRNA of *Mycoplasma haemofelis* and "*Candidatus* M. *haemominutum*", and b) cytologic evaluation of a blood smear looking for evidence of hemoplasma organisms.

In part B, blood samples from 44 cats were submitted to a second laboratory for determination of HM infection using another qualitative PCR assay to evaluate intertest variability. The blood samples included 26 samples from part A (6 positives and 20 negatives) and 18 samples from cats with historical clinical or subclinical HM infections. Where sample volume permitted, the PCR testing was repeated (16 of 44 samples at the first laboratory and 40 of 44 samples at the second laboratory) to assess the intratest variability for both PCR assays.

For part A, the prevalence of subclinical HM infection was 12% (7/58) in the SPCA population and 4% (2/57) in the client-owned population. Within the SPCA population, *M. haemofelis* was found in 5% (3/58) and "*Candidatus M. haemominutum*" in 7% (4/58) of cats. The two client-owned cats were infected with *M. haemofelis*. There was no statistically significant difference in the infection rate between the two populations (p=0.16). Across both populations, no risk factors for HM infection were identified (sex, age, neuter status, breed, retroviral status, external parasitism).

For part B, there was substantial agreement between the two independent PCR assays for *M. haemofelis* (κ=0.601 95%CI 0.32–0.89) as well as for "*Candidatus M. haemominutum*" (κ=0.70 95%CI 0.40–0.99). Both PCRs performed well with zero intratest variability respectively.

There is no difference in the subclinical HM infection rates between the SPCA and client-owned cat populations evaluated. In using PCR testing for HM infection, results between laboratories show substantial agreement, but not perfect agreement. This highlights the need for continued attention to standardization of PCR testing.

ABSTRACT #10

DETECTION OF CANINE DISTEMPER VIRUS RNA FROM BLOOD AND CONJUNCTIVAL SWABS COLLECTED FROM HEALTHY PUPPIES AFTER ADMINISTRATION OF A MODIFIED LIVE VACCINE. JH Burton, JK Veir, L Pearce, JR Hawley, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Reverse transcriptase polymerase chain reaction (RT-PCR) assays are now being used to detect canine distemper virus (CDV) RNA in samples from dogs to aid in the clinical diagnosis of infection. However, little information exists on whether administration of modified live CDV containing vaccines affects the test results. The objective of this study was to determine the duration of detectable levels of CDV RNA in blood and conjunctival cells from healthy puppies vaccinated with a modified-live CDV containing vaccine.

Of the 12 six week old puppies selected for study, six were from a bitch with a high CDV antibody titer (1:512) against CDV and six were from a bitch with a low CDV antibody titer (1:64). On day 0, the puppies from the bitch with the high CDV titer were seropositive and the puppies from the bitch with the low CDV titer were seronegative. A modified-live CDV containing vaccine was administered SQ on day 0 and blood and conjunctival swabs were collected on days 0, 2, 5, 7, 10, and 14. Fluorescent antibody (FA) staining of smears made from the conjunctival swabs and a conventional CDV RT-PCR on RNA extracted from whole blood and conjunctival swabs were performed on samples from all time points.

Positive FA test results were detected in one seronegative puppy on day 0, five seronegative puppies and five seropositive puppies on day 2, one seronegative puppy on day 5, and five seronegative puppies and one seropositive puppy on day 10. CDV RNA was not amplified from conjunctival swabs of any puppy on any day. CDV RNA was amplified from blood of three seronegative puppies on day 7.

Current CDV diagnostic test results are not specific for the disease and so positive CDV FA results on conjunctival smears and conventional CDV RT-PCR results on blood should be cautiously interpreted, especially in recently vaccinated, previously naïve dogs.

ABSTRACT #11

DETECTION OF CANINE PARVOVIRUS DNA FROM BLOOD AND FECES COLLECTED FROM HEALTHY PUPPIES AFTER ADMINISTRATION OF MODIFIED LIVE VACCINE. JH Burton, JK Veir, AK Morris, JR Hawley, MR Lappin. Department of Clinical Sciences, Colorado State University, Ft. Collins, CO.

Polymerase chain reaction (PCR) assays are now being used to detect canine parvovirus (CPV) DNA in samples from dogs to aid in the clinical diagnosis of infection. However, little information exists on whether administration of modified live CPV containing vaccines affects the test results. The objective of this study was to determine the duration of detectable levels of CPV DNA in blood and CPV antigen in feces from healthy puppies vaccinated with a modified-live CPV containing vaccine.

Of the 12 six week old puppies selected for study, six were from a bitch with a high CPV antibody titer (1:640) and six were from a bitch with a low CPV antibody titer (1:10). On day 0, all puppies were seronegative, regardless of maternal antibody status. A modified-live CPV containing vaccine was administered SQ on day 0 and blood and feces were collected on days 0, 2, 5, 7, 10, and 14. Conventional CPV PCR was performed on DNA extracted from each of the whole blood samples. Two commercially available CPV antigen ELISAs were performed on each of the fecal samples.

CPV antigen was detected in feces of one dog by one of the ELISAs on day 5. CPV DNA was amplified from blood of some dogs on day 2 (8 dogs), day 5 (7 dogs), day 7 (8 dogs), day 10 (6 dogs), and day 14 (12 dogs). CPV antibody titers and CPV PCR results were available for all dogs on days 7, 10, and 14. Of the 26 samples with positive CPV PCR results, 21 were concurrently positive for CPV antibodies (titer range 64–16,384; median titer = 16,384).

The modified live vaccine used here was unlikely to result in positive results in the fecal antigen ELISAs studied. However, the CPV PCR assay could detect CPV DNA in many vaccinated puppies regardless of antibody titer. Positive CPV PCR assay results from blood need to be interpreted cautiously, especially in recently vaccinated, previously naïve dogs.

ABSTRACT #12

DURATION OF INFECTION AND EFFICACY OF DOXYCYCLINE TREATMENT IN DOGS EXPERIMENTALLY CO-INFECTED WITH *ANAPLASMA PLATYS* AND *EHRLICHIA CANIS*. MJ Beall[1], SD Gaunt[2], R Chandrashekar[1], K DeBisceglie[1], B Thatcher[1], PPVP Diniz[3], EB Breitschwerdt[3]. [1]IDEXX Laboratories Inc., Westbrook, ME; [2]Louisiana State University School of Veterinary Medicine, Baton Rouge, LA; [3]North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Rhipicephalus sanguineus is a ubiquitous tick responsible for transmitting *Ehrlichia canis* and most likely *Anaplasma platys* to dogs, as either single or co-infections. The occurrence of co-infection may be difficult to diagnose since thrombocytopenia and anemia result from either infection. The purpose of this study was to evaluate the clinical significance of *A. platys* and *E. canis* co-infection in dogs. Results from the initial phase of the study were reported previously and revealed a more severe thrombocytopenia and anemia in co-infected dogs as compared to single infections with either organism (ACVIM 2007). Results from the chronic phase of the study, which evaluated the duration of *A. platys* and *E. canis* infection and treatment efficacy, are reported here. Six month old female hound-type dogs were inoculated intravenously with Louisiana isolates of *A. platys* and/or *E. canis*. Six groups of six dogs each were evaluated: non-infected controls, *A. platys* infected, *E. canis* infected, *A. platys* and *E. canis* co-infected, *A. platys* infected administered *E. canis* 112 days later, and *E. canis* infected administered *A. platys* 112 days later. Doxycycline treatment (10 mg/kg PO daily × 28d) was initiated at 211 days post-infection (PI), followed by immunosuppression (dexamethasone 0.3 mg/kg IM daily × 5 d) beginning 410 days PI. Molecular evidence of infection was assessed by two independent laboratories using either conventional or real-time PCR on whole blood collected during the study, as well as bone marrow and lymph node aspirates collected at the time of immunosuppression. Thrombocyte counts in dogs infected only with *A. platys* returned to normal by 115 days PI and PCR results were consistently negative after 110 days PI, prior to doxycycline treatment. Most *A. platys*/*E. canis* co-infected dogs (8/9) were also PCR negative in blood for *A. platys* prior to doxycycline treatment. In contrast, all untreated *E. canis* infected dogs (12/12), whether single or co-infected, remained *E. canis* PCR positive in blood for more than a year and had reduced platelet concentrations (mean 125,600/uL). Most of these dogs also tested positive by PCR for *E. canis* DNA in bone marrow (8/12) and lymph node (12/12) following immunosuppression. All *E. canis* infected dogs, both single and co-infected, receiving doxycycline (12/12) were *E. canis* PCR negative in blood and lymph node prior to immunosuppression and had normal thrombocyte concentrations (mean 308,600/uL). Despite immunosuppression, neither *A. platys* nor *E. canis* DNA was detected in doxycycline-treated dogs. Although co-infection of *A. platys* and *E. canis* may result in more severe hematologic abnormalities, these experimental infections can be successfully treated with doxycycline.

ABSTRACT #13

ATOVAQUONE AND AZITHROMYCIN FOR THE TREATMENT OF *CYTAUXZOON FELIS*. AJ Birkenheuer[1], LA Cohn[2], MG Levy[1], EB Breitschwerdt[1], HS Marr[1]. [1]North Carolina State University—College of Veterinary Medicine, Raleigh, NC. [2]University of Missouri—College of Veterinary Medicine, Columbia, MO.

Cytauxzoonosis is an emerging infectious disease of domestic cats in the United States. Historically *Cytauxzoon felis* was believed to be uniformly fatal in domestic cats. However, over the past decade there have been scattered reports of cats with and without anti-protozoal drug therapy surviving *C. felis* infections. Atovaquone and azithromycin has recently been demonstrated to be the most effective therapy for the treatment of canine babesiosis caused by *Babesia gibsoni*, and organism related to *C. felis*. The purpose of this study is to describe the survival rate of cats with cytauxzoonosis that were treated with atovaquone and azithromycin combination therapy.

Twenty-two cats from AR, NC, and MO with naturally occurring cytauxzoonosis (infections confirmed by microscopy and/or poly-

merase chain reaction) were treated with atovaquone (15 mg/kg PO TID) and azithromycin (10 mg/kg PO Q24). Additional supportive care varied between cases, but all cases were treated with intravenous fluids and most with heparin. Sixty-four percent (14/22) of the cats survived to discharge and 36% (8/22) died or were euthanized. At least 6 of the cats that died did so within several hours of admission. Detailed clinical and laboratory data were not available for all cases. Long-term follow-up information was available for six cats and they all are alive and clinically normal at least 19 months after discharge. Each of the veterinarians that participated in treatment of cats for this study had prior experience in treatment of cytauxzoonosis with either supportive care alone or supportive care plus imidocarb dipropionate. Prior to the beginning of this study, survival of *C. felis* infected cats from their practices was a rare occurrence.

Survival of these cases cannot clearly be attributed to the antiprotozooal therapy, but atovaquone and azithromycin combination therapy appears to be a promising treatment option for cytauxzoonosis. Cats that survive cytauxzoonosis can have complete resolution of signs and excellent long-term prognosis. A controlled study comparing the efficacy of atovaquone and azithromycin to imidocarb dipropionate is ongoing.

ABSTRACT #14

COMPARISON OF TWO DRUG PROTOCOLS FOR CLEARANCE OF *CYTAUXZOON FELIS* INFECTIONS. LA Cohn[1], AJ Birkenheuer[2], E Ratcliff[3]. [1]University of Missouri – College of Veterinary Medicine, Columbia, MO[2]. North Carolina State University—College of Veterinary Medicine, Raleigh, NC[3]. Fairgrove Veterinary Hospital, Fairgrove, MO.

Although infection of domestic cats with *Cytauxzoon felis* is usual fatal, some cats survive. The piroplasms stage of the protozoal pathogen may persist in the surviving cats indefinitely, as it does in the bobcat reservoir host. The purpose of this prospective, randomized clinical trial was to compare the efficacy of two treatment protocols for the eradication of parasites in persistently infected cats. A colony of cats with multiple survivors of *C. felis* was identified. Nine cats positive for *C. felis* by PCR were randomly divided into two groups. All cats were treated topically with fipronil q 3 weeks throughout the study period. Imidocarb dipropionate was administered to the first group (4 mg/kg IM twice, 2 weeks apart) and atovaquone (15 mg/kg PO q 8 hr) and azithromycin (10 mg/kg PO q 24 hours) were administered to the second group for 10 days. Blood was obtained from each cat immediately before treatment and again at 1, 6, and 8 weeks after treatment. Stained smears were examined microscopically, and samples underwent PCR testing for *C. felis* DNA using previously described methods. Cats that failed to clear the infection at either 6 or 8 weeks were "crossed over" and the alternative treatment administered with identical follow-up.

Four cats were initially treated with imidocarb and 5 with atovaquone/azithromycin. All cats treated with imidocarb remained persistently infected at all 3 time periods afterwards. Of the cats treated with atovaquone/azithromycin, 2 cats tested negative at 1, 6, and 8 weeks; 1 cat was positive at 1 week only, another at 6 weeks only, and yet another at 8 weeks only. After the 4 cats initially treated with imidocarb were then treated with atovaquone/azithromycin, 1 cat tested negative at 1, 6, and 8 weeks, 1 cat tested negative at 1 week and was lost to follow-up, and 2 cats tested positive at 1 week but negative at weeks 6 and 8. The two cats that initially "failed" treatment with atovaquone/azithromycin went on to receive imidocarb, and one cat tested positive at 1, 6, and 8 weeks while the other tested negative on all 3 post-treatment dates.

Our results demonstrate that although neither treatment completely eliminates parasitemia due to *C. felis* infection, the parasite burden is greatly decreased by treatment with atovaquone/azithromycin as compared to imidocarb dipropionate. It is unclear if persistently infected cats can serve as competent reservoirs for infection when bitten by a tick. If so, a marked reduction in the circulating parasite load might reduce the likelihood that surviving cats infect naïve ticks. Certainly, imidocarb dipropionate does not effectively reduce parasitemia in persistently infected cats.

ABSTRACT #15

SERUM THYMIDINE KINASE CONCENTRATIONS IN NORMAL VERSUS TUMOR-BEARING DOGS. Selting KA[1] and Thamm DH[2]. [1]University of Missouri, Columbia, MO.[2]Animal Cancer Center, Colorado State University (ACC-CSU), Fort Collins, CO.

Proliferation indices have been histochemically evaluated in tumor tissue and can predict biologic behavior more precisely than tumor grade in some cases. Thymidine kinase (TK) is a soluble biomarker present in S-phase of a salvage pathway for DNA synthesis, and can be measured in serum. TK activity correlates with stage, prognosis, and relapse in dogs and humans with lymphoma. The purpose of this study was to compare TK concentrations among dogs with various tumors and to healthy dogs.

Serum samples previously stored at −80 °C at the ACC-CSU were assayed: transitional cell carcinoma (TCC, n=18), osteosarcoma (n=15), lymphoma (n=14, with 9 B-cell, 3 T-cell, and 2 null cell), and hemangiosarcoma (n=18). Sera from age-matched clinically normal dogs comprised a control population (n=33). An ELISA using AZT as a TK1 substrate was used. Comparisons among groups were made using 1- and 2-tailed student T-tests as appropriate.

TK activity in normal canine serum ranged from 0 to 5.6 U/L (mean, 2.8 U/L). An upper limit of normal (mean+2SD) was established at 6.16 U/L. TK activity was significantly higher than control ($p<0.0001$) in dogs with hemangiosarcoma and lymphoma (mean+/−SD = 34.2+/−36.5 and 59.0+/−43.4, respectively). There was a significant difference between B- and T- cell lymphoma (77 vs. 1.2 U/L, $p=0.0035$). In 22%, 13%, 71%, and 80% of dogs with TCC, osteosarcoma, lymphoma, and hemangiosarcoma, respectively, TK activity was above the normal range.

TK activity may be a useful for the early detection of canine lymphoma and hemangiosarcoma, and may help differentiate between lymphoma immunophenotypes in dogs.

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

ABSTRACT #16

CORRELATION OF SURVIVIN AND KI-67 IMMUNOREACTIVITY IN CANINE URINARY BLADDER TISSUES. W Velando Rankin, CJ Henry, SE Turnquist, JR Turk, JW Tyler, ME Beissenherz, JA Green. University of Missouri-Columbia, College of Veterinary Medicine, Columbia, MO.

Survivin is an inhibitor of apoptosis that we have previously identified in canine urinary bladder transitional cell carcinoma (TCC), normal bladder, and cystitis tissues. The purpose of this study was to correlate survivin expression with cell proliferation (assessed by Ki-67 immunoreactivity) in canine urinary bladder tissues.

Murine anti-Ki-67 antibody was used on formalin-fixed, paraffin-embedded TCC, normal urinary bladder, and cystitis samples that had been previously evaluated for nuclear and cytoplasmic survivin immunoreactivity. The total number of epithelial cells and cells positive for Ki-67 were manually counted in ten 400× fields per sample; counts were expressed as a percentage of total cells.

TCC samples without nuclear survivin (n=8) had an average Ki-67 score of 18% compared to 31.2% for those samples with nuclear survivin (n=11) (P=0.02). Cystitis tissues without nuclear survivin (n=11) had an average Ki-67 score of 7.1% compared to 44.6% in those with nuclear survivin (n=12) (P<0.001). Normal bladder tissues lacked nuclear survivin immunoreactivity. Tumor samples without (n=12) cytoplasmic survivin and those with cytoplasmic survivin (n=7) had no significant difference in average Ki-67 scores (23% versus 27.2%, P=0.5). Normal tissues without (n=26) and with (n=16) cytoplasmic survivin had average Ki-67 scores of 2.2% and 2.7% (P=0.4). Only one cystitis sample had cytoplasmic survivin.

Urinary bladder samples with nuclear survivin have higher Ki-67 immunoreactivity compared to those without nuclear survivin. Prospective studies are warranted to determine if nuclear survivin is associated with more aggressive disease and its potential as a prognostic tool or therapeutic target in canine urinary bladder TCC.

ABSTRACT #17

EXPRESSION OF GALECTIN-1, 3, AND 7 IN CANINE HEMANGIOSARCOMA. L Parshley, M Kiupel, D Sledge, T Allen, E McNiel. Michigan State University, East Lansing, MI.

Galectins are a family of carbohydrate binding proteins that have been shown to have diverse biological activities. Recent evidence points to a role for galectins in tumorigenesis through multiple mechanisms including angiogenesis, metastasis, and immune system evasion. To date there are limited data available for galectins and their role in canine cancers. The purpose of this study was to examine the expression levels and the patterns of expression of galectin-1, 3, and 7 in canine hemangiosarcoma (HSA).

Expressions of galectin-1, 3, and 7 were elevated in normal canine spleen and splenic hemangiosarcoma using western blot analysis and immunohistochemistry.

Semi-quantitative analysis of the western blots did not indicate significant expressional differences for galectin 1 or 3 in HSA and normal spleen. Expression of galectin 7 was variable but without significant pattern between tumors and normal spleen. Immunohistochemistry indicated no staining of tumor cells for galectin-1 and 7, whereas 15–20% of tumor and normal cells had weak cytoplasmic staining for galectin-3. There was no difference between individual tumors in galectin-3 staining. Inflammatory cells, particularly histiocytic cells, and reactive fibroblasts within in normal tissues and tumors had strong cytoplasmic signals for galectin-3. The stroma of tumor and normal tissues had weakly positive staining for galectin-1 without appreciable difference between individual tumors or normal tissue. Our data do not support a role for these galectins in canine hemangiosarcoma tumorigenesis, which is in contrast to our observations in canine epithelial malignancies.

ABSTRACT #18

URINE PROTEIN CREATININE RATIO IN BERNESE MOUNTAIN DOGS OF HIGH OR LOW RISK OF DEVELOPING MALIGNANT HISTIOCYTOSIS. L Nielsen, M Aronsson, E Persson, F McEvoy, A Lundorff Jensen, A-T Kristensen. Department of Small Animal Clinical Studies, LIFE, University of Copenhagen, Denmark.

Neoplastic conditions can result in proteinuria due to cancer related antigenic stimulation and subsequent glomerulonephropathy.

The aim of this study was to establish the normal urine protein creatinine ratio (UPC) in a population of healthy Bernese mountain dogs (BMD) and to assess if there was a difference in UPC in dogs of higher risk of developing malignant histiocytosis (MH) compared to dogs with a low risk of developing this neoplasia. Thirty-one healthy BMD aged 4–6 years, half of which came from families with a history of neoplasia, were screened for MH. The dogs were examined by blood work, urinalysis including culture and sensitivity, thoracic radiography, and abdominal ultrasonography. The kidneys were examined specifically by UPC and by the resistance index. The UPC was performed by the enzymatic method.

Parameters were investigated using a multivariate analysis and linear correlations in SAS 9.0.

None of the dogs were diagnosed with renal disease or MH and there was no correlation between a family history of neoplasia and an elevated UPC. However most of the dogs had a UPC between 0.6 and 1 with the mean and standard deviation being 0.8−/+ 0.41, which is higher than that observed in published studies, including studies in other BMD.

The higher UPC could be explained by different methodology in the creatinine measurements or be due to slow progressive age related changes in the kidneys. Repeated UPC measurement in the same dogs to confirm proteinuria and further investigations of UPC in BMD affected by MH are warranted.

ABSTRACT #19

CANINE CUTANEOUS MAST CELL TUMORS: ASSOCIATIONS WITH SEX, NEUTER STATUS, AND BREED. CR White[1], E Procter-Gray[2], AE Hohenhaus[1], J Kelsey[2]. [1]The Animal Medical Center, New York, NY, [2]University of Massachusetts Medical School, Worcester, MA.

This study evaluated dogs with cutaneous mast cell tumors (MCT) seen at the Animal Medical Center (AMC) with regard to signalment and American Kennel Club (AKC) breed group.

This was a case-control study of dogs with grade 2 or 3 MCT (cases) diagnosed histologically at the AMC in 1997–1998 and 2005–2006. Cases (N=252) were identified by searching the AMC Pathology Department database. Controls (N=1608) were randomly selected from all dogs seen at the AMC during the 2 time periods.

Intact female (FI) dogs had a significantly lower relative risk for developing MCT when compared to spayed females (FS) (OR=6.36, 95%C.I [3.46–11.72], p<.001), castrated males (MC) (OR=4.23, 95%C.I. [2.26–7.92], p<.001), and intact males (MI) (OR=2.22, 95%C.I. [1.14–4.32], p=.02). Pairwise comparisons using the Scheffe adjustment for multiple comparisons showed that the 4 categories fell into 3 groups with respect to odds of MCT: FS> MC > intact dogs of both sexes. The odds ratio (OR) of having a MCT according to breed was examined for the 25 most popular breeds at the AMC relative to mixed breed dogs. Adjusting for age, sex, and year, Boxers had the highest OR of MCT. Yorkshire Terriers (OR=0.28, 95% C.I. [0.08–0.92], p=.04) had significantly lower odds of MCT than mixed breeds. When odds ratios for MCT were examined according to AKC grouping of breeds, the sporting (OR=2.50, 95% C.I. [1.64–3.79], p<.001) and working (OR=1.89, 95% C.I. [1.15–3.11], p=.01) groups were found to have a significantly increased OR for developing MCT. Logistic regression showed that within the AMC canine population the odds of MCT in dogs 6–10 years of age (OR=5.16, 95% C.I. [3.57–7.45], p<.001) and dogs >10 years old (OR=3.34, 95% C.I. [2.24–4.97], p<.001) were significantly higher than among dogs 0–5 years of age.

Breed	Odds Ratio	95% Confidence Interval	P-value
Boxer	5.26	2.55–10.87	<.001
Labrador	3.64	2.18–6.08	<.001
Pug	2.73	1.29–5.79	.009
Golden Retriever	2.14	1.15–4.00	.017

This study identifies a previously unreported association between neuter status and MCT. It also confirms previously reported breed predilections for the development of MCT and identifies specific AKC breed groups with higher odds of this tumor.

ABSTRACT #20

Abstract withdrawn.

ABSTRACT #21

HYDROPULSION TO BIOPSY AND DEBULK NASAL TUMORS. EA Ashbaugh[1], BC McKiernan[2], CJ Miller[1], B Powers[3]. [1]Wheat Ridge Veterinary Specialists, Wheat Ridge, CO; [2]Southern Oregon Veterinary Specialty Clinic, Medford, OR; [3]Colorado State University, Fort Collins, CO.

Intranasal neoplasms of dogs and cats account for approximately 1% of all neoplasia in these species. Rhinoscopy accompanied with histopathology is the most definitive method to confirm a diagnosis of nasal neoplasia. It has been shown that rhinoscopic obtained biopsies can miss the diagnosis of nasal tumor up to almost 17% of the time. A relatively noninvasive technique to obtain a larger portion of mass tissue would help to increase the specificity of nasal biopsy. A technique for nasal hydropulsion is described here that allows for collection of a large portion of nasal tissue mass. This technique also has value by immediately improving clinical signs while awaiting histology results and treatment options.

Between January 2006 and January 2007, 24 patients (17 dogs and 7 cats) were evaluated with rhinoscopy and a nasal mass confirmed at Wheat Ridge Veterinary Specialists. Rhinoscopy of the anterior and posterior nasal cavities using both a 2.7-mm multipurpose rigid telescope and a 5.0-mm flexible bronchoscope is performed. Once a nasal mass is confirmed, hydropulsion is performed as described: One side of the nares is digitally occluded. A 20–60 cc syringe containing room temperature sterile saline is inserted in the contralateral past the alar fold and sealing it with digital pressure. Typically a large catheter tip syringe is used except for small cats

and dogs where a regular leur tip syringe could only be fitted into the nares. The saline (20–60cc volume) is forcefully infused, or hydropulsed, into the nasal cavity. This process is repeated on the contralateral nares. Tissue is collected from the wet laboratory table or the oropharynx and preserved in 10% buffered formalin for histopathological evaluation. The oropharynx is suctioned and cleaned thoroughly after the procedures The patient is then recovered routinely.

Hydropulsion was attempted in all 24 patients with a visible nasal mass. A diagnostic sample was successfully dislodged from the nasal cavity in 22 of 24 nasal tumors (92% success rate). Cats had a 100% success rate of diagnostic specimen obtained whereas dogs success rate was slightly lower at 88%. Nasal tumors that were identified include adenocarcinoma (5), other carcinomas (8), lymphoma (2), osteosarcoma (1), other sarcomas (5), and one spindle cell tumor. Two cats and three dogs had hydropulsion performed multiple times in order to alleviate recurring clinical signs of obstructive nasal breathing and epistaxis. The symptom free interval ranged from four months to fifteen months for these patients. In the 2 cases where hydropulsion was unsuccessful, endoscopic pinch biopsies or true cut biopsies (muti-lobulated osteosarcoma) were obtained. Postoperative complications included sneezing, mild postoperative epistaxis, and rarely, temporary retrobulbar swelling with subsequent bupthalmia.

Hydropulsion allows for minimally invasive debulking of some tumors, providing clinical relief and biopsy collection to direct further therapies.

ABSTRACT #22

SUBCUTANEOUS VERSUS MUCOSAL (INTRANASAL) ALLERGEN-SPECIFIC RUSH IMMUNOTHERAPY IN EXPERIMENTAL FELINE ASTHMA. TM Lee-Fowler, LA Cohn, AE DeClue, CR Reinero. University of Missouri, College of Veterinary Medicine, Columbia, MO.

Allergen specific immunotherapy addresses a dysregulated (Th2) immune response to aeroallergens. Previously, subcutaneous rush immunotherapy (SC RIT) was shown to dampen eosinophilic airway inflammation in experimental feline asthma; however, side effects were noted. In humans, mucosal immunotherapy has an improved safety and efficacy profile compared with SC RIT. In this study, we hypothesized that IN RIT would be as efficacious as and safer than SC RIT.

Twelve cats were sensitized and challenged with bermuda grass allergen (BGA) and randomly received SC or IN RIT. RIT was given over 2 days (doses of 20–200 mcg of BGA) followed by 200 mcg BGA weekly as maintenance. Adverse reactions were recorded. Bronchoalveolar lavage fluid (BALF) % eosinophils and BALF IL-4 and IFN-g concentrations were measured before RIT (Day 1) and at months 1, 3, and 6 (M1, M3, M6).

Twelve adverse reactions were seen with SC RIT vs. 6 with IN RIT; however, all were mild and self-limiting. BALF % eosinophils decreased after RIT in both groups (mean±SEM, SC RIT D1 62±12, M6 9±4; IN RIT D1 54±9, M6 14±6). The BALF IL-4:IFNg ratio decreased in both groups (mean±SEM, SC RIT D1 1.6±.4, M6 1.2±.3; IN RIT D1 2.4±.2, M6 1.0±.2).

Both protocols dampened airway eosinophilia and altered the Th2:Th1 cytokine profile locally; IN RIT had fewer adverse events. Either could be considered for treating allergic asthma.

ABSTRACT #23

BERMUDA GRASS ALLERGEN IMMUNOTHERAPY EXERTS CROSS PROTECTION IN HOUSE DUST MITE SENSITIZED CATS WITH EXPERIMENTAL ASTHMA. CR Reinero, TM Lee, LA Cohn, R Cohen, AE DeClue. University of Missouri, College of Veterinary Medicine, Columbia MO.

Allergen-specific immunotherapy relies on identification of the specific allergen(s) to which animals have been sensitized. Administration of Bermuda grass allergen-specific rush immunotherapy (BGA-RIT) in cats sensitized and challenged with BGA has previously been shown to confer protection (i.e., dampen eosinophilic airway inflammation). Since it may be difficult to determine which specific allergens are responsible for inducing the asthmatic phenotype in cats with naturally developing asthma, we undertook a study to evaluate if administration of RIT using a different allergen to which the animal has been sensitized would have beneficial effects on the asthmatic phenotype. We hypothesized that administration of BGA-RIT to cats sensitized to house dust mite allergen (HDMA) would at least in part blunt eosinophilic airway inflammation. Nine cats were enrolled: group 1 (n=3) sensitized to BGA, group 2 (n=4) sensitized to HDMA, group 3 (n=2) placebo (saline) sensitized. Cats received weekly aerosol challenges of BGA, HDMA, and saline, respectively, for the duration of the study. All cats received subcutaneous BGA RIT over a 2 day period (dose escalation to 200 mcg BGA), followed by weekly sc injections of BGA (200 mcg). Bronchoalveolar lavage fluid (BALF) was collected prior to BGA-RIT (D0) and at month 1 (M1). Cytospins of the BALF were made and differential counts performed. Results showed that the BALF eosinophil % decreased in cats sensitized to both BGA and to HDMA (mean±SEM, group 1 D0 26±12, M1 4±1; group 2 D047±15, M1 16±3; group 3, D0 1±1, M1 1±1). Additional cats are being enrolled into this study. Preliminary results suggest that immunotherapy using one allergen exerts beneficial cross protection to asthmatic cats sensitized to an unrelated allergen.

ABSTRACT #24

DOSE EFFECTS OF FLUTICASONE PROPIONATE IN AN EXPERIMENTAL MODEL OF FELINE ASTHMA. LA Cohn, AE DeClue, RL Cohen, CR. Reinero. University of Missouri – College of Veterinary Medicine, Columbia, MO.

Feline asthma is an inflammatory lower airway disorder affecting up to 1% of cats. Treatment relies on administration of corticosteroids; inhaled corticosteroids may result in control of airway inflammation with fewer systemic effects than oral or injectable preparations. Currently, there is no evidence as to the appropriate dose of inhaled corticosteroid to control airway inflammation in cats. The goal of this research was to compare efficacy of 44 μg, 110 μg, or 220 μg fluticasone propionate delivered by inhalation twice daily for 21 days on eosinophilic airway inflammation in cats with experimentally-induced asthma. We hypothesized that the 44 μg would control airway inflammation equally as well as higher dosage formulations.

Six kittens were sensitized to Bermuda grass allergen to create an asthmatic phenotype; a minimum of >15% eosinophils on airway lavage was documented prior to treatment. Cats were randomized to one of three treatment groups, and then received the fluticasone dose appropriate for the group (44 μg, 110 μg, or 220 μg) twice daily for 21 days. Evaluation included bronchoalveolar lavage (BAL) with enumeration of eosinophils at baseline and after completion of the 21 day treatment. After a four week wash-out period, cats were crossed-over to another dose group, and the procedures repeated until each cat had received all three dose regimens. Results were expressed as means ±SD. Base line treatments were compared by ANOVA. Pre and post treatment eosinophil percentage was compared via paired T-test.

Usable data were obtained for only 5 cats for the 44 μg and 220 μg doses due to either inadequate baseline eosinophilia or poor sampling. Airway eosinophils were not different between dose treatment groups at baseline (p=0.225; 44 μg dose 25.5± 14.4% eos; 110 μg dose 41.0± 26.8% eos; 220 μg dose 22.3± 12.7% eos). After treatment, airway eosinophilia was reduced in all treatment groups as compared to pretreatment values (44 μg dose 6.6±4.6% p = 0.021; 110 μg dose 8.3±9.2% p = 0.018; 220 μg dose 4.8±4.1% p = 0.01). In conclusion, it appears that all three dosages (44, 110, and 220 μg/cat BID) of inhaled fluticasone were able to significantly diminish airway eosinophilia. Studies should be performed to evaluate clinical efficacy of 44 μg/cat BID fluticasone in cats with naturally occurring feline bronchopulmonary disease.

ABSTRACT #25

COMPARISON OF CULTURE AND POLYMERASE CHAIN REACTION FOR THE DETECTION OF *MYCOPLASMA* SPECIES IN CANINE AND FELINE RESPIRATORY TRACT SAMPLES. A Cruse, W Ratterree, S Sanchez, A Koenig. University of Georgia, Athens, GA.

Mycoplasma species commonly colonize the respiratory tract of normal dogs and cats and have also been shown to be a respiratory pathogen. Culture is the gold standard for identifying *Mycoplasma*

sp; however, the organism is notoriously difficult to grow. PCR is faster and does not rely on organism viability for identification. The purpose of this study was to compare results of PCR and culture for the detection of *Mycoplasma* species in respiratory tract samples.

Records of the University of Georgia Veterinary Teaching Hospital and the Athens State Veterinary Diagnostic Lab were searched for dogs and cats with respiratory disease that had a Mycoplasma PCR and *Mycoplasma* culture (MC) performed on the same day from the same respiratory specimen. Data collected included site and method of sample collection and results of PCR, MC, other cultures, and cytology.

Results showed moderate agreement between PCR and culture for the detection of *Mycoplasma* (24 of 30 samples; kappa coefficient 0.59). Sensitivity and specificity of PCR were 81.8% and 78.9%, respectively, using MC as the gold standard. There were no significant differences between proportion of false positives (13%) or false negatives (7%) (p=0.4142). The six discordant results were further characterized as four PCR+/MC− and two PCR−/MC+ samples. Sixty-seven percent (4/6) of the discordant results were obtained on mailed samples.

PCR compared favorably to culture for the identification of *Mycoplasma* spp. in respiratory tract samples obtained from dogs and cats with respiratory disease. Discordant results occurred more commonly in mailed samples.

ABSTRACT #26
RADIOLOGICAL CHANGES IN FOXES (*VULPES VULPES*) EXPERIMENTALLY INFECTED WITH *ANGIOSTRONGYLUS VASORUM*. Willesen JL[1], McEvoy FJ[1], Webster P[2], Monrad J[2], Jensen AT[3], Svalastoga EL[1], Koch J[1]. [1]Dept of Small Anim. Clin. Sci. [2]Centre of Exp. Parasitol. [3]Dept. of Nat. Sci., University of Copenhagen, Copenhagen, Denmark.

Clinical signs of canine pulmonary angiostrongylosis (CPA) are primarily from the respiratory tract and are caused by the presence and migration of adult worms and the first-stage larvae of *Angiostrongylus vasorum* in the pulmonary arteries and parenchyma. The clinical assessment is often supported by thoracic radiographs. The objective of the study was to investigate the effect of age and worm burden on the development of radiological manifestations of CPA and develop an objective radiological score of pulmonary changes in CPA using an experimental fox model.

Thirty-four foxes were grouped after age (young and adult), infective dose (50 and 200 third-stage larvae) and controls. Ten weeks after inoculation, the radiographic studies were performed. Subsequently, the foxes were euthanized and burdens of adult worms established. Radiographs were assessed blinded by two of the authors following a predefined scoring system. Also, the type of lung pattern and anatomic location in the lung field were recorded. Based on these parameters, the radiologists scored each set of radiographs in one of four categories according to severity. Mean and 95% confidence interval (CI) of worm count were calculated. A Fisher's exact test was performed to determine differences in variables across dose and age groups. Inter- and intra-observer agreement was tested using kappa statistics. Worm count and radiological score were analyzed using linear regression, and one way ANOVA tested the relationship between worm burden and age or dose. The study was conducted under a Danish experimental animal licence.

The score for pulmonary changes differed significantly between control and infection groups. Radiological differentiation between high and low-dose groups was possible if data from the accessory and cranial lung only were considered. A linear relationship could be shown between the radiological score versus worm count. The mean worm count of the young, high-dose foxes was significantly higher than in all the other infection groups ($p < .0001$). Inter-observer variation in classifying the pattern of pulmonary changes was poor to moderate (κ-values of 0.12 to 0.53). Despite these inter-observer differences in the pattern of pulmonary changes, the overall radiological score for lung pathology was in agreement with inter-observer κ-values of 0.62.

In conclusion, an objective, radiological scoring system was developed that may allow reliable, reproducible assessment of the severity of pulmonary changes in an experimental *A. vasorum* fox model. The anatomical distribution of lesions is more severe and more frequent in the peripheral and caudal areas of the lung, and supports previous data. This is similar to the changes reported in dogs with spontaneous CPA. Finally, the finding of a relationship between age and severity of lesions and number of adult worms recovered seems to indicate an age related immunity in the development of CPA.

ABSTRACT #27
BALLOON-EXPANDABLE METALLIC STENT PLACEMENT FOR BENIGN NASOPHARYNGEAL STENOSIS IN 4 DOGS AND 3 CATS. A Berent, C Weisse, M Rondeau, A Reiter. Matthew J. Ryan Veterinary Hospital of The University of Pennsylvania, Philadelphia, PA.

Nasopharyngeal stenosis (NPS) is a pathologic narrowing within the nasopharynx caudal to the choanae, resulting in a variable degree of inspiratory stertor. This can occur as a congenital anomaly or be secondary to an inflammatory condition, surgery, trauma, or a space-occupying lesion. Traditional therapy involves surgery or serial balloon dilatation procedures. The purpose of the present study was to describe a novel, minimally invasive technique and clinical outcomes following balloon-expandable metallic stent (BEMS) placement for the treatment of benign NPS in veterinary patients.

Four dogs and three cats were diagnosed via computed tomography and rhinoscopy. Using fluoroscopy and retroflex rhinoscopy, a BEMS was advanced over a guidewire through the nares. The stenotic lesion was then dilated to restore patency.

All seven patients presented with severe inspiratory stertor and evidence of upper airflow obstruction. All patients had immediate resolution of signs after stent placement. The procedure took a median of 42 minutes (range 22–70 minutes). One patient had stricture in-growth into the stent resulting in stenosis recurrence; another patient with a very caudal stenosis needed the caudal aspect of the stent trimmed because of hairball entrapment and exaggerated swallowing four months after stent placement. All animals lacked signs of discomfort, had immediate resolution of stertor, and 6/7 were breathing normally at the time of this report (1–20 months after stent placement).

Transnasal BEMS placement represents a short, safe, non-invasive and effective treatment in animals with nasopharyngeal stenosis.

ABSTRACT #28
THE UTILITY OF NT-proBNP TO DIFFERENTIATE CARDIAC AND RESPIRATORY CAUSES OF DYSPNEA IN CATS. G Wess, P Daisenberger, J Hirschberger. Clinic for Small Animal Internal Medicine, LMU University of Munich, Germany.

In clinical practice it is necessary to differentiate cardiac and respiratory causes of dyspnea, as treatment of cardiac and respiratory cases is very different. This differentiation however is often not easy and expensive, but important for further decisions on diagnostics and therapy. Measurement of B-type Natriuretic Peptide concentrations (BNP) is helpful in distinguishing cardiac from noncardiac causes of dyspnea in dogs and humans. Previous canine studies have shown elevated BNP and NT-proBNP in dogs with congestive heart failure. BNP is synthesized as a prohormone proBNP, secreted into the blood stream, and cleaved into N-Terminal ProBNP (Nt-proBNP) and BNP. Nt-proBNP has no physiological activity, but is more stable than BNP and therefore easier to measure.

The purpose of this prospective study was to evaluate the utility of Nt-proBNP to differentiate cardiac and respiratory causes of dyspnea in cats. A healthy control group was used to obtain reference values for Nt-proBNP.

Nt-proBNP was measured in plasma samples from 74 cats using an ELISA antibody assay (VETSIGN Feline CardioSCREEN Nt-proBNP, Guildhay Ltd, UK). The cats were classified according to echocardiography and thoracic x-rays into one of the following groups: clinical healthy (control) group (n=33, mean age 4.6 years), cats with dyspnea due to respiratory causes (n=21, mean age 8.7 years) and dyspnea due to cardiac causes (n=20, mean age 8.7 years), Cardiac causes were decompensated hypertrophic cardiomyopathy (n=14), restrictive cardiomyopathy (n=4), and DCM (n=2).

There was no significant difference ($p < 0.001$) between the control group (mean Nt-proBNP 120+/− 107 pmol/l) and the respiratory

group (mean Nt-proBNP 170+/− 143 pmol/l). Nt-proBNP values of cats with dyspnea due to cardiac causes were significantly ($p < 0.001$) higher than in the other groups (mean Nt-proBNP 686+/− 368 pmol/l). Using a cut-off value of 277 pmol/l Nt-proBNP had a sensitivity of 95.0% and a specificity of 84.6% for the differentiation between cardiac and respiratory causes of dyspnea in cats.

In conclusion, this feline ELISA Nt-proBNP assay was helpful in the diagnosis of CHF in cats and had a very high sensitivity and good specificity to differentiate between cardiac and respiratory causes of dyspnea.

ABSTRACT #29

DOPAMINE ANTAGONIST STIMULATION TEST IN HORSES (EFFECT OF DOSE AND TESTING INTERVAL ON PLASMA ENDOGENOUS ACTH). LP Jackson, JE Sojka, GE Moore, BD Denton, MA Miller. Purdue University, West Lafayette, IN.

Equine pituitary pars intermedia dysfunction (PPID) is a chronic progressive disease that occurs secondary to lack of dompaminergic inhibition on pituitary melanotrophs. It was our hypothesis that administration of a dopamine antagonist, domperidone, would result in an exaggerated increase in endogenous adrenocorticotropic hormone (ACTH). A pilot study demonstrated that ACTH levels are significantly elevated after domperidone administration in PPID horses, but the appropriate test dosage and interval have not been determined.

The response of ACTH to different doses of domperidone was evaluated in 8 horses (5 PPID and 3 control). While horses were initially classified by clinical signs, definitive classification was made by pituitary histopathology. Blood was collected immediately prior to an 8 AM dose of domperidone and every 2 hours for 8 hours following one of 4 doses (control, 0.5 mg/kg, 1.25 mg/kg, and 5 mg/kg PO). Blood was collected in silicon coated EDTA tubes. Endogenous ACTH concentration (pg/ml) was determined using a chemiluminescent immunoassay. Receiver operating characteristic (ROC) curves were generated to quantify test accuracy, and area-under-the-curve (AUC) was calculated for the different domperidone doses and time points.

There was an increase ACTH in the PPID horses 2 and 4 hours post domperidone administration. The median (range) baseline ACTH in PPID horses was 53.8 pg/ml (15, 528) and 25.6 pg/ml (15.5, 30.9) in control horses. Following an oral 1.25 mg/kg dose of domperidone the median (range) ACTH was 145 pg/ml (29.7, 303) in horses with PPID versus 23.4 pg/ml (12.7, 105) in control horses at 2 hours, and 150.5 pg/ml (48.1, 365) in horses with PPID versus 33 pg/ml (26, 38.8) in control horses at 4 hours. After an oral 5 mg/kg dose of domperidone the median (range) ACTH was 144.5 pg/ml (80.9, 662) in horses with PPID versus 28.9 pg/ml (12.1, 63.4) in control horses at 2 hours and 170.5 pg/ml (43.6, 808) in horses with PPID and 29 pg/ml (13.5, 57.9) in control horses at 4 hours (Table 1).

In conclusion, the changes in ACTH in response to the medium (1.25 mg/kg) and high dose (5 mg/kg) domperidone were able to differentiate PPID horses from control horses at both 2 and 4 hours post domperidone administration.

Table 1. Area under the ROC curve (95% CI) at 2 and 4 hrs

Time	Dose	AUC (95% CI)
2 hr	1.25 mg/kg	0.899 (0.631–1.000)
	5 mg/kg	1.000 (1.000–1.000)
4 hr	1.25 mg/kg	1.000 (1.000–1.000)
	5 mg/kg	0.944 (0.790–1.000)

ABSTRACT #30

THE RELATIONSHIP OF INFILTRATIVE LEUKOCYTES TO ADIPOSE TISSUE CHEMOKINE EXPRESSION AND INSULIN RESISTANCE IN DIET-INDUCED OBESITY IN HORSES. TA Burns[1], R Carter[2], J McCutcheon[2], R Geor[2], JK Belknap[1]. [1]The Ohio State University, Columbus, OH. [2]Virginia Tech University, Blacksburg, VA.

Equine metabolic syndrome is characterized by obesity (generalized or regional adiposity), insulin resistance, and increased risk of laminitis. In obesity in other species, fat depots function as "inflammatory organs" in which the production of chemokines such as monocyte chemoattractant protein (MCP) isoforms results in infiltration of the adipose tissue by macrophages. This abnormal milieu of macrophages and adipocytes is reportedly responsible for the production of inflammatory molecules including proinflammatory cytokines and plasminogen activator inhibitor-1 (PAI-1); whereas as the inflammatory molecules can be produced by both adipocytes and macrophages, the majority of PAI-1 is reported to be produced by macrophages. The release of these proinflammatory cytokines and PAI-1 into the circulation contributes to the decrease in insulin sensitivity observed in this syndrome. In a companion study of equine diet-induced obesity, 12 Arabian horses were documented to undergo an ~20% increase in bodyweight and a marked decrease in insulin sensitivity after being fed 200% of daily digestible energy requirements for 4 months. However, in samples of subcutaneous adipose tissue obtained before and after weight gain, we found minimal evidence of abnormal proinflammatory cytokine expression in the face of the marked decrease in insulin sensitivity. As these data are in contrast to findings in human obesity studies, and the presence or role of macrophages in adipose tissue in equine metabolic syndrome has not been previously determined, the present study investigated events concerning monocyte recruitment and macrophage presence in adipose tissue in horses with obesity-related insulin resistance. In biopsy samples of nuchal crest subcutaneous fat obtained from the 12 Arabian horses prior to and following weight gain, mRNA concentrations of MCP-1, MCP-2, and PAI-1 were quantified via real-time quantitative PCR. Formalin-fixed adipose tissue was processed for immunohistochemistry using an anti-CD13 primary antibody (surface marker for cells of myeloid lineage) for quantification of leukocytes pre and post weight gain. No CD13-positive cells were noted in any of the adipose tissue samples. However, the expression of MCP-2 and PAI-1 within adipose tissue increased ($P < 0.05$, Mann-Whitney U test) after weight gain, coincident with the decrease in insulin sensitivity. The results of this study suggest that the adipose tissue becomes a source of inflammatory mediators in the horse, but that unlike findings in human obesity, adipocytes may be the primary source of inflammatory mediator expression, minimally influenced by monocyte infiltration. However, other fat depots, including visceral adipose tissue, must be examined prior to generalizing these results to all fat depots in the horse.

ABSTRACT #31

VALIDATION OF A COMMERCIAL ENZYME IMMUNOASSAY FOR DETECTION OF THE PRESENCE OF *CLOSTRIDIUM DIFFICILE* TOXINS IN FECES OF HORSES WITH ACUTE DIARRHEA. CE Medina-Torres, JS Weese, H Staempfli. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Clostridium difficile has been implicated as the causative agent of acute diarrhea in adult horses and foals. A fast and accurate diagnosis of *C. difficile*-associated disease (CDAD) is of the outmost importance for an adequate therapeutic intervention. While the cell cytotoxicity assay (CTA) is considered the "Gold Standard", it is neither readily available nor conductive to routine testing, so enzyme immunoassays are typically used. The *C. DIFFICILE* TOX A/B II™ ELISA (TECHLAB® Inc; 2001 Kraft Drive, Blacksburg, VA 24060-6358) has been validated in humans, but has been reported to have low sensitivity and specificity in dogs. While this test is widely used in horses, it performance on equine feces is unclear.

The objective of the present study was to determine the performance of the *C. DIFFICILE* TOX A/B II™ ELISA, and validate its use as a diagnostic tool for the detection of *Clostridium difficile* toxins in feces of horses with acute diarrhea.

Fecal samples were collected and processed prospectively from hospitalized horses with acute diarrhea for which the treating clinician requested *C. difficile* toxin testing. Samples were stored at 4 °C within 2 hours of collection, and processed within the following 2 weeks. The ELISA was performed in parallel with the CTA.

Out of a total of 72 fecal samples tested, 19 (26.4%) were positive using the CTA. Sixteen (84.2%) CTA positive samples were positive on the ELISA, and 2 (3.8%) CTA negative results were positive on the ELISA. No significant difference was observed between the ELISA test and the "gold standard" using the McNemar's test [OR = 0.66 (95% confidence interval (CI) = 0.08, 4.28); 2-tailed p = 1.00], indicating that the probability of the ELISA being positive or negative was the same as that of the gold standard. The ELISA had a sensitivity of 84% [(CI = 61, 96%); median unbiased estimates (MUE) = 84%], a specificity of 96% [(CI = 87, 99%); MUE = 96%] and positive and negative predictive values of 89% [(CI = 67, 98%); MUE = 88%] and 94% [(CI = 85, 94%); MUE = 96%], respectively. A good agreement between the ELISA and the CTA was observed [Kappa = 0.82 (CI = 0.67, 0.97) (p-value under Ho of no agreement between tests is <0.0001)].

In conclusion, the overall performance of the test was adequate, being similar to that reported for human samples and superior to that reported for canine samples. The high level of agreement between the ELISA and the CTA indicates that the *C. DIFFICILE* TOX A/B II™ ELISA test is an adequate diagnostic tool and a reliable, practical test for the clinical diagnosis of CDAD in horses.

ABSTRACT #32

EFFECTS OF INTRARECTALLY ADMINISTERED OMEPRAZOLE PASTE ON GASTRIC JUICE PH IN ADULT HEALTHY HORSES. CL Rand, S Stanley, N Pusterla. School of Veterinary Medicine, University of California, Davis, CA.

Gastric ulcers in horses have been associated with stress, prolonged periods without feed, and low gastric fluid pH. Omeprazole is a proven anti-ulcer medication used in veterinary medicine for treatment of equine gastric ulcer syndrome. Horses have previously been shown to have an increase in gastric fluid pH following administration of both the oral and intravenous formulations of omeprazole. However, use of the oral formulation of omeprazole is often not possible in horses suffering from gastric reflux or neurologic conditions that prevent them from swallowing, and the intravenous formulation of omeprazole is expensive and can only be administered in a hospital environment. The goal of this study was to evaluate the effects of a commercially available omeprazole paste given intrarectally on the gastric juice pH of healthy adult horses.

Ten healthy adult horses were randomly assigned to one of two groups: 6 horses were assigned to the treatment group receiving 4.4 mg/kg omeprazole paste intrarectally for 5 consecutive days and 4 horses were assigned to the control group. Horses were fasted for 12 hours prior to gastric fluid collection. Gastric fluid was collected from each of the horses via nasogastric tube prior to (day 0) and 4 hours following the last day (day 5) of omeprazole administration. The gastric fluid pH was analyzed immediately following collection via a pH meter. Nonparametric analysis was used to compare the two groups against one another, as well as against baseline (day 0) measurements. Levels of significance were set at $p<0.05$.

Gastric juice pH was low in all study horses on day 0 and varied from 1.46 to 4.52 (mean±SD=3.45±1.3) for the treatment group and 2.14 to 4.46 (3.52±1.0) for the control group. On day 5, the gastric juice pH remained low in 4 treated horses (range 1.8 to 2.66), while 2 horses showed an increase in gastric pH ($pH>5.0$) that persisted for 24 hours following final intrarectal omeprazole paste administration. The gastric juice pH of all control horses remained low on day 5 (range 1.7 to 3.91, 3.0±1.0). No significant differences ($p>0.05$) in gastric juice pH were found when groups were compared against one another on day 0 and day 5 as well as against baseline.

In conclusion, the study has shown that omeprazole paste, when administered intrarectally, causes an increase in gastric juice pH in only 33% of the treated horses. Reasons for this variability could be related to proximity of defecation following drug administration, the formulation used in this study, or differing rates of absorption through the rectal mucosa. Further work is warranted at this time to determine if a different formulation of omeprazole would result in enhanced ability to suppress gastric acid secretion and increase gastric juice pH when administered intrarectally.

ABSTRACT #33

DETERMINATION OF 24-HOUR URINE PROTEIN EXCRETION AND CORRELATION WITH RANDOM-SAMPLE URINE PROTEIN:CREATININE RATIO IN EQUIDS. B. Uberti, DB Eberle, GE Moore, BM Pressler, JE Sojka. Purdue University, West Lafayette, IN.

Normal urine protein excretion has been determined in a variety of species, including humans, dogs, and cats. Excess urine protein loss of renal origin may occur due to primary renal diseases affecting the glomeruli, or secondary to systemic inflammatory diseases, presumptively secondary to inflammation. Proteinuria may be a useful early marker of inflammatory disease. The purpose of this study was to determine 24-hr urine protein excretion in normal equids and determine how well this value correlates with random-sample urine protein:creatinine ratio (UPC).

Urine was collected from 6 random-source adult mares and 6 adult female ponies. All animals were normal based on physical examination at the time of enrollment in the study, barren, and remained healthy throughout the study period. For 24-hr urine collection periods, urine was collected via an indwelling Foley catheter attached to a disposable closed collection system. After placement of the catheter the bladder was completely emptied. Urine was then evacuated at 4-hr intervals by means of a 1-way valve, volume was measured, and aliquots were frozen at −20 °C until later analysis. Ten percent of the urine collected from each of the 4-hr time points was pooled to create a representative 24-hr pooled sample. Single spot urine samples were also collected from the 6 mares on 3 different days using rigid mare urine collection catheters. Urine protein and creatinine were measured by standard methodology.

All results obtained from horses and ponies were sufficiently similar such that the 12 animals were evaluated as a single group; 24-hr urine protein excretion ranged from 403.7 to 3144.2 mg (mean 1451.2 mg; median 1513.5 mg; SD 771.2 mg). UPC in pooled samples showed excellent correlation to 24-hr total protein excretion values ($R^2=0.913$). UPC values measured from aliquots of each of the 4-hr time points likewise showed excellent correlation with 24-hr protein excretion and minimal change over the 24-hr period, with values fluctuating maximally by 156.8% from the pooled UPC value. UPC results from urine collected on different days likewise remained relatively consistent, with results changing maximally by 170.1% from the pooled UPC value; however, all values remained within normal values reported in other species.

This study provides a preliminary reference range for 24-hr urine protein excretion in equids, and establishes the reliability of UPC as a clinical estimator of 24-hr protein excretion. Future studies will increase the power of the results reported here, and investigate the use of the UPC as a diagnostic test and prognostic indicator in various systemic inflammatory diseases of equids.

ABSTRACT #34

THE PREVALENCE OF PLASMA TRANSFUSION REACTIONS AND THE PRESERVATION OF CLOTTING FACTORS, ANTI-THROMBIN, AND PROTEIN C IN EQUINE PLASMA. EM Wilson[1], SJ Holcombe[1], JG Hauptman[1], M Brooks[2]. [1]Michigan State University College of Veterinary Medicine, East Lansing, MI. [2]Comparative Coagulation Laboratory, Comparative Coagulation Section—Animal Health Diagnostic Laboratory, Cornell University, Ithaca, NY.

The hypothesis of this study was that acceptable levels of clotting factors, anti-thrombin (AT), and Protein C would be preserved in equine plasma following collection and storage and that the prevalence of plasma transfusion reactions would low and similar to other species.

The method of plasma harvest is as follows. Ten horses were anesthetized and blood was collected into sterile 5-liter fluid bags containing 250 ml of sodium citrate 4%. Once 25 liters of blood were harvested the horse was humanely euthanized. The blood was stored at 20° F for 48 hours in an upright position to allow separation of red blood cells via gravity sedimentation. The plasma was decanted into sterile 3-liter bags and stored at −20° F.

Three plasma samples were collected from each horse; S0 was collected prior to exsanguination and stored at −70° F for 72 hours, S1 was collected when the plasma was decanted and stored at −70° F for 24 hours. Both sample were shipped to the Comparative

Coagulation Section of the Diagnostic Laboratory at Cornell University. The S90 sample was collected at plasma decant and stored for 90 days at −20° F and then shipped to the Comparative Coagulation Section of the Diagnostic Laboratory at Cornell University. Each plasma sample was analyzed for coagulation factors VII though XII, Protein C, and AT. Coagulation factors were measured using factor deficient substrate and modified APTT assay. Antithrombin and Protein C activity were analyzed using chromogenic substrate assay method. No equine reference values exist for Protein C such that the assay was performed using a human standard. Data were analyzed using a one-way ANOVA with repeated measures, $p < 0.05$. Prevalence of plasma reactions was estimated by evaluating medical records from 46 consecutive horses that received plasma transfusions. The activity of factors IX, X, AT, and Protein C significantly decreased from time S0 to time S1. The activity of factor XI significantly decreased from S1 to S90. However, the stability of factors VII, VIII, IX, XI, XII, Protein C, and AT was maintained within the reference range following processing and 90 days of storage at −20° F. Only the activity of factor X significantly decreased below the reference value. The prevalence of plasma transfusion reactions was 11% (5/46 horses). Reactions included hives (n=1), tachycardia and pyrexia (n=2), tachypnea (n=1), and severe pruritus and swollen eyes (n=1). None of the reactions was fatal and all occurred during plasma administration.

Clotting factors, AT, and protein C were well preserved following harvest and storage of plasma. Prevalence of reactions to fresh frozen plasma was low and similar to other species, with tachycardia and pyrexia being the most common clinical signs.

ABSTRACT #35

SYSTEMIC CALCINOSIS IN 5 HORSES. J Tan[1], SJ Valberg[1], M Sebastian[2], G Davis[3], L Goehring[4], M Harland[5], J Kelly[6], L Kuebelbeck[6], J Newton[5], J Reimer[7], B Waldridge[5]. [1]University of Minnesota, St Paul, MN. [2]Livestock Disease Diagnostic Center, Lexington, KY. [3]Okotoks Animal Clinic, Okotoks, AB. [4]Colorado State University, Fort Collins, CO. [5]Auburn University, Auburn, AL. [6]Surgi-Care Center for Horses, Brandon, FL. [7]Rood and Riddle, Lexington, KY.

Systemic calcinosis is a syndrome of calcium deposition in the connective tissue of organs including lungs, kidneys, stomach, heart, and skin, not previously described in horses. The purpose of this study was to characterize the signalment, history, clinical signs, muscle biopsy findings, and postmortem findings of 5 horses with potential systemic calcinosis. Records from the Neuromuscular Diagnostic Laboratory at the University of Minnesota were searched to identify horses with biopsy findings of calcified myofibers (Von Kossa stains) and postmortem findings of dystrophic calcification in other organs.

Five Paint or Quarter Horses fit the criteria and ranged in age from 8 months to 6 yrs (median 3.5 yrs). Duration of clinical signs ranged from 3 to 17 days, and in 3 of the 5 horses, respiratory illness was reported in conjunction with clinical signs. Common clinical signs were lethargy (5/5), inappetence (2/5), epaxial and gluteal atrophy (3/5), and stiffness (3/5) or recumbency (2/5). Hyperfibrinogenemia (5/5), hyperphosphatemia (5/5), a Ca*P product of greater than 70 (4/5), and high serum CK activity (1,880–950,000 U/L) were consistently present. Treatment consisted of nonsteroidal antiinflammatories and antibiotics, and frequently included intravenous fluids, dexamethasone, and furosemide. All horses deteriorated despite treatment and developed complications such as colitis (2/5), thrombophlebitis (2/5), laminitis (1/5), respiratory distress (1/5), or prolonged recumbency (1/5). Muscle biopsy findings included acute myofiber necrosis, macrophage infiltration, anguloid atrophy, centrally located myonuclei, rare multinucleated giant fibers, and scattered fibers with dense calcium deposition. All horses were euthanized. Postmortem findings revealed calcification of muscle (5/5) and kidney cells (5/5). Calcification was also present in the heart (3/5), lungs (3/5), liver (1/5), and vascular tunica intima (1/5). Two forms of systemic calcification occur in humans as a consequence of 1° or 2° hyperparathyroidism or elevated Ca*P product. In calciphylaxis, dystrophic calcification occurs in the tunica media of blood vessels, resulting in tissue ischemia and necrosis. In contrast, systemic calcinosis results in calcification of connective tissue and cells within multiple organs. Corticosteroids are suggested to exacerbate calcification. We hypothesize that in some horses, hyperphosphatemia from rhabdomyolysis or hyperparathyroidism may trigger systemic calcinosis. Prospective analysis of parathyroid hormone concentrations is indicated in horses with hyperphosphatemia and calcified fibers in muscle biopsy. Until this condition is better understood, the prognosis remains guarded.

ABSTRACT #36

PHARMACOKINETICS OF PERGOLIDE IN NORMAL MARES. A Wright, L Beard, R Gehring, H Coetzee. College of Veterinary Medicine, Kansas State University, Manhattan, KS.

Pars pituitary intermedia dysfunction (PPID) is a common equine geriatric disease that affects approximately 14% of the aged equine population. Pergolide mesylate is the recommended treatment of choice for PPID. However, there are no pharmacokinetic data about the use of pergolide in horses. The objective of this study was to determine the pharmacokinetics of oral pergolide in normal horses.

Six healthy mares (with normal hair coats and a normal dexamethasone suppression test) between the ages of 3–17 years of age were included in the study. Mares were administered either placebo or pergolide (0.01 mg/kg) in a randomized crossover design, with a washout period of approximately 14 days. Mares were fasted for 8 hours prior to administration of the placebo/pergolide. Heparinized blood samples were collected at baseline, and at 0.1, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 hr after administration of placebo/pergolide. Plasma was separated by centrifugation and frozen at −80 °C until analysis. Pergolide concentrations were determined by HPLC coupled with electrospray ionization tandem mass spectroscopy. The lowest limit of quantitation (LLOQ) was 0.5 ng/ml.

Results of noncompartmental pharmacokinetic analysis revealed that pergolide is rapidly absorbed with a short time to peak concentration (median T_{max} = 0.415 hr). The mean maximum concentration (C_{max}) was 4.05 ng/ml ±2.02 ng/ml and the mean terminal half life ($T_{1/2}$) was 5.86 ± 3.42 hr. The area underneath the curve (AUC) was 14.08 ± 7.46 h*ng/ml. The calculated mean apparent volume of distribution (Vd/F) was 3,082 ± 1,354 ml/kg. The calculated mean apparent oral clearance (CL/F) was 1,204 ml/kg/hr. The pharmacokinetic results indicate that pergolide is more rapidly absorbed and the peak plasma concentrations are much higher than reported in humans. The terminal half life of pergolide in horses (5.86 hr) is much shorter than reported in humans (21 hr). However, the rapid estimated rate of elimination in this study may not a be true reflection of the elimination phase due to the relatively high LLOQ of this assay. Future recommended studies are necessary to determine concentrations and efficacy at steady state.

ABSTRACT #37

VENOUS PLASMA L-LACTATE IS A PROGNOSTIC INDICATOR OF SURVIVAL IN NEONATAL FOALS. M Miskovic, M Lévy, GE Moore. Purdue University, West Lafayette, IN.

Plasma L-lactate (lactate) can be used in the assessment of hydration, acid-base, and oxygenation status of critically ill neonatal foals. While many factors contribute to the final outcome of survival, earlier information about the overall systemic status of each foal may help to formulate a more accurate prognosis on an individual basis. Our objective was to assess the use of plasma L-lactate concentration and the change in lactate over time as prognostic indicators for development of septicemia and survival to discharge in the population of neonatal foals seen at our referral facility.

Foals less than 2 weeks old admitted to Purdue University Veterinary Teaching Hospital from January 2005 through July 2006 were enrolled in this prospective, observational study. Information for a minimum database was collected at admission and a sepsis score calculated. Venous blood was drawn for plasma lactate concentration measurement at admission, 12–16 hours later, and 36–40 hours later. Foals were classified according to development of septicemia and survival to discharge. The Wilcoxon rank sum test was used to compare lactate concentrations between septicemic (sepsis score $\geq$ 11) and nonsepticemic (sepsis score $<$ 11) foals and between foals that survived and those that did not survive to discharge.

Multivariate logistic regression was used to create separate models for predicting septicemia and nonsurvival. Receiver-operator characteristic curves were generated to suggest cut-off points for lactate concentrations to predict nonsurvival.

Septicemic foals had higher lactate concentrations at admission ($P < 0.001$) and at 12–16 hours ($P = 0.007$) than nonsepticemic foals; there was no difference in the change in lactate concentrations over time. Nonsurviving foals had higher lactate concentrations at admission ($P = 0.001$), 12–16 hours ($P = 0.001$), and 36–40 hours ($P = 0.005$) than surviving foals; there was no difference in the change in lactate concentrations over time. Lactate concentration was not a significant predictor of septicemia in a multivariate model, based on sepsis scores. In another multivariate model, increasing lactate concentrations were associated with a 1.65-fold increase in the chance of death.

A lactate concentration cut-off of ≥ 5.3 mmol/L is suggested to predict nonsurvival in neonatal foals, but a useful cut-off point for lactate could not be established for predicting septicemia. Venous blood L-lactate concentrations should be assessed early in the treatment of critically ill neonatal foals to assist in formulating a prognosis for survival.

ABSTRACT #38

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS DYSFUNCTION IN CRITICALLY ILL NEONATAL FOALS. KA Hart[1], NM Slovis[2], MH Barton[1]. [1]University of Georgia College of Veterinary Medicine, Athens, GA. [2]Hagyard Equine Medical Institute, Lexington, KY.

Dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis occurs frequently in critically ill humans, particularly patients with sepsis and septic shock, and has been shown to have a significant impact on survival in human critical care. However, the prevalence and significance of HPA axis dysfunction in critically ill neonatal foals are not well characterized. The purpose of this study was to assess HPA axis function in foals < 7 days of age at the time of admission to tertiary referral centers and to determine correlations between HPA axis dysfunction and indicators of disease severity and outcome. Basal plasma endogenous ACTH and total serum cortisol concentrations were measured with chemiluminescent assays in 71 foals, and a synthetic ACTH stimulation test (measurement of serum total cortisol concentration before and 90 minutes after intravenous administration of 100 µg synthetic ACTH) was performed in 59/71 foals. HPA axis dysfunction was defined as 1) an inappropriately low basal serum cortisol concentration and 2) an inadequate increase in serum cortisol concentration (delta cortisol) after administration of synthetic ACTH. Specifically, an inappropriately low basal cortisol concentration was defined as a basal cortisol concentration less than the lowest cortisol concentration (mean − 1 standard deviation) achieved after administration of a physiologic dose (10 µg) of synthetic ACTH to healthy age-matched foals. An inadequate delta cortisol was defined as a delta cortisol less than the mean delta cortisol achieved in healthy age-matched foals using the same synthetic ACTH stimulation protocol. Data were analyzed using chi-square and Student's t-tests with significance set at $P<0.05$.

Using these two criteria independently, HPA axis dysfunction was diagnosed in 49% (35/71) and 51% (30/59) of foals, respectively. Seventy-six percent of foals (54/71) met criteria for sepsis (sepsis score $\geq$11 and/or positive blood culture); HPA axis dysfunction was diagnosed in 37% (20/54) and 41% (22/54) of septic foals, respectively, with the above criteria. In septic foals, low delta cortisol was correlated with nonsurvival (death or euthanasia for prognostic reasons during hospitalization; $P=0.04$). In addition, 90% of the septic foals with an increased basal serum cortisol concentration and a low delta cortisol did not survive, as compared to a nonsurvival rate of only 9% in foals with a high basal cortisol concentration and an appropriate delta cortisol ($P=0.02$). Finally, septic foals with a low delta cortisol were more likely to have multiple organ failure ($P=0.03$) and shock ($P=0.04$) than were septic foals with an appropriate delta cortisol. These findings suggest that HPA axis dysfunction occurs with significant prevalence in critically ill foals and that septic foals with concurrent HPA axis dysfunction are more likely to have shock and multiple organ failure, and are less likely to survive.

ABSTRACT #39

BACTEREMIA IN EQUINE NEONATAL DIARRHEA. AR Hollis, JE Palmer, PA Wilkins. New Bolton Center, Kennett Square, PA.

Although studies have investigated bacteremia in the critically ill neonatal foal, none specifically investigated bacteremia in neonatal foals with diarrhea. These animals are likely to have a compromised gastrointestinal tract and be at increased risk of bacteremia, perhaps with different organisms than in foals with other disease conditions.

Records of all neonatal (<30 d of age) foals presenting with diarrhea between January 1990 and September 2007 were examined. Foals that developed diarrhea > 12 hours after admission were excluded. Thus 153 records were available for inspection with 133 admission blood culture results recorded; 66 foals (50%) were bacteremic at admission, with 75 isolates. Blood culture from a further 18 foals (14%) grew *Corynebacterium* spp., interpreted as skin contamination. Nine foals (14%) had two or more organisms isolated. One foal had 5 different organisms, interpreted as contamination. Forty-eight foals (36%) had no growth. Excluding *Corynebacterium* spp., 43 isolates (57%) were gram negative organisms, and 32 isolates (43%) were gram positive organisms. *Enterococcus* spp. (22 isolates, 29%) were most common, many resistant to multiple antimicrobials, followed by *Pantoea agglomerans* (13 isolates, 17%).

The high prevalence of bacteremia, dominated by *Enterococcus* spp. and *Pantoea agglomerans* isolates, contrasts previous studies of critically ill foals. Bacteremia appears to be more common in neonatal foals with diarrhea than other critically ill neonatal foals. Foals with diarrhea may be predisposed to gram positive or gram negative bacteremia with a multiply resistant pathogen early in the clinical course. Decisions regarding antimicrobial selection should be made with these differences in mind.

ABSTRACT #40

SERUM IL-6 AND IL-10 CONCENTRATIONS IN NORMAL AND SEPTIC NEONATAL FOALS. AJ Burton[a], B Wagner[b], HN Erb[b], DM Ainsworth[a]. [a]Dept of Clinical Sciences and [b]Dept of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Previously it was reported that compared to surviving septic foals, nonsurviving foals had a 35-fold increase in IL-10 (Pusterla *et al*., AJVR, 2006) and a 15-fold increase in IL-6 (Gold *et al*., JVIM, 2007) expression in their PBMC. As gene expression profiles can be time-consuming, we sought to determine if serum [IL-6] and [IL-10] in foals would aid in the diagnosis and prognosis of septicemia.

A prospective study of septic neonatal foals admitted to the Cornell University (CU) Equine Hospital during the spring of 2007 was performed. Septicemia was confirmed in 7 foals using a combination of blood culture results and sepsis scores. Blood samples were collected from foals at the time of admission (T0) and again 24 (T24) and 48 (T48) hours later. All foals were treated with antibiotics, plasmam and supportive therapiesm and outcomes were determined. Blood samples from age-matched control foals (n=15), born at the CU Equine Park (2007), were obtained at 12–72 hours of age (T0) and again 24 (T24) and 48 (T48) hours later. Serum and colostral [IL-6] and [IL-10] were also measured in samples obtained from the dams. [IL-6] was measured using an ELISA and [IL-10] was measured using a Luminex immunoassay system. Group differences were detected using a Wilcoxon rank sum test with a Bonferroni correction applied to the p value.

Relative to the controls, septic foals had significantly lower serum [IL-6] at all three time points ($p = 0.0313$) and a trend towards higher [IL-10]. The single septic foal that died had a 5,106-fold higher [IL-10] at T0 compared to survivors ($n = 6$). Serum and colostral [IL-10] in the dams were undetectable in 10 out of 13 samples. Dam serum and colostral [IL-6] were high in 18 out of 18 samples. These results are in contrast to our previous work where gene expression of IL-6 in PBMC was studied and the increased IL-6 mRNA expression in septic foals suggested production of this cytokine in response to the disease. Here, we found an increased serum [IL-6] in healthy compared to septic foals, which was likely to be the result of maternal transfer of high [IL-6] to the healthy neonates. This could be due to localization of IL-6 in PBMC rather than in serum of septic foals. Our results also suggest that IL-6 is partly derived from colostrum, whereas IL-10 was not transferred with the colostrum in

most foals and is produced by the foal's own immune system. This is the first report of serum [IL-6] and [IL-10] measurement in neonatal foals, and preliminary data suggest that elevated serum [IL-10] but not [IL-6] are associated with a poor prognosis.

ABSTRACT #41
BLOOD GLUCOSE CONCENTRATIONS IN CRITICALLY ILL NEONATAL FOALS. Hollis AR,[1] Furr MO,[2] Magdesian KG,[3] Axon JE,[4] Ludlow V,[5] Boston RC[1] and Corley KTT[6]. [1]New Bolton Center, Pennsylvania; [2]Marion DuPont Scott Equine Medical Center, Virginia; [3]University of California at Davis, California; [4]Scone Veterinary Hospital, Australia; [5]Royal Veterinary College, UK; [6]Anglesey Lodge Equine Hospital, Ireland.

Critical illness is associated with hyperglycemia in humans, and a greater degree and duration of hyperglycemia are associated with non-survival. Hypoglycemia is also seen in critically ill humans and is associated with nonsurvival. This may be true in the critically ill foal. The objective of this study was to investigate the association of blood glucose concentrations with survival, sepsis, and the systemic inflammatory response syndrome (SIRS) in critically ill neonatal foals.

Blood glucose concentrations at admission (515 foals) and 24 hours (159 foals), 36 hours (95 foals), 48 hours (82 foals), and 60 hours (45 foals) after admission were analyzed. Logistic regression analyses were performed to investigate the association of glucose concentrations with survival, sepsis, a positive blood culture, or SIRS. At admission, 29% of foals had blood glucose concentrations within the reference range (75.6–131.4 mg/dL), 36% were hyperglycemic, and 34% were hypoglycemic. Foals that did not survive to hospital discharge had lower mean blood glucose concentrations at admission, as well as higher maximum and lower minimum blood glucose concentrations in the first 24 hours of hospitalization, and higher blood glucose at 24 and 36 hours. Foals with blood glucose concentrations less than 2.8 mmol/L (50.4 mg/dL) or greater than 10 mmol/L (180 mg/dL) at admission were less likely to survive. Hypoglycemia at admission was associated with sepsis, a positive blood culture, and SIRS.

Derangements of blood glucose concentration are common in critically ill foals. Controlling blood glucose concentrations may therefore be beneficial in the critically ill neonatal foal, and this warrants further investigation.

ABSTRACT #42
TREATMENT OF NEONATAL FOALS WITH IMMUNOSTIMULANTS ENHANCES PHAGOCYTIC CELL ACTIVITY AGAINST *EX VIVO* INFECTION WITH *RHODOCOCCUS EQUI*. C. Ryan, S. Giguère. University of Florida, College of Veterinary Medicine, Gainesville, FL.

The objective of this study was to determine the effect of immunostimulants on neutrophil and macrophage activity against *ex vivo* infection with *R. equi*. Seventeen neonatal foals were treated with Zylexis®, EqStim®, or saline on days 7, 9, and 15 of life. Peripheral blood mononuclear cells and BAL cells were collected on day 7 (pre-treatment), 19, 31, and 43. Neutrophil phagocytosis and oxidative burst in response to *R. equi* infection were assessed using a flow cytometric assay. Intracellular proliferation of *R. equi* within macrophages was assessed by light microscopy for samples collected on days 7 and 19.

Neutrophils from foals treated with Zylexis had a significantly greater ability to phagocytize opsonized *R. equi* and had higher oxidative burst on day 19 and day 31 (posttreatment) compared to baseline values ($P < 0.05$). On day 31, foals treated with Zylexi® had significantly greater phagocytosis and oxidative burst than foals treated with EqStim ($P<0.05$). The effect of Zylexis on neutrophil function was no longer detectable on day 43. There was no significant effect of time on phagocytosis and oxidative burst in control foals and in foals pretreated with EqStim. Treatment with EqStim resulted in significantly less intracellular proliferation of *R. equi* within monocyte-derived and BAL macrophages on day 19 compared to control foals ($P<0.05$) but not compared to foals treated with Zylexis.

In conclusion, treatment of neonatal foals with Zylexis enhances the activity of neutrophils whereas EqStim enhances activity of macrophages following *ex vivo* infection with *R. equi*.

ABSTRACT #43
PROGNOSTIC INDICATORS FOR SURVIVAL OF DOWNER COWS MANAGED WITH A FLOTATION TANK SYSTEM IN A REFERRAL HOSPITAL. AJ Burton[a], DV Nydam[b], TL Olivett[a], TJ Divers[a]. [a]Dept of Clinical Sciences and [b]Dept of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Recently, the use of flotation tank devices has improved the management of downer cows both in the field and in hospital settings. The population of downer cows sent to or occurring in a referral hospital is challenging to manage as they have often been down for a long time and/or may be critically ill. Maintenance of a cow in a float tank can be costly, labor intensive and may be stressful for the patient. Our aim was to enable clinicians and owners to make informed decisions about whether or not to float a cow. The objective of this study was to evaluate prognostic indicators within the first 24 hours of hospitalization and flotation of the downer cow.

A retrospective study of all cows admitted to the Cornell University Farm Animal Hospital between January 1, 1997 and December 31, 2007 and managed with a float tank (Aqua Cow Rise System®) for recumbency was performed. Both cows that were admitted as downers and those that became downers during hospitalization were included. Data on signalment, history, biochemical parameters, stall and tank behavior, and outcome was collected. Outcome was defined as survival to discharge. Data was analyzed using Wilcoxon rank sum and Chi square tests. Alpha was set at .05.

Of 46 total cows, 17 (37%) survived and 29 (63%) died or were euthanized. There was no significant difference between survivors and nonsurvivors in median weight, age, stage of lactation, number of days down prior to floating or having prior abdominal surgery. The longest time down prior to flotation in a survivor was 7 days. Median creatinine kinase (CK) concentration on admission was not significantly higher in the non-survivors (5284 U/L) compared to the survivors (4402 U/L). The highest admission [CK] recorded in a surviving cow was 68,545 U/L. Being able to back out without falling after the first flotation attempt was significantly associated with an increased chance of survival ($p < 0.001$). Cows that did not eat in the tank were 1.9 times more likely to die than those that had a good appetite ($p = 0.03$). Cows that stood square in the tank on the first flotation attempt were 2.6 times more likely to survive than those that were asymmetric or unable to stand ($p = 0.02$). This study has identified some objective parameters within the first 24 hours of flotation, associated with survival to discharge. Used in context with the individual's history, clinical picture and financial value, these findings will assist logical decision making with respect to floating of downer cows.

ABSTRACT #44
PHARMACOKINETIC PARAMETERS OF CEFTIOFUR CRYSTALLINE FREE ACID IN NONLACTATING DOMESTIC GOATS. Elizabeth Doré, John A. Angelos, Joan D. Rowe, Scott Wetzlich, Lisa A. Tell. School of Veterinary Medicine, University of California, Davis, CA.

The objective of this study was to describe the pharmacokinetics of ceftiofur crystalline free acid (CCFA) in the domestic goat (*Capra aegagrus hircus*) using the labelled cattle dosage of 6.6 mg/kg of CCFA after a single subcutaneous injection.

Six non-lactating yearling female goats were used in the study. The goat breeds included Saanen (n=4) and Toggenburg (n=2) and their body weights ranged from 66 to 78 kg. CCFA (Excede, Pfizer, New York, NY) containing 200 mg of ceftiofur/ml in a sterile oil suspension was administered once subcutaneously in the skin fold behind the elbow at a dosage of 6.6 mg/kg. Blood was drawn from the jugular vein at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96, 120,144, 168, 192, 240, and 288 hours after CCFA administration. The blood was allowed to clot and serum was frozen at −80 °C until analysis. Concentrations of ceftiofur and its metabolites were measured using high-performance liquid chromatography. Data were analyzed using compartmental and non-compartmental approaches using a commercial software program (WinNonLin, Pharsight Corporation, Mountain View, CA, USA). Preliminary analysis suggests that the kinetics of CCFA in the domestic goat is best described by a one compartment model. Average (± SD) pharmacokinetic parameters were as follows: area under the concentration time curve (159 h*μg/

ml ± 19), maximum concentration (2.3 μg/ml ± 1.1), time of maximal concentration (26.7 h ± 16.5), and terminal elimination half life (42.2 h ± 16).

ABSTRACT #45

TUBE CYSTOSTOMY FOR TREATMENT OF OBSTRUCTIVE UROLITHIASIS IN GOATS. Elizabeth Doré[1], Lisle W. George[2], Jeanne W. George[3], Omar Maher[1], Christiana M. Drake[4], John A. Angelos[2]. [1]William R. Pritchard Veterinary Medical Teaching Hospital, [2]Department of Medicine and Epidemiology, [3]Department of Pathology, Microbiology and Immunology, [4]Department of Statistics; University of California, Davis, CA.

The objectives of this study were to determine the outcome of the tube cystostomy in combination with urethral flushing for the treatment of obstructive urolithiasis in goats and to determine if urethrotomy negatively affects surgical outcome.

Medical records of male goats that had a tube cystostomy between January 1995 and December 2005 at the University of California were reviewed. Follow-up was obtained by a questionnaire mailed to referring veterinarians or owners and by telephonic communication with owners. Statistical analysis consisted of a survival analysis (Kaplan-Meier) and a log-rank type test to compare the survival between goats that had urethrotomy and goats that did not have urethrotomy. Significance was set at $P < 0.05$.

The study population consisted of 97 goats. Fifteen were intact males; 82 were wethers. There were 39 Pygmy, 13 Alpine, 9 Nubian, 7 LaMancha, and 29 mixed/other breed goats. The age ranged from 7 months to 11 years (median: 3 years). Hospitalization ranged from 1 to 93 days (median: 5 days). Ninety five goats were discharged from the clinic. Follow-up information was available for 73 cases. Fifty-two goats survived without any complications at least 12 months after the procedure, 20 goats had complications before 12 months, and 1 goat was lost to follow-up after 8 months. Of these 52 cases, 23 had a re-occurrence of urolithiasis between 12 and 60 months after the procedure (median: 24 months). Out of these 23 cases, a second tube cystostomy was performed (n=11), owners elected euthanasia (n=9), the calculus was removed manually from the tip of the urethra (n=2), or a perineal urethrostomy was done (n=1). For 3 cases a third tube cystostomy was performed. An urethrotomy was performed in 34 goats, and of these 24 did not have any complications for at least 12 months, 6 had complications before 12 months, and 4 were lost to follow-up. The survival time ranged from 20 days to 106 months (median: 24 months; mean: 34.9 months (SE±7.56)) for goats that had urethrotomy and from 7 days to 114 months (median: 27months; mean: 40.3 months [SE±6.72]) for goats that did not have urethrotomy. There were no statistical difference between the 2 groups ($P = 0.807$).

Tube cystostomy with urethral flushing has a fair to good prognosis for the treatment of caprine urolithiasis. The high risk of re-occurrence should be stressed to clients considering treatment of obstructive urolithiasis. Urethrotomy did not negatively impact outcome and should be considered for removal of some urethral calculi.

Originally presented at the ACVS Symposium, Chicago, IL, October 2007.

ABSTRACT #46

VALIDATION OF THE ADVIA CENTAUR® IMMUNOASSAY FOR THE MEASUREMENT OF BOVINE CARDIAC TROPONIN I. A Varga[1], KE Schober[1], WL Walker[2], J Lakritz[1], DM Rings[1]. [1]Departments of Veterinary Clinical Sciences and [2]Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.

The use of cardiac troponin I (cTnI) as a sensitive and specific biomarker of myocardial injury has been well established in people, small animals, and horses. Prospective studies on the use of cTnI in cattle are lacking. The objective of this study was to validate the ADVIA Centaur®CP Immunoassay System (Bayer Healthcare Diagnostics, Newbury, UK) for the detection of serum bovine cTnI concentrations and to establish a reference range for healthy dairy cows.

Different concentrations of purified bovine cTnI (BiosPacific, Emeryville, CA) were diluted into cTnI-free bovine serum and used to assess sensitivity, precision, linearity, and recovery of the ADVIA Centaur® assay. Intra and inter-assay precision (reported as the mean coefficient of variation [CV]) was measured at serum concentrations of 0.2, 1.0, 10 and 30 ng/ml of cTnI. Intra-assay precision was < 5% and inter-assay precision was < 12%. The assay demonstrated linearity of serial dilutions from 0.05 to 30 ng/ml cTnI. Test recovery ranged from 70% up to 110% depending on the cTnI concentration in serum. No cross reactivity of the assay with homogenized skeletal muscle was observed. Stability of bovine cTnI was determined by analysis of different cTnI concentrations stored at room temperature for 2 days and at 4 °C and −80 °C for 2, 7, and 14 days. A relevant decrease of cTnI concentrations over time was observed when samples were stored at room temperature and at 4 °C. Storage at −80 °C as well as repeated freeze-thaw cycles did not affect cTnI concentrations.

In healthy dairy cows (n=20) serum cTnI concentrations were below the lower limit of detection (0.01 ng/ml) of the assay in 5 and between 0.01 and 0.03 ng/ml in the other 15 cows.

Our preliminary results indicate the ADVIA Centaur® immunoassay developed for use in people may have adequate test performance for the detection of circulating bovine cTnI. This assay may be useful in the early identification of myocardial injury secondary to infectious, toxic, and inflammatory insults in cattle. Studies in dairy cows with myocardial disease are needed to clinically validate our findings.

ABSTRACT #47

ECHOCARDIOGRAPHIC ASSESSMENT OF RIGHT VENTRICULAR SIZE IN CATTLE. GD Hallowell[1] and TJ Potter[2]. [1]Shepshed, Leicestershire, UK [2]Royal Veterinary College, Herts, UK.

Measurements of the right ventricle (RV) taken from standard M-mode views of the left ventricle have been shown to have wide variability and poor repeatability due to the spiral shape of the RV. In this study, novel measurements of RV area and the *trabecula septomaginalis* (TS) using previously reported echocardiographic views are examined. These values may result in better triangulation and more repeatable measurements in clinical patients. The aims of this study were to report normal values for these novel measurements and assess repeatability in adult dairy cows. Eight healthy adult Holstein Friesian cattle (656±11 kg) were recruited and examined on three consecutive days. Standard echocardiographic images were obtained and analysis was performed using three cardiac cycles of the right parasternal long axis projections optimised to show the pulmonary artery (PA), left ventricular outflow tract (LVOT), or left ventricle (LV) and using a short axis projection at the level of either the left ventricle (LV-SA), mitral valve (MV-SA), or left parasternal long axis projections of the right ventricular outflow tract (RVOT). Data was analysed using a repeated measures ANOVA, Student's T-test and repeatability was assessed with intraclass correlation coefficients. TS length and areas from comparable views were compared; if no differences were noted measurements were pooled. Data are displayed in Table 1. Good repeatability (ICC > 0.85) was obtained for all measurements between different measures and different days except for RV diameter from M-mode. These novel measurements for assessment of RV size are reliable and repeatable and may allow for better evaluation of RV enlargement in clinical patients.

Table 1. Echocardiographic measurements pertaining to RV in adult cattle.

Parameter	Mean±SEM	Parameter	Mean±SEM
PA (cm)	5.63±0.06	Total RVOT area (cm^2)–PA and RVOT view	143.9±7.4
Ao (cm)	6.17±0.11	Total RV area (cm^2)–LV-SA view	120.7±8.6
PA:Ao	0.91±0.07	Total RV area (cm^2)–MV-SA view	159.1±6.85
RVDd (cm)°#	3.6±0.16	Area- TS to apex (cm^2)- LVOT and LV views	22.5±2.6
RV TS length (cm) LVOT and LV views	5.2±0.32	Area- TS to TV (cm^2)- LVOT and LV views	37.7±4.6
RV TS length (cm) RVOT and PA views	5.8±0.24	Area- TS to apex (cm^2)- PA and RVOT views	63.7±6.24
Total RV area (cm^2) LVOT and LV views	56.3±3.66	Area- TS to TV (cm^2)- PA and RVOT views	68.2±3.4

°poor ICC between days and #poor ICC (ICC < 0.85) between different measurements.

ABSTRACT #48

APPLICATION OF STRONG ION DIFFERENCE THEORY TO URINE AND THE RELATIONSHIP BETWEEN URINE PH AND NET ACID EXCRETION IN CATTLE. PD Constable,[1] CC Gelfert,[2] M Fürll,[3] R Staufenbiel,[4] H Stämpfli[5]. [1]Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN, USA; [2]Department of Food Animals and Herd Medicine, Veterinärmedizinische Universität Wien, Austria; [3]Medizinische Tierklinik der Universität Leipzig, Germany; [4]Fachbereich Veterinärmedizin, Freie Universität Berlin, Germany; [5]Department of Clinical Studies, University of Guelph, Guelph, Ontario, Canada.

Urinary net acid excretion (NAE) provides the most sensitive clinical insight into acid-base homeostasis in animals. Measurement of urine pH may also have clinical utility in the assessment of systemic acid-base status, particularly in healthy animals, because urine pH is more easily determined than NAE. The objectives of this study were to develop an equation expressing urine pH in terms of independent variables, to derive an equation relating urine pH to NAE, and to apply this new knowledge to determine the role that measuring urine pH should play in the evaluation of systemic acid-base status in healthy and sick cattle.

A physicochemical strong ion approach was applied to develop a general electroneutrality equation that involved urine pH and urinary strong ion difference (SID = difference between strong cation and strong anion concentrations in mEq/l), the urinary concentration of ammonium ($[NH_4^+]$) and phosphate ([P]) in mmol/l, the acidic dissociation constant for $H_2PO_4^-$ (Ka_2), urinary Pco_2, the dissociation constant (K'_1) for carbonic acid (H_2CO_3), and the solubility of CO_2 (S) in urine. We validated the general electroneutrality equation using 327 data points from 11 nonlactating Holstein-Friesian cows that were fed 11 diets of different dietary cation anion difference.

We determined that urine pH in mammals is dependent on 4 variables, urine SID, $[NH_4^+]$, Pco_2, and [P], and 3 constants, Ka_2, K'_1, and S. In cattle, urine pH is dependent on 3 variables (urine SID, $[NH_4^+]$, Pco_2) and 2 constants (K'_1, S) because urine [P] ≈ 0. The relationship between NAE (in mEq/l) and urine pH for cattle was $NAE = [NH_4^+] + 2.5 - 10^{(pH-6.12)}$. A simplified form of the general electroneutrality equation was developed for bovine urine whereby urine $pH \approx 6.12 + \log_{10}([K^+] + [Na^+] + [Mg^{2+}] + [Ca^{2+}] + [NH_4^+] - [Cl^-] - [SO_4^{2-}])$.

We conclude that a change in urine SID, $[NH_4^+]$, Pco_2, or [P] will independently lead to a change in urine pH in mammals. In cattle, urinary $[K^+]$ potassium concentration has the greatest effect on urine pH, with high urine $[K^+]$ producing alkaline urine and low urine $[K^+]$ producing acidic urine. Whole body potassium depletion should be suspected when aciduria is present in sick cattle that are not consuming an acidogenic diet. Urine pH provides an accurate assessment of systemic acid-base homeostasis in healthy cattle only when urine pH is between 6.3 and 7.6. Urine pH should not be used to predict systemic acid-base status in sick cattle because serum electrolyte abnormalities, such as hypokalemia and hypochloremia, are likely to be present.

ABSTRACT #49

PARENTERAL SELENIUM SUPPLEMENTATION BENEFITS FOOT ROT-AFFECTED SHEEP. JA Hall[1], RJ Van Saun[2]. [1]Oregon State University College of Veterinary Medicine, Corvallis, OR. [2]Pennsylvania State University College of Agricultural Sciences, University Park, PA.

The purpose of this study was to determine the clinical effectiveness of selenium (Se) supplementation on foot rot (FR) prevalence and recovery in sheep. A prospective, 15-month, placebo-controlled clinical trial was undertaken in a commercial sheep flock.

FR-affected sheep were randomly divided into 2 groups (n=19). An additional control group of 19 sheep without FR (Control) were identified. Sheep feet were examined, trimmed, and scored for FR using a scale of 0 (no lesions) to 4 (extensive lesions). Half the FR-affected sheep were treated with 5 mg injectable Se (FR-Se) at 1-month intervals for the duration of the study; the other half were given saline injections (FR-Sal). Controls received no treatment. Sheep feet were reexamined, trimmed, and rescored at 3, 6, 9, and 15 months. Sheep were also bled at time 0 and then at 3, 6, and 15 months to assess whole-blood Se concentrations. Whole-blood Se data were analyzed by ANOVA for repeated measures; foot scores were assessed by nonparametric analyses with main effects of month and treatment.

At time 0, Control sheep (255 ng/ml) had higher ($P<0.05$) whole blood Se concentrations compared with FR-Se (205 ng/ml) and FR-Sal (211 ng/ml) sheep. By 6 months, FR-Se sheep (317 ng/ml) had whole blood Se concentrations greater ($P<0.05$) than both Control (281 ng/ml) and FR-Sal (277 ng/ml) sheep. FR-Se ewes showed a faster decline in highest lesion score at 3 ($P=0.05$) and 6 ($P=0.03$) months, a greater decrease in the number of feet with foot score >0 at 6 ($P=0.02$) months, and a tendency for a lower total severity score at 6 ($P=0.07$) months compared with FR-Sal ewes. Sheep with blood Se concentrations <300 ng/ml were at 3.5 times greater risk (1.1–12.1 CI, Odds Ratio) for FR, though this relationship was only significant ($P=0.04$) at 6 months of the study.

In sheep with FR, Se supplementation results in higher whole-blood Se levels and more rapid improvement of foot lesions compared with saline treatment.

ABSTRACT #50

COMPARISON OF TREATMENTS FOR CASEOUS LYMPHADENITIS IN SMALL RUMINANTS: OPENING, DRAINING AND FLUSHING VERSUS INTRALESIONAL OR PARENTERAL TULATHROMYCIN. KE Washburn, W Bissett, V. Fajt, M.Libal, G Fosgate, J Miga, K Rockey. Texas A&M University College of Veterinary Medicine, College Station, TX.

The objectives of our study are to evaluate and compare treatments of caseous lymphadenitis in small ruminants, and to investigate in vitro susceptibility of *Corynebacterium pseudotuberculosis* to tulathromycin.

Client-owned cases are enrolled based on the presence of a peripheral, subcutaneous mass and are randomly assigned to one of three treatment groups. From all cases, lesions are aspirated for bacterial culture and antimicrobial susceptibility, and blood is collected for serum hemolysin-inhibition testing. Treatment groups are as follows: opening, draining and flushing the lesions (A), intralesional tulathromycin (B), and subcutaneous tulathromycin (C). Animals are discharged to owners with specific instructions regarding biosecurity and parameters that warrant re-examination or constitute treatment failure. All cases are re-examined approximately one month from enrollment, unless treatment failure is observed. If lesions are unresolved, they are re-cultured, opened, drained and flushed (A). Follow-up serology is also performed at this time.

Thirty-two cases have been enrolled, of which 24 possessed culture positive *C. pseudotuberculosis* lesions. Minimum inhibitory concentrations for *C. pseudotuberculosis* isolates range from ≤ 1 to 2 μg/ml, with 71.4% ≤ 1 μg/ml. Of 24 culture positive lesions, 20 were resolved in one month. Of the treatment failures, 1 was Group A, 1 was Group B and 2 were Group C.

Data so far indicate that *C. pseudotuberculosis* isolates from clinical cases are sensitive to tulathromycin, although there are no validated breakpoints on which to make that determination, and that there may be acceptable alternative treatments of caseous lymphadenitis rather than opening, draining and flushing the lesions.

ABSTRACT #51

SUSPECTED *CLOSTRIDIUM DIFFICILE*-ASSOCIATED GASTROENTERITIS IN VEAL CALVES. Luis G. Arroyo[1]; Anthony van Dreumel[2]; Reny Lothrop[3]; Henry Staempfli[3]; J. Scott Weese[1]. [1]Departments of Pathobiology and [3]Clinical Studies, Ontario Veterinary College, [2]Animal Health Laboratory, University of Guelph, Guelph, ON, Canada.

Clostridium difficile is an important enteropathogen in humans and several domestic animal species. It has been isolated and its toxin (s) detected in fecal samples of diarrheic and non-diarrheic dairy calves, yet its role in disease is still unclear. The role of this enteropathogen as a cause of disease in veal calves had not been investigated. The objective of this study was to describe the pathological findings of suspected *C. difficile*-associated gastroenteritis infection in veal calves and to characterize *C. difficile* isolates obtained from veal calves.

Six veal calves (age range: 1–18 weeks-old) were submitted from a veal farm operation over a 6 month period for postmortem examination. Gastrointestinal (GI) contents were tested by enzyme immunoassay (EIA) for *C. difficile* toxins A/B and *C. perfringens* enterotoxin, and screened for common enteropathogens associated with neonatal calf diarrhea, including enterotoxigenic *E. coli*, *Salmonella* spp. *C. perfringens*, rotavirus, coronavirus, and parasites. Samples from only 2 calves were cultured for *C. difficile*. *C. difficile* toxins A and/or B were detected in intestinal contents of all calves and no other enteropathogens were identified. Macroscopic lesions were similar in all cases and consisted of fibrinous enteritis, colonic edema, entero-colitis and multiple hemorrhages, dehydration, and pulmonary congestion and edema. Histologically, there were focal areas of mucosal erosion and fibrino-cellular exudates, with colonies of clostridia-like bacilli present in the lumen and on the mucosal surface small intestine and abomasum. There was marked transmural edema and focal areas of hemorrhage in the lamina propria, with congested and thrombosed capillaries. Mesenteric lymph nodes were markedly congested and edematous.

Subsequently, an investigation was performed on the farm. Fecal samples were collected from 24 diarrheic calves at one time point for *C. difficile* culture. Three historical isolates recovered from diarrheic calves 4 years earlier from the same veal farm were included for molecular analysis. PCR-ribotyping was performed and isolates were screened for genes encoding toxins A (*tcdA*), B (*tcdB*) and binary toxin (*cdt*B).

Clostridium difficile was isolated from the 2 initial calves and 22/24 diarrheic calves. Five ribotypes were identified from the 27 isolates (3 historical, 2 postmortem calves and 22 from diarrheic calves), and all were toxigenic. Sixteen (57%) possessed genes *tcdA* and *tcdB*, while the remainder were variant strains. Nine (32%) only possessed genes encoding *tcdB*, while 2 (7.1%) possessed *tcdB* and *cdtB* genes. Overall, the genes encoding *tcdA, tcdB* and *cdtB* were present in 16 (59%), 27 (100%) and 2 (7%) strains, respectively.

This preliminary report supports the potential capacity of *C. difficile* to colonize and cause disease in several species. Further study of diarrheic and normal veal calves is required to elucidate the role of this enteropathogen as a cause of gastroenteritis and diarrhea in veal calves and as a zoonotic pathogen.

ABSTRACT #52

MYCOBACTERIUM AVIUM SUBSP. *PARATUBERCULOSIS* TISSUE INFECTION OF YOUNG DAIRY CATTLE FOLLOWING GRAZING OF PASTURES USING THE "LEADER-FOLLOWER" GRAZING SYSTEM IN A HERD POSITIVE FOR JOHNE'S DISEASE.

ME Fecteau[1], RH Whitlock[1], CD Buergelt[2], RW Sweeney[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.[2]University of Florida, College of Veterinary Medicine, Gainesville, FL.

With increased interest in organic farming and animal welfare, less intensive production systems that employ pasture grazing have gained in popularity. The impact of such practice on the transmission of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) to yearling animals is unknown. The purpose of the study was to investigate the transmission of MAP to yearling animals exposed exclusively through grazing of pasture previously occupied by lactating cattle infected with MAP.

Nine Jersey steers originating from a Johne's disease-positive herd were included. At the age of 15 months, the steers were introduced to pastures heavily contaminated with MAP and remained on those pastures for a total of 10 months. Fecal cultures and fecal RT-PCR testing were performed during pasture season and at slaughter. At slaughter, blood was obtained for MAP antibody detection using ELISA. For each steer, 28 separate samples of intestinal tissue and associated lymph node were collected along the length of the intestinal tract for mycobacterial culture. These samples were processed for MAP culturing on HEYM, using standard methods. Samples of ileum and ileocecal lymph node were examined histopathologically for lesions of paratuberculosis and presence of acid-fast staining organisms.

Fecal RT-PCR was positive in all 9 steers throughout pasture season. At slaughter, 4 steers were RT-PCR positive. Two steers had positive fecal cultures. None of the steers had detectable serum antibodies for MAP. Overall, 6 of the 9 steers had at least one tissue sample positive for MAP organisms. The number of positive samples per animal ranged from 5 (18%) samples to 23 (82%) samples. In the positive animals, the number of colony forming units (CFUs) per sample ranged from 1 CFU to 300 CFUs per sample, and the total number of CFUs per steer for all samples combined ranged from 25 CFUs to 9,337 CFUs. Six of the 9 steers had no histolopathological evidence of MAP infection. The 3 remaining steers were negative for acid-fast staining organisms but individual Langhans' type giant cells and epithelioid macrophages found in the ileal samples were suggestive of an early infection with MAP.

It was initially postulated that these animals were positive for MAP in their feces as MAP was "passing through" following oral consumption on contaminated pasture (i.e., passive shedding). The results of this study, however, suggest that at least 6 of these animals became permanently infected with MAP as each of them was positive for MAP in at least one tissue. We concluded that exposure of yearling cattle to pastures contaminated with MAP can result in permanent infection with MAP, and that age resistance to infection can be overcome by pressure of infection.

ABSTRACT #53

NORMAL ECHOCARDIOGRAPHIC APPEARANCE AND DIMENSIONS IN ADULT ALPACAS.

GD Hallowell[1] and TJ Potter[2]. [1]14, Threadcutters Way, Shepshed, Leicestershire, UK. [2]Royal Veterinary College, Herts, UK.

The aims of this study were to describe the echocardiographic appearance and establish normal cardiac dimensions and time indices for adult Huacaya alpacas, which have not been previously published. Eight healthy male alpacas (72±6 kg) were recruited. Standard images were obtained using a previously described technique through left and right imaging windows. Inter- and intra-operator repeatability was assessed. Echocardiographic examination was performed on each of the alpacas on three separate occasions and the images were evaluated by one operator on three separate occasions and by one operator on one occasion. Statistical tests used included repeated measures and one-way ANOVA and intra-class correlation coefficients (ICC's). Images were obtained in all eight alpacas. Mean (±SD) standard echocardiographic dimensions are displayed in Table 1. There were no significant differences between individual alpacas or from the same alpacas on different days except for pre-ejection period. There was excellent correlation between individuals on different days except for time indices (PEP, MV closure time, RTI) which also had poor ICC's between individual operators' repeated measures and between operators. In conclusion this is a straightforward repeatable technique for acquiring cardiac dimensions in adult alpacas; time indices, however, appear unreliable.

Table 1. Echocardiographic measurements in adult alpacas

Parameter	Mean±SD	Parameter	Mean±SD
PA (cm)	2.31±0.41	RV diameter-diastole (cm)	1.12±0.36
Ao (cm)	2.56±0.43	RV diameter-systole (cm)	0.83±0.32
AoS (cm)	3.04±0.37	IVS-diastole (cm)	1.24±0.28
PA:Ao	0.91±0.04	IVS-systole (cm)	1.68±0.25
LAD (cm)	4.94±0.42	LV diameter-diastole (cm)	5.17±0.75
ET (ms)	0.20±0.06	LV diameter-systole (cm)	3.10±0.72
PEP (ms)*°	0.08±0.03	Free wall-diastole (cm)	1.26±0.39
EPSS (cm)	0.29±0.18	Free wall- systole (cm)	1.67±0.32
MVclosure time (ms)°#	0.10±0.06	Fractional shortening (%)	40.5±8.31
RTI (ms)°#~	0.14±0.05	Left ventricular ejection fraction (LVEF)	0.77±0.09

*indicates there were significant differences between animals, °poor ICC between days and #poor ICC between different measurements performed by same operators and ~poor ICC between different operators.

ABSTRACT #54

THROMBOELASTOGRAPHY (TEG) IN CLINICALLY HEALTHY NEONATAL AND ADULT ALPACAS. D Bedenice[1], EA Rozanski E[1], B Wiinberg B[2]. [1]Cummings School of Veterinary Medicine at Tufts University, Grafton, MA.[2]The Royal Veterinary and Agricultural University, Denmark.

Thromboelastography (TEG) is considered a rapid, reproducible test of hemostatic function in whole blood. This analytical method has been previously evaluated in humans, dogs, and select species to identify and quantify alterations of both hyper- and hypocoagulative disease states. TEG may have similar diagnostic potential in New World Camelids with hemostatic disorders, augmenting traditional coagulation parameters. The objective of this study was to establish a clinically applicable reference interval for reaction time (R), clotting time (K), angle (a), and maximum amplitude (MA) of TEG on citrated whole blood from 21 clinically healthy alpacas.

Ten milliliters citrated whole blood from 15 clinically healthy adult alpacas (1–7 yrs) and 6 neonatal crias (1–14 days) was collected by direct venipuncture. TEG analysis (using recombinant human tissue factor) was performed in duplicate at room temperature, 30 minutes after collection. Prothrombin time (PT) and partial thromboplastin time (PTT) were obtained concurrently.

Mean values of TEG parameters (+/− SD) for adult alpacas and neonatal crias were, respectively, R= 7.7 (2.9), 5.6 (2.2); K= 3.53 (1.43), 2.55 (1.18); α= 48.1° (11.2), 56.2° (10.4); MA= 55.5 (8.35) and 59 (3.21) mm. Mean PT and PTT were 8.6 s (0.6) and 18.9 s (1.9) in adults vs. 9.4 s (0.6) and 18.1 s (1.96) in neonates. A significant difference was not observed between age groups.

Compared to reported TEG results from dogs and horses, alpacas show relatively shorter clotting times (K). Thus, species-specific normal values are necessary for interpretation of TEG parameters.

ABSTRACT #55

IVERMECTIN CONCENTRATIONS IN BLOOD AND CEREBROSPINAL FLUID FOLLOWING INTRAVENOUS ADMINISTRATION TO HEALTHY LLAMAS. Sarel van Amstel, Ashley Portmann, Sherry Cox, Thomas Doherty, Shelley Newman. University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

The response to anthelmintic treatment in cases of meningeal worm (*Parelaphostrongylus tenuis*) remains unpredictable. In a previous study, ivermectin (IVM) was not detected in cerebrospinal fluid (CSF) following subcutaneous administration to healthy llamas. The aim of this study was to determine if a single IV administration of the same dose (2.5 ml/100 pounds bodyweight) of IVM [Ivomec® Injection Sterile 1% Solution (Merial)] results in measurable IVM concentrations in CSF. The IVM dose was added to 1 L 0.9% NaCl and given over 30 minutes to 5 healthy llamas. CSF and blood were collected from indwelling spinal (lumbo-sacral) and jugular catheters respectively, at baseline and 2, 4, 6, 12, 24, 48 and 60 hr after IVM administration. Serum and CSF were stored at −40 °C and IVM concentrations were determined using HPLC.

Immediately after administration of IVM, 3 of the llamas showed transient lethargy and decreased appetite. One llama developed acute neurological signs (paralysis and seizures) 7 days after IVM administration and was euthanased 3 days later, having failed to respond to supportive treatment. Histopathologic examination revealed diffuse myelinic edema in the brain and spinal cord. Although it is possible that these histological changes resulted from IVM administration, IVM was only detected in the CSF at the 4 hr sampling (0.11 ng/ml). Two other llamas developed laboratory changes consistent with septic inflammation in the CSF (increase in nucleated cells predominantly neutrophils) and in one of these cases free and phagocytosed bacteria were present. The CSF returned to normal in both llamas after antibiotic treatment and neither llama had clinical signs at any stage. Results are reported as mean and range.

The results indicate that CSF concentrations of IVM can be achieved following IV administration at the dose used in this study; however, it is not known whether these concentrations are therapeutic in cases of meningeal worm infestation. In addition, because of the possibility of IVM-induced neurological changes, it is recommended that IVM not be administered at this dose IV.

	Serum IVM (ng/ml)	CSF IVM (ng/ml)
2 hr	4751 (2291–7742) [n = 5]	0.258 (0–0.57) [n = 5]
4 hr	491 (102–615) [n = 5]	0.282 (0.1–0.68) [n = 5]
6 hr	298 (86–555) [n = 5]	0.78 (0–2.49) [n = 5]
12 hr	124 (58–222) [n = 5]	0.145 (0–0.44) [n = 4]
24 hr	67 (23–208) [n = 5]	0.115 (0–0.22) [n = 4]
48 hr	38 (10–118) [n = 5]	0.09 (0–0.15) [n = 5]
60 hr	22 (11–48) [n = 5]	0 0 [n = 4]

ABSTRACT #56

CARDIOVASCULAR EFFECTS OF DOBUTAMINE AND NOREPINEPHRINE INFUSION IN HEALTHY, ANESTHETIZED ALPACAS. CJ Vincent, AT Hawley, EA Rozanski, KM Lascola, D Bedenice. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

The objective of this study was to characterize the cardiovascular effects of dobutamine and norepinephrine infusion in isoflurane-anesthetized, healthy alpacas.

Eight adult alpacas (3 females, 5 intact males, 4.1 ± 2.7 years) were evaluated. Initial baseline cardiovascular, respiratory, and metabolic variables were obtained 30 minutes after induction of isoflurane anesthesia. Four treatments (dobutamine at 4 and 8 µg/kg/min; norepinephrine at 0.3 and 1 µg/kg/min) were administered in random order via constant rate infusion over 15 minutes, followed by repeat measurements of values and a 20 minute washout period. Subsequent baseline and post-treatment measurements were similarly repeated until both drugs and dosages were administered to each animal. Baseline data in awake alpacas was obtained 18–24 hours following recovery from anesthesia.

Both norepinephrine and dobutamine significantly elevated cardiac index and arterial blood pressure from baseline. Similar increases in hemoglobin, oxygen content, and oxygen delivery were observed following administration of each drug at either dosage. Only dobutamine, however, reduced relative oxygen consumption while improving overall oxygen balance. Furthermore, heart rate was selectively enhanced by dobutamine and systemic vascular resistance by norepinephrine. Norepinephrine infusion resulted in dose dependent changes in cardiovascular variables.

This study shows that both dobutamine and norepinephrine are appropriate choices to improve cardiac index, mean arterial pressure, and thus overall oxygen delivery in alpacas experiencing isoflurane induced hypotension. The lower infusion rates of both dobutamine (4 µg/kg/min) and norepineprhine (0.3 µg/kg/min) are recommended to avoid potential arrhythmogenic effects and excessive vasoconstriction, respectively.

ABSTRACT #57

EXPRESSION OF SEROTONIN, TRANSFORMING GROWTH FACTOR-BETA1, AND EXTRACELLUAR MATRIX SIGNALING MOLECULES IN MYOXOMATOUS MITRAL VALVE TISSUE. MA Oyama[1], SV Chittur[2], JE Rush[3], BW Keene[4]. [1]Department of Clinical Sciences, University of Pennsylvania, Philadelphia, PA, [2]Center for Functional Genomics, State University of New York at Albany, Albany, NY, [3]Department of Clinical Sciences, Tufts University, North Grafton, MA, [4]Department of Clinical Sciences, North Carolina State University, Raleigh, NC.

The etiology and pathogenesis of canine myxomatous mitral valve disease (MVD) are poorly understood. Using oligonucleotide microarrays, we have previously reported increased expression of various serotonin (5HT) and transforming growth factor-beta1 (TGF-B1) signaling molecules in myxomatous canine valve tissue. We have also shown that canine interstitial valve cells demonstrate increased downstream mitogen activated protein-kinase (MAP-K) activity after exposure to in vitro 5HT, suggesting that serotonin and TGF-B1 may have the capacity to mediate both the proliferative and degenerative changes seen in MVD. Based on these results,

we sought to further characterize the expression of key signaling components of the 5HT and TGF-B1 pathways and extracellular matrix in affected canine mitral valves. Mitral valve leaflets from 7 dogs that were euthanized or died due to severe MVD were excised, snap frozen in liquid nitrogen, and stored at −70C until processing. Mitral valve tissues from 5 healthy dogs that were euthanized for an unrelated study were collected as controls. Total RNA was isolated and analyzed for integrity and quality. Transcription was evaluated by real-time reverse transcriptase quantitative PCR (rt-qPCR) using a commercial system (SybrGreen RT-qPCR, Applied Biosystems). Fifteen different transcripts were analyzed, including various 5HT-related molecules (5HT-receptor subtypes 1A, 1B, 2A, and 2B, and 5HT transporter), TGF-B1-related molecules (TGF-B1, TGF-B1 receptor, SMAD-2, and TAK-1), and extracelluar matrix components (MMP-1, 2, and 9, cathepsin K, collagen A, and fibronectin). Results were expressed in units of relative expression. For the 5 5HT-related molecules, transcriptional activity was detected in 33 of 35 MVD samples (94%) vs 9 of 25 control samples (26%) ($P<0.0001$). Affected valves had significantly increased 5HT-receptor-1B as compared to control (MVD 29.8 vs control 0.0; $P<0.05$). For TGF-B1 components, no significant difference was found in the presence or absence of expression or relative expression levels between MVD and control. For extracellular matrix components, transcriptional activity was detected in 40 of 42 MVD samples (95%) vs 19 of 30 control samples (63%) ($P=0.0005$). Affected tissue had significantly increased MMP-9 transcription as compared to control (MVD 98.5 vs control 0.0; $P<0.01$). Expression of 5HT and extracellular matrix components is more prevalent in affected mitral valves, and 5HT signaling may play a role in the pathology of canine MVD.

ABSTRACT #58

SERUM SEROTONIN CONCENTRATION IS ELEVATED IN DOGS WITH DEGENERATIVE MITRAL VALVE DISEASE. JW Arndt[1], MA Oyama[1], JM Connolly[2], CA Reynolds[1], RJ Levy[2]. [1]Department of Clinical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. [2]Department of Pediatric Cardiology Research, The Children's Hospital of Philadelphia, Philadelphia, PA.

Little is known concerning the molecular mechanisms involved in degenerative mitral valve disease (DMVD). In humans, elevated serotonin (5-HT) is associated with development of valvular lesions. Canine mitral valve cells demonstrate dose-dependent 5-HT-mediated ERK1/2 signaling, suggesting a possible link with canine DMVD. We sought to measure serum 5-HT concentration in dogs with DMVD, dogs predisposed to DMVD (small breed dogs weighing <10 kg and without a murmur), and healthy large breed control dogs. Measurement of 5-HT was performed using a competitive ELISA (IB89527, Immuno Biological Laboratories, Inc., Minneapolis, MN), which was validated for canine use. Seventy-nine dogs were enrolled (27 affected, 24 predisposed, and 28 controls), with 17/27 affected and 15/24 predisposed dogs being Cavalier King Charles Spaniels (CKCS). The assay demonstrated acceptable linearity ($r=0.96$), parallelism ($P>0.05$), and recovery (mean=100.9%). Analysis revealed significantly higher mean serum 5-HT in affected dogs vs. control dogs (affected, 650.6 [SD=232.9] ng/ml vs. control, 474.3 [210.4]; $P<0.05$), and in predisposed vs. control dogs (predisposed, 765.9 [322.8] vs. control, 474.3; $P<0.001$). Subgroup analysis revealed that predisposed CKCS had greater mean serum 5-HT than predisposed non-CKCS (CKCS, 903.9 [321.5] ng/ml vs. non-CKCS, 536.0 [153.7]; $P=0.004$). We conclude that dogs with clinically apparent DMVD as well as CKCS that are predisposed to DMVD have elevated serum 5-HT. Our results suggest that 5-HT may play a role in the development of DMVD in small breed dogs, and in particular in the CKCS. Further studies involving the relationship between 5-HT, DMVD, breed, and platelet number, morphology, and function are warranted.

ABSTRACT #59

GENETIC ASSOCIATION OF THE A31P AND A74T POLYMORPHISM IN THE MYBPC3 GENE AND HYPERTROPHIC CARDIOMYOPATHY IN MAINE COON CATS. G Wess, C Schinner, K Weber, K Hartmann. Clinic for Small Animal Internal Medicine, LMU University of Munich, Germany.

Hypertrophic cardiomyopathy (HCM) is the most common feline cardiac disease and is inherited as an autosomal dominant trait. The A31P and A74T single nucleotide polymorphisms (SNPs) in the MYBPC3 gene are thought to be causative mutations in Maine Coon cats. However, in many investigators' experience phenotypes often differ from genotypes. Echocardiographically confirmed HCM positive Maine Coon cats may have negative genetic test results, and vice versa. Veterinarians are therefore often unsure which recommendations they should give breeders with various test results. The aim of this study was to evaluate the association of the A31P and A74T SNPs and HCM in Maine Coon cats and to validate the clinical use of both genetic tests. The presence of these SNPs in other breeds was assessed as well.

83 Maine Coon cats (mean age 71.3 months) and 68 cats of other breeds (mean age 102.4 months) were prospectively phenotyped using echocardiography as phenotype-healthy or phenotype-HCM. Inclusion criteria were a minimum age of 24 months for males and 36 months for females. The HCM-phenotype was defined as a left ventricular wall thickness of more than 6 mm in diastole measured in a 2D image using a VIVID 7 (General Electrics). Cats with hyperthyroidism or hypertension were excluded. Taqman® genotyping assays were used for genotyping. The potential impact of the SNPs was assessed with PolyPhen software.

Of the 83 Maine Coon cats, 21.7% were positive and 78.3% negative for A31P. None of the other breeds had a positive A31P SNP. From the genotype-positive cats, 83.3% were phenotype-healthy and 16.7% were phenotype-HCM. From the genotype-negative cats, 86.1% were considered phenotype-healthy and 13.9% phenotype-HCM. 79 Maine coons were tested for the A74T SNP and 35.4% were genotype-positive. The A74T SNP was present in other breeds as well. 78.6% of the Maine Coon genotype A74T SNP positive cats had a normal ultrasound examination. The genotype-negative cats were considered as phenotype healthy in 88.2% and as HCM in 11.8%.

There was no significant allele frequency difference between HCM and healthy cats for both SNPs. Echocardiographic HCM positive cats were genotype-negative in 75% concerning the A31P and in 50% concerning the A74T SNP. None of the genetic tests could provide a useful predictive value of disease outcome. Computer based protein analysis of the evaluated SNPs indicated the potential impact of both SNPs to be ''benign''. The use of presently available genetic tests for selective breeding appears to be questionable according to these study results. The gold standard for the diagnosis of HCM remains the annually echocardiographic examination.

ABSTRACT #60

EFFECT OF RENAL DYSFUNCTION ON N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE; A CANINE BIOMARKER FOR HEART DISEASE. MK Schmidt[1], CA Reynolds[2], AH Estrada[1], R Prošek[1], HW Maisenbacher[1], MM Sleeper[2], MA Oyama[2]. [1]University of Florida College of Veterinary Medicine, Gainesville, FL.[2]University of Pennsylvania College of Veterinary Medicine, Philadelphia, PA.

N-terminal B-type natriuretic peptide (NT-proBNP) is secreted from the cardiac ventricles in response to volume expansion or pressure overload. This peptide has previously been shown to distinguish cardiac and noncardiac causes of dyspnea in humans and dogs. In humans with kidney disease, BNP concentration can be elevated due to decreased renal clearance, and clinical interpretation of these markers must take renal function into account. The aim of this study is to determine the effects of kidney dysfunction on serum NT-proBNP concentrations in dogs. In total, 28 dogs were examined by physical examination, echocardiography, renal panel, non-invasive blood pressure measurement, urinalysis, and serum NT-proBNP assay (Canine CardioCare, Veterinary Diagnostics Institute, Irvine CA). Twenty-two healthy control dogs and 6 dogs with renal disease but without a heart murmur, valvular, or primary myocardial disease were recruited. Mean NT-proBNP in control dogs was 282 pmol/L, range of 179 to 578 pmol/L. Compared with controls, NT-proBNP was significantly increased in dogs with kidney dysfunction (mean=1069 pmol/L; range 179–2071 pmol/L; $P=0.0004$). These results suggest that renal insufficiency may false-

ly increase serum NT-proBNP; however, a high degree of variability was present within the kidney dysfunction group. Optimal NT-proBNP cut-off values for the diagnosis of heart disease or heart failure in dogs with concurrent renal dysfunction should be investigated.

ABSTRACT #61
EFFECTS OF ATORVASTATIN ON ENDOTHELIAL FUNCTION, LIPID PROFILES AND INFLAMMATORY MARKERS IN HEALTHY DOGS AND DOGS WITH CONGESTIVE HEART FAILURE. SM Cunningham, JE Rush, LM Freeman. Tufts Cummings School of Veterinary Medicine, North Grafton, MA.

HMG-CoA reductase inhibitors (statins) improve heart failure class and survival in people with congestive heart failure (CHF), independent of cholesterol reduction. Pleiotropic statin effects include antioxidant and anti-inflammatory properties, antiarrhythmic actions, and improvement of endothelial function. Impaired endothelium-dependent flow-mediated vasodilation (FMD) occurs in people with CHF and is a strong predictor of adverse outcome. The goals of this study were to evaluate the tolerability of atorvastatin in healthy dogs and in dogs with CHF, to determine whether dogs with CHF have endothelial dysfunction, and to evaluate the effects of atorvastatin on markers of endothelial function, systemic inflammation, and oxidative stress in dogs with CHF.

In part I of the study, employee-owned dogs that were healthy based on history, physical examination, CBC, biochemistry profile, and urinalysis (n=11) were treated with atorvastatin and reevaluated on days 14 and 30. Testing on each day included an examination and fasting CBC, biochemistry profile, and C-reactive protein (CRP) concentrations. FMD of the brachial artery was assessed in 6 of 11 dogs on days 0, 14, and 30. Ultrasonographic changes in arterial diameter and blood flow velocities were measured at 10, 15, 30, 45, and 60 sec after a 3-minute period of vessel occlusion. In part II of the study, client-owned dogs with ISACHC Class II or IIIa CHF (n=13) were administered atorvastatin at 2 mg/kg q 24 h for 8 weeks. All dogs were assessed on days 0 and 56 of the study via echocardiography, ECG, blood pressure (BP), and owner-completed FETCH questionnaires. CBC, biochemistry profiles, lipid fractionation, concentrations of 8-$F_{2\alpha}$-isoprostanes, NT-proBNP levels, and CRP were measured at each visit. FMD also was assessed in 5 of 13 dogs on days 0 and 56 of the study. Baseline parameters were compared between control and CHF dogs using Mann-Whitney-U tests, while pre- and post-atorvastatin data were analyzed using Wilcoxon signed ranks tests. At baseline, FMD assessment revealed a trend ($p=0.06$) towards a blunted change in the brachial artery velocity profile at 15 seconds in CHF dogs compared to healthy dogs. Atorvastatin was well-tolerated in control and CHF dogs and did not result in side effects or significant increases in hepatic transaminases or creatine kinase. Reductions in systolic BP ($p=0.04$), total serum cholesterol ($p=0.01$), non-HDL cholesterol ($p=0.002$), and total leukocytes ($p=0.03$) were noted in the CHF group after receiving atorvastatin. There were no significant changes in echocardiographic or FMD parameters or in FETCH scores. Healthy dogs also had a reduction in total cholesterol ($p=0.01$) after atorvastatin administration. Analysis of CRP, isoprostanes, and NT-proBNP concentrations is pending. In conclusion, atorvastatin was well-tolerated at this dose in both groups of dogs and further investigation into the effects of statin therapy in dogs with CHF is warranted.

ABSTRACT #62
TROPONIN CONCENTRATIONS IN PATIENTS WITH MASSES OR TUMORS. G Farace[1], A Beardow[1], C Carpenter[1], K Yeung[1], M Zieba[1], SJ Ettinger[2], SD Forney[2]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA.

During the course of the IDEXX/California Animal Hospital cardiac study we have discovered a number of interesting associations. One such association is the fact that enrolled patients with masses or tumors have higher levels of troponin than similar patients with cardiac disease alone.

Taking all 658 patients currently in the study, normal dogs (n=115) have a mean troponin of 0.34 ng/ml (95% CI 0.22–0.46); those patients with at least some minor echocardiographic changes (n=445) have a mean troponin concentration of 1.29 ng/ml (95% CI 0.97–1.61), while patients with a mass or tumor (n=98) have a mean troponin of 11.71 ng/ml (95% CI 4.99–18.46). Wilcoxon tests show that the group with masses is significantly higher than both the normal dogs and the cardiac disease population ($p<0.0001$). Dividing the cardiac patients into those with minor echocardiographic changes or with asymptomatic disease and those with clinical signs of heart disease or heart failure gives a mean troponin of 0.79 ng/ml (95% CI 0.44–1.13) for the first group and a mean of 1.82 ng/ml (95% CI 1.29–2.36) for the second. Treating the patients with masses in the same manner, we have a group with masses and minor echocardiographic changes or asymptomatic disease and a group with masses and clinical signs of heart disease or heart failure. The means for the two groups are 21.46 ng/ml (95% CI 6.69–36.22) and 5.12 ng/ml (95% CI 1.31–8.92), respectively. In both cases the group of patients with masses is still significantly different from the comparable cardiac group ($p<0.0001$ and $p<0.0026$); therefore severity of cardiac disease does not explain the observed difference. The percentage of patients with a troponin over 2.00 ng/ml in the entire cardiac disease only group is about one-third of that in the group with masses. This gives a 5.4-fold relative risk that elevated troponin is associated with a mass or tumor. Dropping to a 1.00 ng/ml cut-off still gives a 3.5-fold relative risk.

The entire cardiac and masses/tumor groups are similar in terms of N-terminal prohormone atrial natriuretic peptide concentration (1943 vs. 1766 fmol/ml, respectively) and N-terminal prohormone brain natriuretic peptide concentration (1482 vs. 1190 pmol/L respectively). Again this indicates that the underlying cardiac disease is not the cause of the differences in troponin.

Taking into account the location of the mass, it is apparent that troponin is not consistently elevated in the presence of a mass. Only masses located in the heart, spleen, or multiple locations show mean troponin levels over 1.00 ng/ml. Masses in organs such as the lung or liver do not result in a mean troponin greater than 1.00 ng/ml.

Troponin is not consistently elevated in all cases where a mass is present so it cannot be used as a tumor screen; however, troponin levels above 1.00 ng/ml may indicate that the patient has a mass or tumor, and that the mass is likely located in the heart, spleen or multiple sites.

ABSTRACT #63
GREAT DANE DILATED CARDIOMYOPATHY IS ASSOCIATED WITH ALTERED TRIADIN AND CALSTABIN2 TRANSCRIPTION. MA Oyama[1], CR Reynolds[1], M Kuentzel[2], SV Chittur[2]. [1]Department of Clinical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.[2]Center for Functional Genomics, State University of New York, Albany, NY.

Little is known regarding the molecular mechanisms underlying dilated cardiomyopathy (DCM) in the Great Dane. Thus, we performed a widescale evaluation of transcriptional activity from myocardial tissue of affected dogs. cRNA was generated from left ventricular tissues from 3 Great Danes that were euthanized due to refractory DCM and from 3 large breed dogs euthanized for non-cardiac reasons and hybridized to a second generation canine-specific microarray (Canine GeneChip 2.0, Affymetrix). Comparison of transcriptional activity revealed significant downregulation of 24 transcripts and upregulation of 298 transcripts in affected dogs vs control. The two transcripts with the greatest differential expression were triadin (9.07-fold down-regulated) and calstabin2 (FKBP12.6) (61.3-fold up-regulated). Expression of these two transcripts was further investigated using RT-qPCR. Both triadin and calstabin2 are regulators of cardiac ryanodine receptor (RYR2) activity and hence movement of Ca^{2+} within myocardial cells. Triadin is a transmembrane protein that links RYR2 to calsequestrin and is a critical determinant of systolic Ca^{2+} release from the sarcoplasmic reticulum. Triadin deficiency would putatively lead to reduced calsequestrin-RYR2 binding, "leaky" RYR2, and decreased efficiency of excitation-contraction coupling. Calstabin2 binds to the cytoplasmic domain of RYR2 and promotes a closed RYR2 state; thus increased expression of calstabin2 may represent a compensatory response. Further study involving calcium transients, RYR2 function, and the role of triadin and calstabin2 in Great Dane DCM is warranted.

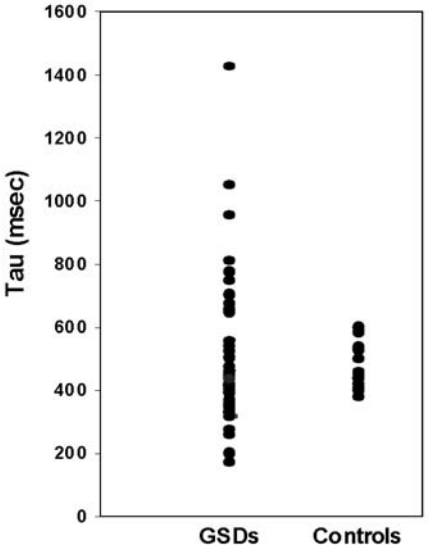
ABSTRACT #64

CARDIOMYOCYTE CALCIUM TRANSIENTS IN GERMAN SHEPHERD DOGS WITH INHERITED VENTRICULAR ARRHYTHMIAS. SA Jesty,[1] BG Kornreich[1], J Cordeiro,[2] C Antzelevitch,[2] NS Moïse[1]. [1]College of Veterinary Medicine, Cornell University, Ithaca, NY. [2]Masonic Medical Research Laboratory, Utica, NY.

Inherited ventricular arrhythmias and abnormalities of repolarization have been documented in German Shepherd Dogs (GSDs). The purpose of this study was to determine the temporospatial aspects of calcium cycling in the ventricular cells of affected GSDs and normal dogs. The hypotheses were that calcium transients in the ventricular cells of affected GSDs would be different from those in normal dogs, and that calcium sparks and early/delayed afterdepolarizations (EADs/DADs) would be more numerous in cells of affected dogs as a result of the calcium cycling abnormalities.

Ten affected GSDs and 10 unaffected dogs were studied. Midmyocardial cells (M cells) were harvested from dogs after euthanasia. Myocytes were loaded with Fluo 3, a fluorophore whose emission spectrum is shifted in the presence of calcium. Myocytes were field stimulated and XT line scan images were recorded using confocal microscopy.

M cells from affected GSDs displayed a high variation in the decay phase of the calcium transient (Tau), compared with normal dogs (Figure). Affected dogs had a mixture of abnormal and normal cells, whereas normal dogs had more consistent calcium cycling across cells. Additionally, the number of calcium sparks, EADs, and DADs was greater in affected GSDs.



These results indicate that abnormalities of calcium cycling, and specifically cell to cell electrical heterogeneity, may be a mechanism contributing to the inherited ventricular arrhythmias in GSDs.

ABSTRACT #65

COMPARISON OF NT-PRO-BNP CONCENTRATION IN CATS WITH ACUTE DYSPNEA FROM CARDIAC OR RESPIRATORY DISEASE. PR Fox[1], MA Oyama[2], K MacDonald,[3] CA Reynolds[2]. [1]Animal Medical Center, New York, NY; [2]University of Pennsylvania, Philadelphia PA; [3]Animal Care Center, Rohnert Park, CA.

Biomarkers are increasingly used to help differentiate causes of acute dyspnea. We aimed to determine whether NTproBNP blood concentrations clinically differentiated respiratory vs cardiac causes of acute dyspnea in cats.

Recruitment required presentation for acute dyspnea. Each cat was evaluated by medical history, physical examination, thoracic radiography, and echocardiography by a board certified cardiologist. Cause of dyspnea was designated cardiac (CHF) or noncardiac (primary respiratory disease). A central laboratory blinded to diagnosis performed serum NTproBNP assays. NTproBNP concentrations were compared by Mann Whitney test. Spearman correlation assessed NTproBNP concentrations vs echocardiographic measurements in CHF cats. Receiver operating characteristic analysis evaluated NTproBNP outcome.

Median NTproBNP was significantly different between cats with dyspnea associated with CHF (n=34) vs respiratory disease (n=22) ($P<0.0001$). Median NTproBNP [interquartile range] was 846 pmol/L [567–1160 pmol/L] for CHF and 52 pmol/L [24–119 pmol/L] for respiratory disease. Correlations (BNP vs echo) were LVPWd (P=0.039, r=0.354), LVd (P=0.113), LVs (P=0.053), %FS (P=073), IVSd (P=0.336), LA (P=0.099), LA/Ao (P=0.12), and vertebral heart score (P=0.995).

NTproBNP >180 pmol/L for dyspnea caused by CHF possessed 94.1% sensitivity, 86.4% specificity, 91.4% positive predictive value, and 90.5% negative predictive value vs respiratory (fitted ROC area under curve, 0.976 [SE=0.0183]). Thus, NTproBNP determination may help differentiate cardiac vs. primary respiratory cause of dyspnea in cats.

ABSTRACT #66

NT-PRO-BNP ASSAY DISTINGUISHES CARDIAC VS PRIMARY RESPIRATORY CAUSES OF RESPIRATORY SIGNS IN DOGS. MA Oyama[1], JE Rush[2], EA Rozanski[2], PR Fox[3], CA Reynolds[1], S Gordon[4], B Bulmer[5], B Lefbom[6], W Brown[7], L Lehmkuhl[8], R Prosek[9], M Lesser[10], M Kraus[11], M Bossbaly[12], G Rapoport[13]. [1]University of Pennsylvania, Philadelphia, PA; [2]Tufts University, North Grafton, MA; [3]The Animal Medical Center, New York, NY; [4]Texas A&M University, College Station TX; [5]Oregon State University, Corvallis, OR; [6]Chesapeake Veterinary Cardiology Associates, Vienna, VA; [7]Veterinary Cardiology Consultants, Novi, MI; [8]MedVet, Worthington, OH; [9]University of Florida, Gainsville, FL, [10]Advanced Veterinary Care Center, Lawndale, CA; [11]Cornell University, Ithaca, NY; [12]Heartsound Consultants, Langhorne, PA; [13]Angell Memorial Animal Hospital, Boston, MA.

In dogs with respiratory signs, differentiation of the underlying etiology (i.e., congestive heart failure [CHF] vs. primary respiratory disease such as pneumonia, chronic airway disease, fibrosis, etc.) is an important step towards accurate diagnosis and treatment. NT-pro-B-type natriuretic peptide (NT-proBNP) is elevated in dogs with CHF, and we sought to evaluate its ability to differentiate cardiac vs. non-cardiac etiology of respiratory signs in a prospective multicenter clinical study. Dogs with respiratory signs (i.e., tachypnea, cough, increased respiratory effort, etc.) that were judged severe enough by the owner to adversely affect the dog's quality of life were recruited from 13 sites. Dogs underwent physical exam, thoracic radiographs, and echocardiogram, after which a board-certified cardiologist categorized etiology of signs as follows: Group 1, CHF; Group 2, primary respiratory disease; Group 3, primary respiratory disease with concurrent heart disease but without CHF; Group 4, equivocal. Serum NT-proBNP assay was performed at a central laboratory blinded to the diagnosis. Complete data was available for 116 dogs. Median NT-proBNP was significantly different between Groups 1 and 2 and between Groups 1 and 3 ($P<0.001$). Group 1, n=62, median NT-proBNP=2445 pmol/L, [interquartile range=1499–3134]; Group 2, n=21, NT-proBNP=413 pmol/L [245–852]; Group 3, n=28, NT-proBNP=510 pmol/L [353–1212]). NT-proBNP >1200 pmol/L possessed 85.5% sensitivity, 81.6% specificity, 85.5% positive predictive value, and 81.6% negative predictive value for identifying dogs with congestive heart failure (Group 1) vs. dogs with primary respiratory disease with or without concurrent heart disease (Groups 2 and 3). Area under the receiver-operating characteristic curve was 0.908 (SE=0.029).

ABSTRACT #67

CIRCULATING NATRIURETIC PEPTIDES CONCENTRATIONS IN CATS WITH RESPIRATORY DISTRESS. D J Connolly, R J Soares Magalhaes, V Luis Fuentes, H M Syme, G Cole, AM Boag. Royal Veterinary College, Herts, UK.

Cats in respiratory distress (RD) can represent a significant diagnostic challenge. The ability to distinguish cardiac from non-cardiac

causes of RD is a vital initial step to achieving an accurate diagnosis and appropriate treatment. It is often not possible to do this on the basis of history and physical examination, and the compromised state of cats with severe RD often limits diagnostic evaluation. Human and canine studies have shown circulating B-type natriuretic peptide concentration to be an accurate predictor of congestive heart failure (CHF), enabling patients with CHF to be differentiated from those with non-cardiac causes of RD (RDNC). In contrast to dogs and humans, relatively little is known about the utility of natriuretic peptide testing in cats. It has been established that circulating NT-proANP and particularly NT-proBNP concentrations measured by ELISA identified cats with heart disease and heart failure. The study aim was to determine if circulating natriuretic peptide (NP) concentration could distinguish cats with RDNC from those with CHF.

The study recruited 91 cats from 1 university teaching hospital and 2 private practices. Serum natriuretic peptide concentrations were measured in 41 cats with RDNC (diseases included asthma, neoplasia, pyothorax, pneumonia, pleural effusion, bronchitis, rhinitis, nasopharyngeal stenosis, nasopharyngeal polyp) and compared to cats with asymptomatic heart disease (AsymHD n=17) and cats with RD due to CHF (RDCHF n=33) using sandwich enzyme immunoassays. The diagnoses in the cats with heart disease included hypertrophic cardiomyopathy, hypertrophic obstructive cardiomyopathy, or both (n=36); restrictive cardiomyopathy (n=10); dilated cardiomyopathy (n=1); mitral dysplasia (n=1); double-chambered right ventricle (n=1); and idiopathic third degree atrioventricular block (n=1). The ability of circulating NP concentrations to distinguish cats with RDNC from those with RDCHF was explored using receiver operator curve (ROC) analysis. The RDCHF group had higher median NT-proANP and NT-proBNP concentrations (1690 and 523 fmol/ml respectively) than the RDNC group (614 and 45 f/mol/ml, $p < 0.05$ and $p < 0.05$, respectively). The area under the curve was 0.88 and 0.96 for the ROC analysis of the diagnostic accuracy of NT-proANP and NT-proBNP concentrations to discriminate RDCHF from RDNC cats. An optimum cut-off concentration of 986 fmol/ml for NT-proANP and 220 fmol/ml for NT-proBNP accurately discriminated RDNC from RCCHF cats with a sensitivity and specificity of 93.8% and 80.3% and 93.9% and 87.8% respectively.

Serum NP concentrations were different in cats with CHF compared with those with respiratory distress of non-cardiogenic origin. Evaluation of circulating NP concentrations may be helpful in the initial approach to cats presenting with respiratory distress.

ABSTRACT #68
CAN A "LOUD" RIGHT-APICAL SYSTOLIC MURMUR PREDICT THE DIAGNOSIS OF CANINE PULMONARY HYPERTENSION? DG Ohad, I Lenchner, T Bdolah-Abram. The Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel.

The index of clinical suspicion of pulmonary hypertension (PHT) is typically low. We tested whether selected findings predict the diagnosis of PHT, as confirmed by a Doppler-derived systolic pressure gradient >35 mmHg between the right ventricle and atrium.

Data from 312 consecutive dogs were retrospectively analyzed to calculate the correspondence ratio between pairs of non-quantitative (historical and/or physical) versus quantitative (Doppler) variables. Sensitivity, specificity, and predictive value indices were computed. The McNemar test was used to seek a trend among cases of disagreement.

The following (in a descending order) variables were more prevalent in PHT (n=96) than in non-PHT (n=216) patients: left apical murmur, cough, right apical murmur, syncope, ascites, dyspnea, a stronger right than left apical murmur, hepatomegaly, and orthopnea. Both left and right apical murmurs were louder in PHT than in non-PHT patients.

A "loud" right apical murmur, whether isolated or combined with non-quantitative variables as ascites or syncope, had a low sensitivity of <44% and a high specificity of >94% as a predictor of PHT. While an isolated stronger right than left apical murmur had a positive predictive value (PPV) of 82% and was 98% specific for PHT, when combined with syncope it had a PPV of 91%, and was 96% specific for PHT. The combination of ascites with a Grade ≥IV/VI right apical murmur had a PPV of 100% and a specificity of 100% for PHT.

A louder-than-expected right apical systolic murmur combined with ascites and/or syncope should highly increase the index of suspicion for PHT.

ABSTRACT #69
TRANSVENOUS COIL EMBOLIZATION OF PATENT DUCTUS ARTERIOSUS IN SMALL DOGS. E Henrich, E Hassdenteufel, N Hildebrandt, C Fischer, M Schneider. Department of Clinical Studies, Small Animal Clinic (Internal Medicine and Surgery), Justus-Liebig-University Giessen, Giessen, Germany.

The occlusion of patent ductus arteriosus (PDA) in small canine patients is challenging for both surgical and interventional therapy. This prospective study examines the feasibility and success of transvenous coil embolization of patent ductus arteriosus in small dogs.

Inclusion criteria were a left-to-right shunting PDA and a body weight equal to or less than 3.0 kg.The presence of congestive heart failure was not considered an exclusion criterion. Patients with additional congenital cardiac diseases were excluded. Under general anaesthesia the right femoral vein was accessed percutaneously using a 4 French introducer sheet. The PDA was catheterized retrogradely with a 4 French wedge catheter, a 4 French multipurpose catheter, and a 0.018 inch guide wire. After switching to an angiographic catheter, the contrast medium was injected into the descending aorta. PDA morphology was classified and the minimal and ampulla diameter of the ductus were measured. A commercial 0.038 inch coil (Detachable Coil for PDA closure, Cook Deutschland GmbH) measuring at least twice the minimal diameter of the PDA was chosen. The coil was placed with approximately 1/2 loop of the coil anchored on the pulmonary side. An echocardiographic follow-up was performed within 24 hours post intervention.

Twenty-one dogs underwent transvenous coil occlusion, with Chihuahua and Yorkshire Terrier being the most common breeds (n=6 and n=5, respectively). The distribution of sex was 14 female and 7 male dogs. The ages of the patients ranged from 1.9 to 83.5 months (median 7.7 months), and the body weight from 1.0 and 2.9 kg (median 1.9 kg). The minimal diameter of the PDA measured 1.2 to 2.4 mm (median 1.8 mm) and the PDA ampulla 2.4 to 5.9 mm (median 4.6 mm). All but one PDA had an aortic ampulla and a pulmonary constriction (Type E, n=16, Type A, n=4). The remaining patient had two constrictions (one on the aortic and one on the pulmonary side, Type D). The coil implantation was successful in all patients. 7 dogs received a coil with a 3 mm loop-diameter while in the other 14 dogs a coil with a 5 mm loop-diameter was implanted. After the coil had been detached, repositioning of the pulmonary loop of the coil with a 4 French wedge catheter became necessary in one dog. Two dogs developed temporary bradycardia after coil implantation requiring medical therapy and 16/21 (76%) dogs had no residual shunting in the follow-up echocardiography after 24 hours.

In conclusion: For an experienced cardiologist, transvenous embolization of patent ductus arteriosus in small dogs is possible using a 4 French catheter and a commercial detachable coil. Arterial access is not essential and the procedure is safe and successful.

ABSTRACT #70
EMBOLIZATION OF LARGE PATENT DUCTUS ARTERIOSUS (> 4.0 mm) IN DOGS WITH A SINGLE POLYESTER FIBERED DOUBLE-HELIX COIL. M Schneider, N Bierent, N Hildebrandt, C Fischer. Department of Clinical Studies, Small Animal Clinic (Internal Medicine and Surgery), Justus-Liebig-University Giessen, Giessen, Germany.

Coil embolization of large patent ductus arteriosus (PDA) is difficult, because coil stiffness is getting lower with increasing loop diameter. To increase coilstiffness a Double-Helix configuration of the coil can be used. The aim of this prospective study was to prove the feasibility of embolization of large PDAs (> 4.0 mm) with a single polyesther fibered Double-Helix coil.

Ten consecutive dogs with an angiographically determinded minimal PDA diameter of > 4.0 mm were included independent on clinical status of congestive heart failure (n=7 NYHA III or IV) or

atrial fibrillation (n=1). Detachable Double-Helix coils designed of two identical strains of stainless steel wire (0.052 inches) with polyester anchored were produced in various diameters (10, 11, 12, 14, 16 mm). Prior to the coil embolization an angiography of descending aorta was performed to classify the PDA morphologic (classification of Krichenko), to measure the PDA minimal and ampulla diameter, and to define the shunt-grade. The shunt-ratio was calculated using the Fick method. The PDA was catheterized in a retrograde manner and a 7 F Mullins sheet was placed through the PDA into the descending aorta. A Double-Helix coil with loop diameter greater than two times the PDA minimal diameter was implanted. Ten minutes after coil embolization calculation of the shunt-ratio and the angiography was repeated. In all dogs a single coil was employed regardless of residual shunting. Clinical and echocardiographic reexaminations were performed within three days after the intervention. M-mode measurements were indexed to body weight by allometric scaling. Data prior and after intervention were compared by student t-test.

German Shepherd dogs (n=4) were overrepresented. The median age was 9.2 month (range 4.3–82.0). The median body weight was 24.0 kg (range 8.1–36.0). The PDA was long and conical (Type E) in seven dogs and showed two constriction (Type D) in three dogs. The minimal PDA diameter on the pulmonic site was 4.8–6.3 mm (median 5.9 mm) and the width of the ampulla was 9.1–18.9 mm (median 15.1 mm). In 9/10 dogs the implantation of the primary selected coil was successful, in the remaining dog the next coil size has to be used. After coil placement the shunt-ratio decreased significantly (mean ± SD: 3.80 ± 0.97, range 2.4–5.3 to 1.38 ± 0.46, range 1.0–2.3, $p < 0.0001$, n=10). Complete closure was documented by angiography in one and by echocardiography in three dogs. Despite of incomplete closure in 7/10 dogs the volume overload was reduced in all dogs, leading to a significant reduction of the maximal aortic blood flow velocity (2.980 ± 0.703 to 2.160 ± 0.635, $p = 0.0016$, n=9) and of the index of left ventricular diastolic diameter (2.259 ± 0.398 to 2.025 ± 0,370, $p = 0.0023$, n=10).

In conclusion, the polyester fibered Double-helix coil has the stiffness to be fixed in a large PDA up to 6.3 mm and produce a reduction of shunt flow.

ABSTRACT #71

MYOTUBULAR MYOPATHY IN A FAMILY OF MANCHESTER TERRIER DOGS. F.L. Robinson[1], A.P. Misizin[1], D.P. O'Brien[2], G.S. Johnson[2], J.E. Dixon[1], G.D. Shelton[1]. [1]School of Medicine, University of California, San Diego, La Jolla, CA. [2]College of Veterinary Medicine, University of Missouri, Columbia, MO.

Three male Manchester Terrier littermates were presented at 2 months of age for weakness and failure to thrive. The dam and sire were clinically normal. The dam was previously bred to a different male with 2 male puppies showing similar clinical signs. Muscle biopsy specimens from all three puppies showed centrally placed nuclei resembling fetal myotubes, or a central clear zone, within most type 1 fibers. Cryostat sections reacted for oxidative enzymes showed attenuated activity at the periphery of the fibers, and dense oxidative staining at the center of the fibers, which lacked myosin ATPase activity. Ultrastructural analysis correlated well with the histochemical findings including myofilaments forming a compact peripheral rim around a central nucleus, or around a central zone, containing mitochondria, glycogen, or dilated membranous profiles. In humans, recessive mutations in the myotubularin (*MTM1*) gene cause X-linked myotubular myopathy. MTM1 is a member of a family of phosophoinositide 3-phosphatases that dephosphorylate phosphatidylinositol 3-phosphate and phosphatidylinositol 3,5-bisphosphate. The canine *MTM1* gene also resides on the X chromosome. Accordingly, *MTM1* was investigated as a possible gene responsible for the described myotubular myopathy in Manchester Terriers. MTM1 protein was analyzed in affected and normal dog muscle. MTM1 protein in detergent extracts was immunoprecipitated with an anti-MTM1 antibody (Santa Cruz Biotechnology) and protein-A agarose, then analyzed by SDS-PAGE and immunoblotting using an anti-MTM1 antibody. MTM1 protein was not detected in the muscle extracts from affected dogs. *MTM1* mRNA analysis of muscle was performed by RT-PCR. Exon 1 containing mRNA was not detected in affected dogs. In conclusion, myotubular myopathy in Manchester Terrier dogs is clinically, histologically, ultrastructurally, and biochemically similar to that of human X-linked myotubular myopathy. The absence of MTM1 protein and exon 1 containing mRNA in muscle tissue of affected dogs suggests that loss of function mutations in this PI 3-phosphatase may be the causative genetic defect. Preliminary data suggests a mutation in exon 1 of the *MTM1* gene.

Originally presented at the World Muscle Society Meeting, Giardini-Naxos, Taormina, Sicily, October 2007.

ABSTRACT #72

MYCOPHENOLATE MOFETIL THERAPY FOR ACQUIRED MYASTHENIA GRAVIS IN DOGS: A COMPARATIVE RETROSPECTIVE STUDY (1999–2007). CW Dewey[1], MF Harb-Hauser[2], S Cerda-Gonzalez[1], JM Levine[3], BL Badgley[1], NJ Olby[4], M Kent[5], N Birnbaum[6], GD Shelton[7]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY; [2]Pet Emergency and Specialty Center of Marin, San Rafael, CA; [3]Texas A&M University College of Veterinary Medicine, College Station, TX; [4]North Carolina State University College of Veterinary Medicine, Raleigh, NC; [5]University of Georgia College of Veterinary Medicine, Athens, GA; [6]Veterinary Internal Medicine Practice of N. Virginia, Manassas, VA; [7]University of California-San Diego, LaJolla, CA.

Acquired myasthenia gravis (MG) is a common autoimmune neuromuscular disorder of dogs, the effective treatment of which can be extremely challenging. Mycophenolate mofetil (MMF) is a lymphocyte-specific immunosuppressive drug that has been used successfully in human autoimmune disorders and transplant recipients. There is anecdotal evidence of MMF efficacy in dogs with various autoimmune diseases. The purpose of this investigation was to retrospectively evaluate the outcome of dogs with serologically confirmed MG that were treated with MMF and compare these MG patients to MG dogs treated with pyridostigmine (PYR) alone.

Myasthenic dogs, 14 treated with MMF (mean dose-18.6 mg/kg BID) and 8 treated with PYR alone (mean dose-1.16 mg/kg BID-TID), were identified. All but 2 MMF dogs also received PYR. Dogs in both groups were of several breeds of both sexes, and all clinical forms (focal, generalized, acute fulminating and paraneoplastic) of MG were represented. Megaesophagus was present in 12/14 (86%) dogs in the MMF group and 7/8 (88%) dogs in the PYR group. At initial diagnosis, acetylcholine receptor (ACh R) antibody titers ranged from 0.96 to 14.4 nM/L (mean-3.30 nM/L) in the MMF group, and from 1.21 to 7.13 nM/L (mean-3.97 nM/L) in the PYR group. Thymoma or lymphoma was identified in 1 MMF dog and 2 PYR dogs. Eight of 14 dogs (57%) treated with MMF achieved clinical remission of MG; 7 of these dogs had megaesophagus, 5 had pneumonia. Three of these 8 dogs (38%) came out of remission during MMF weaning. Two of 8 PYR dogs (25%) achieved clinical and immune remission; 1 of these 2 dogs had megaesophagus and pneumonia. The 1-yr mortality rate (due to MG) was 37.5% for PYR dogs and 28.6% for MMF dogs. Based on a Fisher's exact test ($p<0.05$), the differences between groups in remission rates and mortality rates were not significant.

Results of this study suggest that clinical remission of MG in dogs is more likely to be achieved with MMF than with sole PYR therapy; however, this was not statistically significant. Also, dogs attaining clinical remission with MMF should be monitored closely for disease recurrence during MMF weaning (i.e., sustained immune remission less likely). Further investigation of MMF as a therapy for canine acquired MG with a larger group of dogs is warranted.

ABSTRACT #73

INBORN ERROR OF METABOLISM IN GORDON SETTER PUPPIES: ORGANIC ACID PROFILE AND CANDIDATE GENE SEQUENCING. Gorgi AA[1], O'Brien DP[1], Shelton GD[2], Johnson GS[3]. [1]University of Missouri, College of Veterinary Medicine, Department of Small Animal Medicine and Surgery, Columbia, MO. [2]Comparative Neuromuscular Laboratory, University of California, San Diego, CA. [3]University of Missouri, College of Veterinary Medicine, Department of Veterinary Pathobiology, Columbia, MO.

A fatal, neurologic disease of Gordon Setters called DUNGd by breeders was first reported in the veterinary literature in 2000 as an

autosomal recessive trait. Gait and postural abnormalities and progressive weakness begin at 3–4 weeks of age, progressing to visual deficits and recumbency by 5–6 weeks of age. Serum chemistries and CSF analysis are normal and only subtle and non-specific histopathologic findings are observed at necropsy. Urine samples from three affected puppies were assayed by gas chromatography/mass spectroscopy for organic acids. A distinct pattern of elevated glycine conjugates (3-methylcrotonylglycine, 2methylbutyrlglycine, hexanoylglycine) was observed in all three samples. Urine from one obligate carrier parent and one clinically normal littermate showed a similar pattern, and urine samples from one obligate carrier parent and two clinically normal littermates were normal. Based on the human literature the organic acid findings were most consistent with a mutation in the biotinidase (*BTD*) or holocarboxylase synthase (*HLCS*) genes. Primary methylcrotonyl CoA carboxylase *(MCC)* deficiency has also been reported albeit with much milder clinical signs. Coding regions of the *BTD* and *HLCS* genes have been sequenced and no significant mutations identified. Sequencing of the *MCC* gene is pending.

ABSTRACT #74
BIOMECHANICAL EVALUATION OF TWO INTERNAL FIXATION IMPLANTS USED FOR CANINE CERVICAL SPINE ARTHRODESIS. DG Hicks, MJ Pitts, RS Bagley, A Vasavada, J Simon, AV Chen, FA Wininger. Washington State University, Pullman, WA.

Two fixation devices used to surgically arthrodese the canine cervical spine were evaluated in an in vitro biomechanical study. Twelve cervical spine cadaver specimens (C3-C6) were harvested from skeletally mature neurologically normal dogs. Dual energy X-ray absorptiometry of each spine was obtained to determine bone mineral density for each vertebra. Digital radiographs of the specimens ruled out skeletal abnormalities and confirmed physeal closure. Biomechanical testing of each spine was done as intact specimens and after fixation of the C4-C5 intervertebral space. Spines were randomly distributed between 2 implant treatment groups. Group 1 (n = 6) underwent traditional pin-PMMA fixation by use of positive-profile threaded pins implanted in the vertebral bodies and group 2 (n = 6) underwent screw-bar-PMMA fixation using a novel implant design. In group 2, the construct consisted of cortical bone screws implanted bilaterally in the transverse processes and a reinforcing bar was wired to the screw heads prior to covering with PMMA. Four-point bending was used to approximate a pure bending moment throughout the length of the spine. Each spine was deformed at a constant rate to the same angular deformation. Load deformation curves were recorded during extension during the third through fifth cycles of bending. Stiffness in dorsal bending of unaltered spines was compared to similar measurements in surgically altered spines.

Treated spine specimens were significantly stiffer than controls ($P < 0.0001$) in dorsal bending (estimated mean difference [treatment – control] = 0.098 Nm/degrees, CI: [0.074, 0.122]). There was no statistical difference in stiffness between the traditional pin-PMMA fixation and the novel screw-bar-PMMA fixation ($P > 0.05$).

ABSTRACT #75
SAFETY AND PHARMACOKINETICS OF 4-AMINOPYRIDINE DERIVATIVES IN DOGS. NJ Olby[1], JW Humphrey[1], M Papich[1], N Parke[1], K Spinapolis[1], PM Mehta[1], T Harris[1], R Shi[2], D Smith[2]. [1]North Carolina State University, Raleigh, NC; [2]Purdue University, West Lafayette, IN.

The myelin sheath is critical for secure axonal conduction but demyelination is a consequence of many different pathological processes within the nervous system. Loss of myelin can lead to leakage of potassium from exposed potassium channels in the axonal membrane, blocking axonal conduction and producing neurological deficits. 4-Aminopyridine (4-AP) is a potassium channel blocker that restores conduction to demyelinated axons and has been shown to improve neurological function following spinal cord injury. However, it produces unwanted side effects such as tremors and seizures at doses close to the effective dose. In order to develop safer and more effective alternatives to 4-AP, derivatives have been tested in *in vitro* and *in vivo* models of spinal cord injury in guinea pigs. Three drugs showed promise as therapeutic agents: methyl-, ethyl- and n-(4-pyridyl)-t-butyl-carbamate derivatives (MC, EC and t-BC). The aims of this study were to determine the safe range of doses of these drugs in dogs and to evaluate their pharmacokinetic properties.

Normal Beagle dogs aged between six and 18 months were used. A complete blood cell count and serum biochemistry panel were performed prior to and 24 hours after drug administration. Each dog had a jugular catheter placed to facilitate blood sampling at regular intervals for the pharmacokinetic studies. Blood samples were heparinized at the time of sampling and stored for drug concentration measurements using high performance liquid chromatography (HPLC). Following drug administration, vital parameters and behavior were recorded hourly for 6 hours, then at lower frequencies for the subsequent 18 hours. Drugs were administered to two dogs at a time at doses extrapolated from the work in guinea pigs; on each subsequent testing session the dose was doubled until either adverse effects were noted or the dose exceeded the effective dose.

None of the drugs produced adverse effects in any of the dogs at any of the doses evaluated. HPLC measurement of plasma levels showed that the MC and EC derivatives were very similar to 4-AP in their absorption and elimination. The MC derivative was administered at doses of 0.5 to 6 mg/kg and the EC derivative was administered at doses of 0.5 to 2 mg/kg. Peak blood levels were reached within 1 to 3 hours and there was little detectable drug remaining by 24 hours after administration. The t-BC derivative was far more potent than the other compounds when tested *in vitro* and so was administered at doses ranging from 0.015 mg/kg to 0.15 mg/kg. It was absorbed rapidly reaching peak levels within 30 to 60 minutes of administration. In all three drugs, plasma concentrations higher than those that restored conduction *in vitro* were achieved without causing adverse effects. We conclude that these derivatives are safe alternatives to 4-AP and phase 1 clinical trials are warranted. The lack of adverse effects may reflect poor penetration of the blood brain barrier and so measurement of CSF levels is indicated.

ABSTRACT #76
PHASE 1 CLINICAL TRIAL OF 4-AMINOPYRIDINE DERIVATIVES IN DOGS WITH CHRONIC MYELOPATHIES. NJ Olby[1], N Parke[1], K Spinapolis[1], JW Humphrey[1] PM Mehta[1], T Harris[1], M Papich[1], R Shi[2], D Smith[2]. [1]North Carolina State University, Raleigh, NC; [2]Purdue University, West Lafayette, IN.

4-Aminopyridine, a potassium channel blocker, has been shown to improve neurological function in chronic canine spinal cord injury and has recently received FDA approval for the treatment of multiple sclerosis. However, it also produces side effects such as tremors, anxiety and seizures. Carbamate derivatives of 4-AP were developed by a group at Purdue University and tested for their ability to restore conduction to the injured spinal cord *in vitro*. Effective drugs were then tested in an *in vivo* model of spinal cord injury in the guinea pig. Three derivatives compared favorably with 4-AP in these models and were shown to be safe in dogs. The aim of this study was to perform a phase 1 clinical trial of 4-AP derivatives in dogs with chronic myelopathies.

Dogs were recruited from the patient population of the NCSU College of Veterinary Medicine. Study participants had to have chronic, stable paraplegia due to an acute spinal cord injury, or to suffer from degenerative myelopathy. All cases underwent a full diagnostic workup and appropriate surgical treatment if indicated. In the first phase of the study the dogs were treated with 4-AP. The dose of 4-AP was titrated over a period of 4 days to determine the maximum, safe dose. Dogs were videotaped during a neurological examination and when walking on a non-slip surface each day of the dose titration. Once the safe dose was established, the dog was sent home on a placebo controlled, blinded trial with the owner for a period of 2 weeks; they received placebo for one week and 4-AP for the other week. The owners kept a daily log of neurological status and noted all changes in their dogs. At the end of the trial the owners were asked to identify which week the dog was on 4-AP. The trial was then repeated with one of the derivatives.

The methyl-carbamate (MC) and n-(4-pyridyl)-t-butyl-carbamate (t-BC) derivatives were evaluated. The MC derivative did not produce any discernable effect in two dogs in which it was tested. Both dogs showed a slight improvement with 4-AP but the owners

were unable to detect an effect of MC during the blinded phase of the trial. The t-BC derivative appeared to produce a clear improvement in pelvic limb function in dogs with chronic paraplegia due to acute disc herniations during dose titration and all owners correctly identified the week during which the drug was being administered. In contrast, 4-AP did not produce a discernable effect in these dogs. The t-BC derivative was administered to one dog with degenerative myelopathy and no effect was noted. Several owners noted that their dogs had increased anxiety when receiving 4-AP but this complication was not reported with either of the derivatives. We conclude that the t-BC derivative may show promise as a safe drug for the therapy of chronic canine spinal cord injury. Recruitment of dogs to the phase 1 clinical trial is ongoing and if the results continue to show promise, larger phase 2 and 3 clinical trials should be undertaken.

ABSTRACT #77
MAGNETIC RESONANCE IMAGING CONTRAST ENHANCEMENT OF THE TRIGEMINAL NERVE IN 42 DOGS WITHOUT EVIDENCE OF TRIGEMINAL NEUROPATHY. R Pettigrew, T Schwarz, H Rylander. University of Wisconsin Veterinary Teaching Hospital, Madison, WI.

Medical records and brain magnetic resonance imaging (MRI) were reviewed retrospectively from 2002–2007 in order to establish the incidence of trigeminal nerve contrast enhancement in dogs with an otherwise normal MRI and no clinical evidence of trigeminal neuropathy. Only cases without abnormalities detected on blood work, CSF analysis or MRI, and where the neurological examination did not reveal any brainstem or trigeminal nerve abnormalities, were included in the study. The MRI of 42 dogs were evaluated by a board certified radiologist and board certified neurologist. The trigeminal nerve was divided into 3 regions identified as A, B, and C. The total number of evaluations performed by the reviewers was 126. Only in 3/126 (2.4%) of evaluations could the trigeminal nerve not be identified. Region A was the only region where the trigeminal nerve could not be visualized (n=3). In 120/126 or 95% of these evaluations, contrast enhancement was observed. In region A 34/42 (81%) of the dogs showed contrast enhancement. In region B 42/42 (100%) of the dogs had contrast enhancement of the trigeminal nerve. In region C 41/42 (98%) of dogs had contrast enhancement of the trigeminal nerve. The intensity of contrast enhancement was considered less than what was seen in the pituitary gland in 105/126 (83%) of the evaluations. MRI contrast enhancement of the trigeminal nerve was seen in a majority of dogs with no clinical evidence of trigeminal nerve pathology.

ABSTRACT #78
HEREDITARY CEREBELLAR CORTICAL DEGENERATION IN SCOTTISH TERRIERS. G Urkasemsin[1], NJ Olby[1], PM Mehta[1], JS Bell[2]. [1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC. [2]Tufts Cummings School of Veterinary Medicine, North Grafton, MA.

Hereditary cerebellar degenerative diseases have been described in several breeds of dog. These progressive neurodegenerative disorders cause neurological deficits that reflect gradual loss of cerebellar and, in particular, of Purkinje neurons. Details of a Scottish Terrier with a cerebellar cortical degenerative disease have been reported and the problem appears to be becoming more prevalent within the breed worldwide. This paper reports the clinical and histopathological features, and mode of inheritance of this disease in 58 affected Scottish Terriers.

Pedigrees and medical records of affected dogs were obtained and results of diagnostic workup were recorded. A full neurological workup included neurological examination, routine blood work, diagnostic imaging of the brain, and cerebrospinal fluid (CSF) analysis. Videotapes of the dogs' gait when walking, running, and negotiating steps were evaluated. Owners were interviewed by telephone to obtain details of the onset and progression of their dog's signs, and were subsequently contacted every 3 months to document progression. A definitive diagnosis was reached using histopathology. Following euthanasia, the brain was removed, placed in 10% buffered formalin, embedded in paraffin and sections cut and stained for histopathologic evaluation. Sections from an age matched normal Scottish Terrier were used for comparison. A pedigree analysis was performed to determine the mode of inheritance.

Fifty-eight dogs were identified from 5 different countries; diagnosis was confirmed at necropsy in 9 of these dogs. Affected dogs showed onset of gait abnormalities from 2 months to 5 years of age, with the majority showing signs in the first year of life. Owners first noted clumsiness of the pelvic limbs, particularly when running. While rate of progression varied, in the majority of dogs, owners reported only slow changes or stabilization of signs and dogs were not euthanized because of neurological deterioration. Evaluation of the videotapes revealed dysmetria of all 4 legs causing difficulty in negotiating steps. When running, some dogs were unable to control the caudal half of the body, causing pronounced bouncing of the hindquarters. Subtle atrophy of the cerebellum was evident on MRI. Microscopically, there was a significant depletion of granular and Purkinje neurons, thinning of the molecular layer, and gliosis. Distribution of the degeneration was not uniform; in general the dorsal half of the cerebellum was more severely affected. Pedigree analysis suggested autosomal recessive inheritance of this disorder in Scottish Terriers.

In conclusion, hereditary cerebellar degeneration is emerging as a problem in the Scottish Terrier breed. The clinical phenotype in the majority of dogs is relatively mild and this is reflected in the restricted distribution of the histopathological changes. Genotyping and linkage analysis of families of affected dogs is underway with the aim of identifying the causative mutation.

ABSTRACT #79
GLOBOID CELL LEUKODYSTROPHY IN A LITTER OF KELPIES WITH EPISODIC CEREBELLAR DISEASE. E Beltran[1], LA Matiasek[1], L De Risio[1], C Mellersh[1], SR Platt[2]. [1]Animal Health Trust, Newmarket, UK, [2]University of Georgia, Athens, GA.

Globoid cell leukodystrophy (GLD) is a rare, autosomal recessive lysosomal storage disease that results in progressive degeneration of white matter of the CNS and PNS in several dog breeds. The disease is caused by mutations in the gene encoding for the lysosomal enzyme galactosylceramidase (GALC), which results in an accumulation of psychosine (galactosylsphingosine), which is highly toxic to oligodendrocytes and Schwann cells. The genetic muation for GLD has been identified in West Highland White Terriers, Cairn Terriers, and Irish Setters. The clinical signs associated with this disease are variable and may reflect a multifocal syndrome, often presenting as ascending pelvic limb paresis, lower motor neuron signs, cerebellar signs or (partial) seizure activity. Episodic cerebellar signs have not been reported with this disorder in dogs. We report the presentation and diagnosis of GLD in Kelpies.

Three affected dogs (one female and two males) from a litter of eight were all presented at the age of 14 months with a 9 to 11-month history of episodic paroxysmal events. Video footage of the events depicted cerebellar ataxia of the head, trunk and limbs, hypermetria and hypertonia of the pelvic limbs, and intermittent decerebellate rigidity. They occurred about every 3 weeks and were of 5 minutes duration on average. In between the episodes the dogs were neurologically normal. The frequency and severity of these events were non-progressive after 12 months from diagnosis. Hematology and serum biochemistry, MRI of the brain, CSF analysis (including PCR for canine distemper virus, Toxoplasma gondii, and Neospora caninum), electrodiagnostic testing and pelvic limb muscle biopsies were unremarkable. A skin biopsy was taken for fibroblast culturing from all three dogs. In two patients there was no detectable activity of GALC, and GALC activity was reduced in the remaining dog by more than 50% compared to healthy controls. The parents and siblings were reported to be clinically unaffected. Investigations to identify the genetic mutation leading to this untypical presentation of GLD in Kelpies are currently underway.

ABSTRACT #80
LINKAGE ANALYSIS IN AMERICAN STAFFORDSHIRE TERRIERS WITH HEREDITARY CEREBELLAR CORTICAL DEGENERATION. N.J. Olby[1], T. Harris[1], P.M. Mehta[1], M. Breen[1], R. Thomas[1], R. Myers[2], D. Nielsen[2]. [1]College of

Veterinary Medicine, [2]Department of Genetics, North Carolina State University, Raleigh, NC.

A hereditary neurodegenerative disease affecting primarily the cerebellum has emerged within the American Staffordshire Terrier breed. The late onset of clinical signs and recessive nature of the disease have resulted in widespread dissemination of the causative mutation in the breeding population of dogs. The purpose of this study was to genotype families of affected dogs and to perform linkage analysis to identify the disease locus.

DNA samples and pedigrees were obtained from affected dogs and their relatives by dissemination of information through the Staffordshire Terrier Club of America. Affected individuals and their parents and siblings were genotyped with a genome-wide panel of 315 canine fluorochrome labeled microsatellite markers (representing ~10cM resolution), organized into 69 multiplex PCR groups (MSS-2). PCR fragments were analyzed on an ABI-3700 automated Genetic Analyzer (Applied Biosystems), with amplified fragments visualized by incorporation of a fluorescently labeled PCR primer (Applied Biosystems). Results were analyzed with GeneMapper 3.7 software (Applied Biosystems). Linkage analyses were performed using the "lm_bayes" program from the computer package Morgan. This method utilizes a Markov-chain Monte Carlo (MCMC) approach for estimating pedigree likelihoods using the genotype and phenotype information provided.

DNA samples were obtained from over 60 affected American Staffordshire terriers and 120 of their normal relatives. Forty-eight dogs were genotyped with the MSS-2 panel of microsatellites; positive LOD scores were obtained on seven different chromosomes but the only significant linkage was found on CFA9. Additional microsatellites located in the linked region on CFA9 at approximately 1cM intervals (details obtained from the UC Davis Canine Linkage Map) were genotyped in 92 dogs. In these dogs, the disease locus mapped to CFA9 with a maximum LOD score of 9.4. The linked region extended over the first 23-Mb of the chromosome, but there was a peak of linkage extending over a distance of approximately 3-Mb from markers FH2263 to CAP09S. This region contains the genes for two isoforms of a voltage dependent calcium channel gamma subunit (*CACNG1* and *4*). The gamma subunit is implicated in both calcium channel and glutamate receptor function. We conclude that cerebellar cortical degeneration in American Staffordshire Terriers maps to CFA9, a region containing a viable candidate gene for the disease. Sequencing of the gene for this calcium channel subunit is underway in affected and normal dogs.

ABSTRACT #81

ELECTROENCEPHALOGRAPHY OF THE DEVELOPING FELINE BRAIN. Melissa Lewis,[1] Wenge Ding,[1] Colette Williams,[2] Charles Vite[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. [2]School of Veterinary Medicine, University of California, Davis, CA.

Electroencephalography (EEG) measures ongoing electrical activity in the brain and offers a way to non-invasively characterize brain function. EEG represents a useful tool for examining cerebrocortical development in young animals; however, limited data are available among certain species, including felines. Previous studies of EEGs in kittens are limited in number and vary widely with respect to how recordings were performed making broad generalizations and comparisons difficult. Various types of sedation used in different studies further complicate the picture of the developing feline brain because pharmacologic effects on the electrical activity recorded by EEG must be considered. We characterized the normal post-natal maturational process of the feline brain with regard to EEG by describing a standardized sedation protocol and recording EEGs with subcutaneous electrodes at discreet postnatal ages from two to 24 weeks of age.

Twenty five minute EEG recordings were performed in 20 cats at intervals of 2, 4, 6, 8, 12, 16, 20, and 24 weeks of age. Surface electrodes were placed on the head in a standard arrangement. The positioning of electrodes over appropriate brain regions was verified by post-mortem exam of one euthanized cat. Cats were sedated with medetomidine hydrochloride and butorphanol and reversed with atipamezole. Sedation protocol limited significant motion artifacts and was not associated with any adverse effects. Recordings were visually reviewed for the character of background electrical activity. Computer-aided analysis was used to perform frequency spectral analysis and to determine absolute and relative power of the background activity.

Analysis of background frequency, amplitude, and absolute power showed strong age-related electrical changes. Quantitative calculation of absolute power showed minimum values in 2 week old kittens within each frequency range reached maximum values in 6 to 8 week old cats, and declined steadily until reaching a plateau in 20 to 24 week old cats. There was no correlation between age and relative power. Our data show that age is an important consideration when interpreting EEG data in a young cat.

EEG recordings were also made in developing cats with the neurodevelopmental disorder Niemann-Pick type C. Affected cats showed abnormal spike and spike/wake activity as well as decreased normal transients compared to unaffected cats. Recordings also indicate age-related electrical changes with regard to amplitude and frequency of background activity.

ABSTRACT #82

MINIMALLY-INVASIVE EXCISIONAL BRAIN BIOPSY AND INTRACRANIAL BRACHYTHERAPY CATHETER PLACEMENT IN DOGS. RA Packer, LJ Freeman, AE Fauber, MA Miller, WB Morrison. Purdue University School of Veterinary Medicine, West Lafayette, IN.

A novel technique for minimally-invasive excisional brain biopsy and intracranial placement of a balloon-tipped brachytherapy catheter was evaluated in 5 purpose-bred dogs. CT-guidance was used to plan biopsy trajectory to a predetermined target with reference to a localizer grid affixed to a craniotomy stand. The procedure was performed through a 1 cm skin incision and 6 mm burr hole using a 9-gauge biopsy device. Five cylindrical samples measuring 3–4 mm in diameter by 7–12 mm in length were removed over 5 cycles of the vacuum-assisted ATEC® breast biopsy and excision system [Hologic Inc., Indianapolis, IN], leaving approximately a 1 cm^3 resection cavity. A GliaSite® [Proxima Therapeutics, Inc., Alpharetta, GA] balloon-tipped intracranial catheter was placed through the burr hole into the resection cavity to simulate 125-I liquid brachytherapy, and explanted after 7 days.

Four of 5 dogs had favorable outcomes, and were evaluated for 4 weeks post-operatively. One dog died within hours due to misguided biopsy. Neurological deficits were unilateral, focal and mild/moderate. Three dogs developed proprioceptive deficits; 2 had menace deficits, 2 developed circling, and 1 had horizontal nystagmus and reduced response to noxious nasal stimulation. Neurological status improved throughout the study period.

Histologic quality of the biopsy specimens was excellent. Histologic healing response of the brain at necropsy 4 weeks post-operatively was narrowly confined to the margins of the biopsy defect and catheter trajectory.

This simple technique can be used to target lesions and obtain high-quality tissue samples efficiently, with minimal morbidity. Clinical trials and adjunctive studies are underway.

ABSTRACT #83

THE PHARMACOKINETICS OF LEVETIRACETAM IN HEALTHY DOGS FOLLOWING SINGLE AND MULTIPLE ORAL DOSES. SA Moore, KR Muñana, MG Papich, J Nettifee-Osborne. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

The use of levetiracetam (LEV) in veterinary medicine is increasing for both refractory and newly diagnosed epileptic patients. Although the pharmacokinetics after a single dose in dogs has been reported, the pharmacokinetics after multiple doses is unknown. The objective of this study was to measure the pharmacokinetics of LEV after an oral dose in dogs, and to determine if the pharmacokinetics would change after repeated dosing. Six healthy dogs were administered a single oral dose of LEV (20.8–22.7 mg/kg). Blood samples were collected at baseline and intermittently for 24 hours after a single oral dose, and again after 6 days of Q8h dosing. Plasma LEV concentrations were measured by HPLC. Pharmacokinetic data was analyzed using a compartmental model. Peak (C_{MAX}) concentration occurred in 0.8 hours (T_{MAX}) with absorption $T_{1/2}$ of

12 min after the first dose. Minimal accumulation occurred over 6 days. After multiple doses, the C_{MAX} was 61.42 ug/ml ± 11.48, compared with 55.19 µg/mL ± 11.72 after the first dose. The elimination $T_{1/2}$ was 3.58 h ± 0.79 and absorption $T_{1/2}$ was 0.19 h ± 0.17. Plasma trough levels were variable depending on the time of day they were collected (18.42 ± 5.16 morning trough vs 12.57 ± 4.34 mid-day trough), suggesting a nocturnal difference in excretion. After multiple doses, the pharmacokinetics did not change appreciably, indicating that multiple doses of LEV does not alter its own pharmacokinetics. Administration of LEV at 20 mg/kg orally Q8h produced plasma drug concentrations consistently within the therapeutic range established for LEV in human medicine.

ABSTRACT #84

EFFECT OF TIME OF SAMPLE COLLECTION, INITIAL DOSE, WEIGHT AND DURATION OF THERAPY ON SERUM PHENOBARBITAL LEVELS IN DOGS WITH EPILEPSY. R. Monteiro, T.J. Anderson, G. Innocent, N.P. Evans and J. Penderis. Institute of Comparative Medicine, Faculty of Veterinary Medicine, University of Glasgow, UK.

Phenobarbital (Pb) is the most commonly drug used in the management of epilepsy. Pb is primarily metabolised in the liver via the hepatic microsomal enzyme system. Longer term Pb therapy results in induction of the hepatic microsomal enzyme system, increasing the rate of metabolism and resulting in higher doses of oral Pb in order to maintain the same serum Pb level. Moreover, body mass is inversely proportional to the metabolic rate which then influences the turnover rate at the cellular level and thus the dose required in different individuals.

The aim of this study was to assess the relationship between initial label recommended doses of Pb and the proportion of dogs reaching therapeutic serum level; the relationship between weight and dose required to reach therapeutic serum level, the significance of timing of blood collection in relation to serum Pb level in dogs on different doses; and to investigate the effects of duration of treatment on the serum Pb level relative to the dose administered.

Data was collected retrospectively from the Vétoquinol UK Pb monitoring voucher scheme (n=2021). Recorded data included signalment, weight, total daily dose, time of sampling (trough or non-trough), length of treatment and serum Pb level. For statistical purposes the trough and non-trough groups were divided into a total daily dose of 2–5 mg/Kg, ≥5–8 mg/Kg and ≥8 mg/Kg; the length of treatment was grouped as ≤2 weeks (w), 2-≤4w, 4-≤ 12w, 12w-≤ 1year and >1 year; and the weight analysis included all dogs divided as ≤ 10 Kg, 10–30 kg and > 30 Kg within the therapeutic range that had serum Pb level checked between 2 and 4w after initiation of therapy.

The results revealed that from dogs started on Pb therapy at the manufacturer's recommended dose (2–5 mg/kg/day), 90 out of 220 reached therapeutic serum level at first monitoring, but if started at 5–8 mg/kg /day, 40 out of 50 reached therapeutic levels ($P < 0.001$); of the 247 dogs evaluated for starting dose in relation to weight a highly significant inverse relation was found between weight and dose/Kg/day ($P<0.001$);at a total daily dose of Pb of <8 mg/kg the ratio of serum level to total daily dose for the trough group (n=799) was only 2.7% lower than that of the non-trough group (n=464, $P = 0.3029$), but at >8 mg/kg the trough group (n=166) was significantly lower (17.8%, p=0.01114) than the non-trough group (n=86); and therapy duration of <2w the ratio was 18.31 (n=106), at 2–4w 16.94 (n=214, P=0.17), at 4–12w 16.46 (n=310, P=0.05), at 12w to 1-year 15.96 (n=315, P=0.01), and at >1-year 16.29 (n=609, P=0.02).

In conclusion, higher initial doses are required to reach therapeutic level and choice of initial dose should take into consideration body weight. In dogs on higher daily doses of Pb, consistency of timing of blood sampling is important to ensure accurate comparison between serum Pb levels. Animals on a long term therapy will need adjustment of dose throughout treatment.

ABSTRACT #85

TSH MEASUREMENT IN SENIOR CATS – A PROSPECTIVE STUDY. J. Wakeling, J. Elliott, H. Syme. Royal Veterinary College, London, UK.

Hyperthyroidism is a common disease of older cats and is insidious in onset. In a retrospective study most hyperthyroid cats were found to have had undetectable TSH concentrations (<0.03 ng/ml) 1–3 years prior to diagnosis. Also, euthyroid cats with TSH <0.03 ng/ml reportedly have a higher frequency of thyroid adenomas and/or hyperplastic nodules than cats with TSH ≥0.03 ng/ml. The purpose of this prospective longitudinal study was to document the frequency with which hyperthyroidism is diagnosed in cats with differing TSH measurements.

Senior cats (>8 years old) presenting for routine health checks were recruited. A complete history, clinical examination, systolic blood pressure, urinalysis, biochemistry, total thyroxine (tT4) and TSH measurement (DPC canine TSH assay) were performed. Cats diagnosed with hyperthyroidism on initial exam (tT4 >55 nmol/l) were excluded but all other cats were included irrespective of their actual health status when examined. Cats were re-examined every 6 months (or more often if they were not healthy) with tT4 and TSH concentrations determined at least annually. The probability of hyperthyroidism being diagnosed at follow-up was compared in cats with TSH <0.03 ng/ml or TSH ≥0.03 ng/ml, by Fisher's Exact test at first annual check-up (maximum 14 months) and by Kaplan Meier analysis with Log-Rank testing over the total period of follow-up (maximum 3 years). Comparisons between groups were made by the Mann-Whitney test.

Of 106 cats recruited to the study, 17 were diagnosed with concurrent disease (mild chronic kidney disease n=10; hypertension n=6; diabetes n=1). There was no significant difference in tT4 measurements between cats with TSH <0.03 or ≥0.03 ng/ml at baseline (p=0.14). Cats with TSH <0.03 ng/ml were more likely to be diagnosed with hyperthyroidism within a year (p<0.001; see table). Of the 13 cats diagnosed with hyperthyroidism within one year, 12 had baseline TSH concentration <0.03 ng/ml and 1 cat had baseline TSH of 0.08 ng/ml. Over the total follow-up period cats with baseline TSH <0.03 ng/ml were significantly (p<0.001) more likely to develop hyperthyroidism with a median estimated (95% confidence interval) time to diagnosis of 441 (44–838) days. All cats that became hyperthyroid had a TSH concentration <0.03 ng/ml at, and 6 months prior to, diagnosis.

Baseline	First Annual check-up			
TSH ng/ml	Hyperthyroid TSH < 0.03 ng/ml	Euthyroid TSH < 0.03 ng/ml	Euthyroid TSH ≥ 0.03 ng/ml	Lost to follow up
≥0.03 (n = 75)	1	11	49	14
<0.03 (n = 31)	12	9	5	5

These data show that although TSH is commonly undetectable (<0.03 ng/ml) in geriatric cats, many of these cats subsequently develop hyperthyroidism. Measurement of TSH by the DPC canine TSH assay may be useful as part of routine feline senior health programmes to assess the risk of incipient hyperthyroidism.

ABSTRACT #86

LONG-TERM FOLLOW-UP OF GLOMERULAR AND TUBULAR KIDNEY FUNCTION IN HYPERTHYROID CATS AFTER TREATMENT WITH RADIOIODINE. I van Hoek[1], HP Lefebvre[2], K Peremans[1], E Meyer[1], S Croubels[1], E Vandermeulen[1], H Kooistra[3], JH Saunders[1], D Binst[1], S Daminet[1]. [1]Ghent University, Ghent, Belgium. [2]École Nationale Vétérinaire de Toulouse, Toulouse, France. [3]Utrecht University, Utrecht, The Netherlands.

Treatment of feline hyperthyroidism may influence different functions of the nephron. The objectives of this study were to evaluate long-term effects of radioiodine (^{131}I) treatment on glomerular and tubular function in hyperthyroid (HT) cats and to evaluate the potentially predictive value of renal parameters for development of chronic kidney disease (CKD).

Inclusion criteria were clinical signs compatible with hyperthyroidism, increased serum total thyroxin (TT4) concentration and increased thyroidal uptake of $^{99m}TcO_4^-$. The exclusion criterion was azotemia before treatment. Antithyroid drugs had to be discontinued at least 3 weeks prior to inclusion. One day before and 1, 4, 12 and 24 weeks after ^{131}I, serum concentrations of TT4, creati-

nine and urea, urine specific gravity (USG), urinary protein/creatinine ratio (UPC) and blood pressure (BP) were determined. Glomerular and tubular function was evaluated by glomerular filtration rate (GFR) measured with the plasma exo-iohexol clearance test (van Hoek et al., JVIM 2007;21:950) and by urinary retinol binding protein/creatinine ratio (RBP/c) (van Hoek et al., JIM 2008;329:208), respectively. Results were analysed with Student's t-test and with ANOVA.

Twenty-two cats were included and divided at 24 weeks after ^{131}I in group A (n=16) without evidence of CKD and group B (n=6) with CKD (IRIS stage II). Serum TT4 and GFR decreased significantly until 4 weeks after ^{131}I for the whole group ($P<.001$) and group A ($P<.001$) but only until 1 week after ^{131}I in group B ($P<.001$). Serum creatinine increased significantly from 1 week until 12 weeks after ^{131}I ($P<.001$) in the whole group, until 24 weeks after ^{131}I in group A ($P<.001$), and only until 4 weeks after ^{131}I in group B ($P<.001$). Serum urea and USG did not change significantly after ^{131}I for the whole group ($P=.224$ and $P=.216$) or for group A ($P=.787$ and $P=.249$) and B ($P=.107$ and $P=.539$) separately. There was a significant decrease until 4 weeks after ^{131}I in UPC for the whole group ($P<.001$), group A ($P<.001$) and group B ($P=.029$) and in RBP/c for the whole group ($P=.001$) and group A ($P<.001$), but in group B RBP/c ($P=.149$) did not change significantly over time after ^{131}I. Blood pressure decreased significantly for the whole group from 4 until 24 weeks after ^{131}I ($P=.022$), but did not change significantly in group A ($P=.082$) or group B ($P=.302$) separately. Group A had significant higher serum TT4 concentration ($P=.003$), USG ($P=.001$) and GFR ($P=.009$) before ^{131}I compared to group B. All cats from group B had a USG $<$ 1.035 before ^{131}I.

This study is the first to show a prolonged effect of ^{131}I treatment on glomerular function measured with GFR and tubular function measured with RBP/c in HT cats maintaining a healthy kidney function, but not in patients developing CKD. Pretreatment serum TT4 concentration, USG and GFR could potentially be predictive parameters for development of CKD after ^{131}I treatment.

ABSTRACT #87

PREVALENCE OF ANTI-MYELOPEROXIDASE ANTIBODIES IN METHIMAZOLE-TREATED HYPERTHYROID CATS. Robarge ME, Anderson KA, Pressler BM. Purdue University, West Lafayette, IN.

As with cats, people with hyperthyroidism are frequently treated with oral antithyroidal drugs. A significant percentage of these patients develop anti-neutrophil cytoplasmic autoantibodies (ANCA), most commonly specific for the neutrophil granule protein myeloperoxidase (MPO). Cats with hyperthyroidism are often treated with the same antithyroidal drugs, and may develop clinical signs that mimic those of people with ANCA-associated diseases. Normal cats administered propylthiouracil develop anti-MPO antibodies (using a recombinant human MPO [hMPO] ELISA), and our laboratory has determined that the feline and human MPO sequences are 85% homologous. We therefore hypothesized that a subset of cats with hyperthyroidism treated with methimazole develop anti-MPO ANCA.

Aliquots were collected from all serum samples submitted to the Purdue University Clinical Pathology Laboratory for T4 measurement. Aliquots were maintained at −80 C until testing. Samples included those from newly diagnosed hyperthyroid cats, cats currently or historically treated for hyperthyroidism, and cats suspected of being hyperthyroid but whose T4 serum concentrations were within the reference range. Presence of anti-hMPO antibodies was evaluated by ELISA. In brief, plates were coated with hMPO, blocked with a commercial blocking agent (Superblock T20; Pierce), and incubated with 1:50 serum dilutions in the blocking agent. Alkaline phosphatase-conjugated goat-anti-cat antibody (KPL Laboratories) was added, and optical density was measured at 1 hr after administration of substrate. Background reactivity versus coat buffer alone-coated wells was subtracted for each patient sample, and an internal control of anti-hMPO primary antibody (Dako) served as both a positive control and to adjust for inter-plate variability. Immunoreactivity was defined as an optical density above the mean plus two standard deviations of the non-hyperthyroid cat patients.

Samples from 112 hyperthyroid (treated and untreated) and 85 non-hyperthyroid cats were evaluated. Seven of 112 hyperthyroid cats (6.3%) vs. 5 of 85 (5.9%) non-hyperthyroid cats were immunoreactive against hMPO; these results were not statistically different (two-sample Wilcoxon rank-sum test, $p>0.05$). When hyperthyroid cats were subcategorized based on treatment modality all cats with anti-hMPO immunoreactivity were found to have been treated with methimazole (4 of 39; 10.3%), although insufficient numbers of cats have been tested to reach statistical significance.

These preliminary results suggest that a subset of hyperthyroid cats do develop anti-hMPO antibodies, although whether this is a unique feature of hyperthyroidism or treatment with methimazole is unclear. However, because human and feline MPO are only 85% homologous, a significant number of antibodies may not be detected using the assay reported here. Future studies are being developed using a recombinant feline MPO protein.

ABSTRACT #88

EFFECT OF HYPOTHYROIDISM ON REPRODUCTION IN BITCHES. DL Panciera[1], BJ Purswell[2], KA Kolster[2]. [1]Department of Small Animal Clinical Sciences and [2]Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Numerous reproductive abnormalities, including irregular interestrous period, anestrus, and infertility, have been attributed to hypothyroidism. We previously documented normal fertility but higher periparturient pup mortality in bitches with hypothyroidism for a median duration of 19 weeks. The purpose of this study was to evaluate reproductive function in these same bitches after more prolonged hypothyroidism.

Fourteen multiparous bitches were studied. Hypothyroidism was induced in 8 dogs by administration of 1 mCi/kg ^{131}I. Hypothyroidism was confirmed by finding serum T4 concentrations before and 4 hours after IV administration of human recombinant TSH that were $<$ 5 nmol/L. Six bitches were euthyroid, untreated controls. Dogs were evaluated daily for signs of estrus and were bred by 1 of 2 males when serum progesterone $\geq$5 ng/ml. Ultrasonographic examinations were performed weekly beginning 21 days after ovulation to confirm pregnancy and monitor for fetal resorption. Interestrous interval, gestation length, strength and duration of contractions during whelping, time between pups, number of live pups and stillbirths, viability of pups at birth, weight of pups, and periparturient mortality were recorded. The Student's t-test was used to compare differences between control and hypothyroid bitches.

All hypothyroid dogs had signs of hypothyroidism present for at least 40 weeks. Breeding took place a mean of 56 weeks after ^{131}I administration. No difference in interestrus interval or gestation length, or strength of contractions during whelping was noted between control and hypothyroid dogs. All 6 control and 4 of 8 hypothyroid bitches became pregnant. Fetal resorption was documented in 1 hypothyroid and 1 control bitch. The number of pups, puppy viability, and birth weight were significantly lower in hypothyroid dogs. Periparturient mortality, interval between delivery of pups, and duration of uterine contraction were greater in hypothyroid bitches. There were 8 stillborn pups from 4 litters, all from hypothyroid bitches.

Prolonged, severe hypothyroidism results in decreased fertility and increased periparturient mortality compared with euthyroid bitches. The results of this study show progression of reproductive abnormalities with more prolonged hypothyroidism when compared with a previous study of these bitches.

ABSTRACT #89

ADRENAL FUNCTION IN CRITICALLY ILL PUPPIES WITH PARVOVIRAL DIARRHEA. JP Schoeman[1], ME Herrtage[2]. [1]Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa, [2]Department of Veterinary Medicine, University of Cambridge, United Kingdom.

The adrenal response to critical illness and its role in prognostication is an important issue in human medicine. A positive association between high basal serum cortisol and adverse outcome has been demonstrated in human and canine illness. The association

of serum ACTH-stimulated cortisol and delta cortisol concentrations with outcome in critical illness is less clear.

This prospective, case controlled study was conducted on 63 puppies with parvoviral diarrhea. The diagnosis was confirmed by detection of viral particles on fecal electron microscopy. Seventeen healthy puppies were used as controls. Blood samples were obtained in each dog at admission prior to treatment and daily thereafter until death or discharge from the hospital. Immediately after the basal samples were drawn, each dog was injected daily with 5 ug/kg of ACTH (tetracosactrin) intravenously. A second sample was taken 1 hour later for serum ACTH-stimulated cortisol measurement and the calculation of delta cortisol. Cortisol concentrations were determined by a commercial canine radioimmunoassay kit (Coat-a-count®, DPC, CA). Dogs were retrospectively assigned to two groups: survivors (n=50) and non-survivors (n=13). Hormone concentrations between the survivors and non-survivors were compared with the Mann Whitney U test for non-parametric data. Significance was set at $p < 0.05$.

Median day 1 (D1) basal cortisol and ACTH-stimulated cortisol was significantly higher in patients than in controls (259 vs. 77 nmol/L) and (393 vs. 295 nmol/L); $P < 0.01$ for both. Median delta cortisol was lower in patients (68 vs. 203 nmol/L). In nonsurvivors vs. survivors, D1 basal cortisol was significantly higher (539 vs 234 nmol/L), $P < 0.05$. ACTH-stimulated cortisol did not differ significantly (459 vs. 380 nmol/L); $P = 0.2$, therefore delta cortisol was significantly lower in nonsurvivors (−60 vs 128 nmol/L); $P < 0.05$. On day 3 (D3), basal cortisol was still higher in non-survivors, but not significantly so (189 vs. 68 nmol/L); $P = 0.18$, yet ACTH-stimulated cortisol was significantly higher in nonsurvivors (417 vs. 345 nmol/L); $P < 0.05$. In contrast to D1, delta cortisol was not significantly lower in nonsurvivors on D3 (179 vs. 256 nmol/L); $P = 0.2$.

This study confirmed the previously described association between high basal serum cortisol concentrations and mortality in parvoviral diarrhea dogs. Low delta cortisol was also associated with mortality on D1. However, the D3 delta cortisol was not significantly lower in nonsurvivors compared to survivors. This study highlights the important limitation of designating a patient as adrenal insufficient on the basis of a one-off ACTH stimulation test in the early stages of acute illness. During this stage basal cortisol production is close to maximum and delta cortisol would be low, without necessarily indicating adrenal insufficiency. The serial sampling in this study provided novel insights on the adrenal response and re-enforces the need to consider basal-, delta- and ACTH-stimulated cortisol in the evaluation of adrenal reserve in canine critical illness.

ABSTRACT #90

URINARY CATECHOLAMINE AND METANEPHRINE TO CREATININE RATIOS IN DOGS WITH PHEOCHROMOCYTOMA.

P.H. Kook[1], S. Quante[1], P. Grest[2], C.E. Reusch[1]. [1]Clinic for Small Animal Internal Medicine, [2]Institute of Pathology, Vetsuisse Faculty, University of Zurich, Switzerland.

Pheochromocytomas (PHEO) are neuroendocrine tumors arising from chromaffin cells of the adrenal medulla or extra-adrenal paraganglia. In people, diagnosis crucially depends on biochemical evidence of production of the secretory product of the tumor. Widely used biochemical tests include measurements of urinary catecholamines and its metabolites. In veterinary medicine, biochemical testing is difficult to perform due to limited availability of techniques and lack of established reference ranges, and PHEO is most commonly identified as an incidental finding at necropsy. Measurement of urine catecholamine concentrations has been documented only in one dog with PHEO; however results were inconclusive. Recently, we reported on catecholamine- and metanephrine:creatinine (crea) ratios of spot urine samples in healthy dogs. The purpose of this study is to evaluate the biochemical diagnosis of PHEO in 6 dogs with histologically confirmed disease.

Between October 2004 and July 2007, all dogs with ultrasonographic evidence of adrenal gland enlargement and clinical suspicion of PHEO were enrolled into the study. The final criterion for inclusion was histopathological confirmation. Ten healthy client-owned dogs served as controls. During the study period 11 client-owned dogs were evaluated and histopathology confirming PHEO was available in 6/11 dog. Urine sampling procedures varied in dogs with PHEO. In one dog, urine was collected by the owner on day 7 (d7) after discharge from the hospital. In five dogs urine was collected during the initial work-up. Additional urine specimens were sampled in two dogs on day 2 (d2), resp. on days 6 (d6) and 7 (d7) after discharge by their owners. Results were compared to timely corresponding values from 10 healthy dogs collected in a veterinary hospital (*t0*), and 1 resp. 7 days later at home (*t1*, *t7*). Urinary free catecholamines comprising epinephrine, norepinephrine, and dopamine and urinary fractionated metanephrines comprising free and conjugated metanephrine and normetanephrine were separated and quantitatively determined by high pressure liquid chromatography with electrochemical detection using commercial reagents (BIO-RAD, Munich, Germany). Values were expressed as ratios to crea (nM:mM).

Normetanephrine:crea ratios were consistently increased compared to controls, ranging from 103 to 6430 (*t0*: 14–91; median 59.5, *t1*: 32–100; median 57.5, and *t7:* 21–79; median 55).

Four dogs had increases well above (414; resp. 243 on d2, 476, 925, and 6430 (d7)) even the highest results of healthy dogs at *t*0, whereas 2 dogs showed only smaller increases: (161) and (157 resp. 103 on d6, and 131 on d7). Highest normetanephrine : crea ratios were found in bilateral PHEO (2/6). All other ratios showed more overlap.

In conclusion, urinary normetanephrine:crea ratios are useful in diagnosing canine PHEO. In view of our first results these patients should ideally have their urine samples collected in their home environment. Results in one dog suggest that documentation of smaller but persistently increased normetanephrine:crea ratios on repeated measurements can further substantiate the diagnosis.

ABSTRACT #91

PREDICTIVE VALUE OF POSTOPERATIVE PLASMA CONCENTRATIONS OF ACTH, α-MSH AND CORTISOL FOR RECURRENCE OF CUSHING'S DISEASE AFTER TRANSSPHENOIDAL HYPOPHYSECTOMY IN DOGS.

JM Hanson, JA Mol, BP Meij. Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands.

Transsphenoidal hypophysectomy is an effective treatment for Cushing's disease or pituitary-dependent hypercortisolism (PDH) in the dog. However, recurrences occur after initial remission. The aim of this study was to analyze the predictive value of immediate postoperative plasma concentrations of ACTH, α–MSH, and cortisol for recurrence after transsphenoidal hypophysectomy in dogs with PDH.

Transsphenoidal hypophysectomy was performed in 55 dogs with PDH. Plasma concentrations of ACTH, α–MSH, and cortisol were measured before surgery and 1, 2, 3, 4, 5 and 24 to 48 hours after removal of the pituitary gland. The prognostic value of the hormone concentrations for recurrences (disease-free period) was analyzed with univariate Cox proportional-hazard analysis followed by stepwise multivariate analysis.

Forty-eight of the 55 dogs went into remission. Median disease-free period was 569 days. Hypercortisolism recurred in 12 dogs after median 255 days. Postoperative plasma ACTH, cortisol and α–MSH concentrations at 4 and 5 hours after removal of the pituitary gland were significantly associated with recurrence. In multivariate analysis the plasma ACTH concentration remained significant.

It is concluded that immediate postoperative measurement of plasma concentrations of ACTH, α–MSH and cortisol is a valuable tool for evaluation of long-term outcome after transsphenoidal hypophysectomy in dogs with Cushing's disease.

Originally presented at the European College of Veterinary Internal Medicine - Companion Animals Congress, September , 2007.

ABSTRACT #92

SUSTAINED HYPERGLYCEMIA AND HYPERLIPIDEMIA IN CATS: CONTRIBUTION OF INFLAMMATION AND BETA-CELL LOSS TO THE PATHOPHYSIOLOGY OF DIABETES.

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Switzerland.[6]Dept. of Pathol., Technical University of Munchen, Germany.[7]Dept. of Clinical Sciences of Companion Animals, Utrecht University, The Netherlands.

Feline diabetes shares many similarities to human type 2 diabetes mellitus (T2DM), including islet amyloidosis and β-cell loss. Based on cultured rodent and human islet cells, it has been demonstrated that hyperglycemia and hyperlipidemia in T2DM have a negative effect on β-cell viability. Exposure to high glucose or lipid levels induces interleukin expression in cultured pancreatic islets, followed by Fas receptor up-regulation and apoptosis of β-cells. In addition, high glucose levels upregulate islet chemokines and attract neutrophils and macrophages. Inflammatory cells may contribute to pancreatic-islet cell death in T2DM. The objective of this study was to investigate the above findings in vivo in cats.

Eleven healthy cats were infused for 10 days with glucose (n=5) or lipids (n=6) to target their blood concentrations at the approximate level found in untreated feline diabetes (glucose: 450–540 mg/dl; triglycerides: 265–620 mg/dl). As control groups, 10 healthy cats were either infused with saline (n=5) or did not receive infusion (n=5). On day 10, blood samples and pancreatic biopsies were collected. Levels of α_1-acid glycoprotein were measured in plasma samples. Isolated pancreatic islets were used to quantify mRNA transcripts of cytokines (IL-1β, IL-6, TNF-α) and chemokines (IL-8, MCP-1) by real-time PCR. In addition, mRNA transcripts of Fas receptor were measured. To quantify islet neutrophils, pancreatic sections were immunostained with insulin and myeloperoxidase. Pancreatic sections stained with amylin or insulin were used to quantify β-cells in pancreatic islets by morphometric analysis. Statistical differences between groups were determined with nonparametric tests.

Compared to controls, hyperglycemic cats had a 50% reduced number of β-cells per islet surface and an increased number of neutrophils relative to β-cells. More apoptotic cells were noted in the pancreatic islets by light microscopy. In hyperlipidemic cats the number of β-cells and neutrophils did not differ from control cats. Plasma levels of α_1-acid glycoprotein were increased in cats on glucose and lipid infusion. Islet quantities of cytokine, chemokines and Fas receptor transcripts were not different between groups.

Sustained hyperglycemia causes β-cell loss in pancreatic islets of healthy cats, possibly through increased apoptosis. Even though hyperglycemia and hyperlipidemia are accompanied by a systemic inflammatory response, inflammation does not seem to occur in pancreatic islets under the present experimental conditions. The increased number of neutrophils observed in the pancreatic islets of hyperglycemic cats needs to be further explored.

ABSTRACT #93

EFFECT OF HYPERGLYCEMIA AND HYPERLIPIDEMIA ON THE EXPRESSION OF 11β-HYDROXYSTEROID-DEHYDROGENASE AND GLUCOCORTICOID RECEPTOR IN INSULIN SENSITIVE TISSUES. N. S. Sieber-Ruckstuhl[1], E. Zini[1], F. S. Boretti[1], M. Meli[2], M. Osto[3], B. Sigrist[4], M. Franchini[4], P. Linscheid[1], T.A. Lutz[3], C. E. Reusch[1]. [1]Clinic for Small Animal Internal Medicine, [2]Clinical Laboratory, [3]Institute of Veterinary Physiology and Center of Integrative Human Physiology and [4]Institute of Virology of the Vetsuisse Faculty University of Zurich, Zurich, Switzerland.

Type 2 diabetes mellitus is characterized by inadequate insulin secretion and impaired insulin action. Once diabetes mellitus is established, hyperglycemia and hyperlipidemia further impair β-cell function and insulin sensitivity. Increasing evidence in humans and experimental animals suggests that altered tissue cortisol metabolism may additionally promote insulin resistance. Of key importance for the tissue cortisol metabolism is the pre-receptor enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) with its two isoforms (11β-HSD1 and 11β-HSD2). 11β-HSD1 converts the inactive cortisone to cortisol and 11β-HSD2 converts cortisol to cortisone.

The aim of this study was to determine if exposure of cats to high glucose and lipid levels has an influence on the expression of 11β-HSD1, 11β-HSD2 and glucocorticoid receptor (GR) and by this mean on the tissue cortisol metabolism. Eleven cats were infused for 10 days through the jugular vein with glucose (n=5) or lipids (n=6) to clamp their blood concentrations at the level found in untreated feline diabetes (450–540 mg/dl for glucose and 265–620 mg/dl for triglycerides). Ten control cats were infused with saline (n=5) or received no infusion (n=5). At the end of the 10 day period blood samples to measure cortisol and tissue biopsies from omental and subcutaneous adipose tissue, liver and muscle to measure mRNA expression for 11β-HSD1, 11β-HSD2 and GR by real-time PCR were collected.

Lipid infused cats showed significantly higher 11β-HSD1 mRNA expression in omental and subcutaneous adipose tissue than all other cats, significantly lower 11β-HSD2 mRNA expression in omental adipose tissue than all other cats, significantly lower 11β-HSD2 mRNA expression in liver than control cats, significantly lower GR mRNA expression in omental adipose tissue than all other cats and significantly lower GR mRNA expression in subcutaneous adipose tissue than saline and glucose infused cats. Glucose infused cats showed significantly lower 11β-HSD2 mRNA expression in liver than control cats. Saline infused cats showed significantly higher GR mRNA expression in omental fat and significantly higher cortisol blood levels compared to untreated cats. In summary, two-week lipid infusion leads to higher 11β-HSD1 expression and lower GR expression in adipose tissue and lower 11β-HSD2 expression in omental adipose tissue and liver.

Up-regulation of 11β-HSD1 and down-regulation of 11β-HSD2 is expected to result in increased tissue cortisol concentrations. Down-regulation of GR possibly represents a self-protective mechanism of the tissue against increased tissue cortisol levels. In conclusion, these results indicate that hyperlipidemia has a profound effect on the 11β-HSD expression and support the connection between hyperlipidemia, tissue cortisol metabolism and insulin sensitivity.

ABSTRACT #94

THE EFFECT OF HYPOTHYROIDISM ON INSULIN SENSITIVITY IN DOGS. N Inteeworn, DL Panciera, WE Monroe, KE Saker. Department of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Hypothyroidism causes abnormalities in glucose homeostasis in a variety of animal species. It has been documented as a cause of insulin resistance in dogs with concurrent diabetes mellitus. While several studies have been conducted in hypothyroid dogs to determine insulin secretion and glucose concentrations during glucose tolerance tests, parameters of insulin sensitivity have not been determined to date. The purpose of the study was therefore to investigate the effect of hypothyroidism on glucose tolerance and insulin sensitivity in dogs.

Sixteen female mixed breed dogs were studied. Dogs were randomly selected and allocated into two groups. In 8 dogs, hypothyroidism was induced by administration of 1 mCi/kg ^{131}I while the remaining 8 dogs were euthyroid controls. Hypothyroidism was confirmed by finding serum T4 $<$ 5 nmol/L before and 4 hours after IV administration of human recombinant TSH. Experiments were performed on non-anesthetized, fasted dogs in anestrous approximately 12 months after hypothyroidism was induced. The insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) was used to determine insulin and glucose concentrations over a 3-hr time period. Data was analyzed by the Minimal Model Analysis (MINMOD) to calculate basal insulin and glucose concentrations, acute insulin response to glucose (AIRg), insulin sensitivity (S_i), glucose effectiveness (the effect of glucose itself in its own disappearance, S_g) and the disposition index (DI). Student's t-test for unpaired data was used for comparisons between groups. P-values $<$ 0.05 were considered statistically significant.

One dog in the control group was excluded from the analysis due to an exaggerated response to the exogenously administered insulin at 20 minutes. Basal glucose concentrations were mildly, but significantly higher in the control group, whereas basal insulin concentrations were significantly lower. Minimal Model Analysis revealed a lower insulin sensitivity in the hypothyroid group ($P<0.001$), whereas AIRg was higher ($P=0.01$). Glucose effectiveness and disposition index were not statistically different between groups.

The results confirm that hypothyroidism negatively affects glucose homeostasis by inducing insulin resistance. Since the disposition index, defined as the product of insulin sensitivity and secretion remained unchanged, a compensatory increase in insulin secretion as indicated by the increased AIRg occurred in hypothyroid dogs to maintain glucose tolerance. In cases with impaired insulin secretion, such as canine diabetes mellitus, concurrent hypothyroidism can have important clinical implications in the successful management of the disease.

ABSTRACT #95
EVALUATION OF SIX PORTABLE BLOOD GLUCOSE METERS IN DOGS. T Cohen, R Nelson, P Kass, E Feldman. School of Veterinary Medicine, University of California, Davis, CA.

The purpose of this study was to evaluate six portable blood glucose meters (PBGM; AlphaTRAK, One-Touch, Elite XL, Accu-Chek, Precision, Contour) for use in dogs. One hundred fifty-eight venous blood samples were assayed for glucose using all PBGMs in randomized order and by a reference hexokinase method. Results from the PBGMs and reference hexokinase method (HM) were compared.

HM blood glucose concentrations ranged from 41 to 639 mg/dl. There was excellent correlation between PBGMs and HM results (Table). Results were consistently low for 4 PBGMs, compared with HM results. High and low results were common with the AlphaTRAK. The difference in results between PBGMs and HM increased as blood glucose concentrations increased. Differences in results between PBGM and HM were significantly ($p < 0.0001$) less for the AlphaTRAK and One-Touch and significantly ($p < 0.01$) higher for the Contour, compared with other PBGMs. Problems with correct identification of hypoglycemia (<70 mg/dl), normoglycemia (70–120 mg/dl), and hyperglycemia (>200 mg/dl) varied between PBGMs (Table).

Correlation	Percent	Median difference	Percent incorrect	
PBGM	coefficient	low results	from reference (mg/dl)	identification
AlphaTRAK	0.92	55	18	2
One-Touch	0.96	87	21	4
Elite XL	0.97	98	45	16
Accu-Chek	0.96	99	45	16
Precision	0.93	100	49	20
Contour	0.94	99	73	39

Results of this study support use of the AlphaTRAK and One-Touch glucose meters based on significantly closer results with HM.

ABSTRACT #96
HYPERINSULINEMIC-EUGLYCEMIC CLAMPS USING INSULIN DETEMIR AND INSULIN GLARGINE IN HEALTHY CATS. C Gilor, T Keel, KJ Attermeier, TK Graves. University of Illinois College of Veterinary Medicine, Urbana, IL.

Insulin detemir (Levemir®) and insulin glargine (Lantus®) are synthetic long-acting insulins used in human medicine. Reports of the use of Lantus in cats are rare, and the use of Levemir in cats has not, to our knowledge, been reported. In people, Lantus is longer acting and relatively peakless, while Levemir has significantly less within-subject variability. Levemir is also associated with less undesired weight gain and decreased frequency of hypoglycemic events. Because Levemir may prove useful in the treatment of feline diabetes mellitus, we performed hyperinsulinemic-euglycemic clamps in cats, comparing regular insulin (Humulin® R), Lantus and Levemir.

Five young, healthy, purpose-bred cats received subcutaneous injections of 0.5 U/Kg of Humulin R, Lantus and Levemir separately, on 3 different days, at least 1 week apart. Following each insulin injection, the blood glucose concentration, measured every 5 minutes, was maintained at baseline by a constant rate infusion of glucose, and the glucose infusion rate (GIR) was recorded. The duration of action was defined as the time from insulin injection until GIR stabilized at zero. The peak insulin action was defined as the peak GIR. The onset was defined as the time from insulin injection to initial increase in GIR. No adverse reactions were observed with any of the insulin products. The median duration of action of Humulin R was 305 minutes (range: 140–370 minutes). Lantus had a median duration of action of 470 minutes (range: 295–950 minutes). Levemir had a median duration if action of 800 minutes (range 525–915). In 3 cats, the duration of Levemir exceeded the duration of Lantus by 205–505 minutes. In the other 2 cats the duration of action of Lantus was slightly greater than that of Levemir (25 and 35 minutes longer). When compared using the Kruskal-Wallis test the durations of action of the 3 insulins were significantly different ($P = 0.012$). When compared using the Mann-Whitney U test, the duration of Levemir was significantly greater than that of Humulin R ($P = 0.008$), but there was no significant difference between Lantus and Humilin R ($P = 0.095$) or between Lantus and Levemir ($P = 0.31$). The peak effects of Lantus occurred between 120 and 585 minutes. The peak effects of Levemir were less variable among the 5 cats, occurring between 370 and 575 minutes. The median time of onset of Lantus was 80 minutes (range: 50–110 minutes), compared with 135 minutes (range: 80–165 minutes) for Levemir ($P = 0.11$).

In young healthy cats, Lantus may have a more rapid onset than Levemir, but the peak effect of Levemir is somewhat more predictable. The duration of Levemir may be greater in some cats. Investigation of the clinical use of Levemir in diabetic cats is warranted.

ABSTRACT #97
CLINICAL STUDY TO EVALUATE A NEW FORMULATION OF PROTAMINE ZINC INSULIN FOR TREATMENT OF DIABETES MELLITUS IN CATS. GD Norsworthy[1], RC Lynn[2]. Alamo Feline Health Center, San Antonio, TX; IDEXX Pharmaceuticals, Inc., Greensboro, NC.

Treatment of diabetes mellitus (DM) in cats usually requires administration of exogenous insulin. Protamine zinc insulin (PZI VET® Insulin, IDEXX Pharmaceuticals, Inc.) is a long acting beef-pork insulin that is manufactured using pancreatic glands harvested at slaughter. PZI VET insulin is known to be effective in treating cats with DM. Unpublished work in rats with DM showed that a new protamine zinc formulation based on recombinant insulin (PZIR) provided glycemic control that was similar in duration and potency to PZI VET insulin. The purpose of this study was to compare the clinical efficacy of PZIR to that of PZI VET in pet cats with DM.

Fifty pet cats with DM that had stable glycemic control on PZI VET insulin were selected for the study. On Day 0 of the study all cats were switched from PZI VET to PZIR after they were examined and weighed and had blood collected for serum fructosamine. The PZIR was administered at the same dose and dose interval as PZI VET. Cat owners were instructed to make no other changes in the daily diet or routine. Body weight, insulin dose, and serum fructosamine were rechecked on Day 15 and Day 30. Subjective owner impression of the cat's response to therapy was recorded during the 30-day period. On Day 30, the cats were switched back to PZI VET at the same dose as PZIR.

Forty-seven of the 50 cats finished the 30-day study. Three cats were removed from the study; 1 cat went into diabetic remission, 1 cat became fractious, and 1 cat became hypoglycemic. In the remaining 47 cats there were no significant differences in insulin dose, body weight or serum fructosamine at Day 15 or Day 30 when compared to Day 0. PZIR was given twice daily.

Time point	Body Weight (lb)	Fructosamine (μmol/L)	Insulin Dose (units/dose)
Day 0	13.8 ± 2.8	445 ± 91	4.2 ± 3.5
Day 15	13.8 ± 2.9	446 ± 95	4.4 ± 3.4
Day 30	13.7 ± 2.8	432 ± 91	–

Other than one cat with hypoglycemia (mentioned above) and one cat with hyperglycemia, there were no adverse reactions attributable to PZIR. Cat owner observations were consistent with satisfactory glycemic control and our clinical experience with the use of PZI VET insulin for cats with DM.

The results of this study in pet cats are similar to the previous findings in rats with DM. This study in diabetic cats also demonstrates that protamine zinc insulin based on recombinant insulin (PZIR) provides glycemic control that is comparable to that of PZI VET insulin at the same dose and dose interval.

ABSTRACT #98
EFFICACY OF PROTAMINE ZINC RECOMBINANT INSULIN FOR TREATING DIABETES MELLITUS IN CATS. RW Nelson[1], K Henley[2], C Cole[2]. [1]School of Veterinary Medicine, University of California, Davis, CA. [2]IDEXX Pharmaceuticals, Inc, Greensboro, NC.

The objective of this study was to evaluate the effects of a protamine zinc recombinant insulin (PZIR) on control of glycemia in cats with diabetes mellitus. One hundred twenty-six cats with newly diagnosed diabetes and 13 cats with poorly controlled diabetes were treated with PZIR twice daily for 45 days. Control of glycemia was assessed on days 7, 14, 30, and 45 by evaluation of clinical response, body weight, serum fructosamine, and blood glucose concentrations determined 1, 3, 5, 7, and 9 hours after administration of PZIR. Adjustments in dosage of PZIR were made as needed to attain control of glycemia.

PZIR administration resulted in a significant ($p < 0.001$) decrease in 9 hr mean blood glucose, mean blood glucose nadir, and serum fructosamine concentrations and a significant ($p < 0.05$) increase in mean body weight in the 139 diabetic cats at day 45, compared with day 0. 84% of the diabetic cats, including 9 poorly controlled, attained good diabetic control by day 45, based on improvement in clinical signs, physical examination, and blood parameters used to assess control of glycemia. Hypoglycemia was identified in 160 of 690 9-hr serial blood glucose curves involving 87 cats.

Results of this study demonstrate that PZIR is safe and effective for control of glycemia in cats with newly diagnosed and poorly controlled diabetes.

ABSTRACT #99
COMPREHENSIVE PATHOLOGIC EVALUATION OF KIDNEY DISEASE IN DOGS AND CATS: 179 CASES (2005–2007). George E. Lees, Brian R. Berridge, and Fred J. Clubb, Jr. College of Veterinary Medicine, Texas A&M University, College Station, TX.

Nephropathology in human medicine is a well-established subspecialty in which thorough light microscopic (LM) examinations are routinely combined with transmission electron microscopic (TEM) examinations and immunofluorescence (IF) evaluations to adequately characterize the pathologic features of diseases affecting kidneys. Pathologic findings are integrated with clinical findings to render specific morphologic and disease diagnoses and guide appropriate therapy. We hypothesized that taking a similar approach to the pathologic evaluation of renal diseases in dogs and cats would improve diagnosis and treatment of kidney disorders in these animals.

Early in 2005, we established a diagnostic veterinary renal pathology service that (a) provides kits of materials and instructions that enable clinicians to properly obtain and submit specimens of kidney suitable for TEM and IF, as well as histologic (LM) examinations, (b) routinely performs thorough LM, TEM, and IF evaluations, and (c) focuses on identifying pathologic implications for clinical patient management (i.e., diagnosis, prognosis, treatment). Our initial experiences in this activity have yielded some noteworthy observations.

Case submissions increased annually (2005, 19; 2006, 51; 2007, 109) and totaled 179 by the end of 2007. Submissions mainly were from dogs (n, 165) and most were biopsies (154 biopsy and 11 necropsy cases). Submissions from cats (n, 14) were too few to summarize in a meaningful way. Glomerular diseases of various types predominated in most (107/165; 64.8%) of the canine cases, and the remaining cases (58/165; 35.2%) were an eclectic mix of other disorders, including juvenile-onset nephropathies, acute or chronic interstitial nephritis, and acute tubular injury. The biopsy specimens varied in quality, but generally were sufficient for diagnosis; only 9/154 (5.8%) were judged to be inadequate. Importantly, biopsy specimens, which typically are obtained at an earlier stage of disease than necropsy specimens, exhibit a much more diverse and informative range of pathologic changes than is evident in necropsy specimens or is well characterized in the veterinary literature. A membranoproliferative pattern of glomerular injury was the single most common histologic lesion observed, but seemingly had multiple causes, including some for which evidence of glomerular immune-complex deposition could not be demonstrated.

We conclude that thorough nephropathologic evaluations, especially of biopsy specimens that are properly obtained from appropriate patients and correlated with relevant clinical information, often provide previously unappreciated insights about the causes and pathogenesis of kidney diseases in dogs and cats. If they are obtained, prepared, and submitted properly for LM, TEM, and IF evaluations, biopsy specimens are especially informative because they offer excellent tissue quality and often exhibit early stages of disease development, when distinctive diagnostic features are more readily apparent and specific, well-targeted therapeutic interventions are more likely to improve clinical outcomes.

ABSTRACT #100
URINARY N-ACETYL-β-D-GLUCOSAMINIDASE (NAG) INDEX IN CATS WITH VARIABLE AZOTAEMIA AND AS A PREDICTOR OF KIDNEY DISEASE. R.E. Jepson, C. Vallance, H.M. Syme & J. Elliott. Royal Veterinary College, London, UK.

Diagnosis of feline chronic kidney disease is based on the development of azotaemia and inadequate urine concentrating ability by which time substantial damage to the kidney will already have occurred. NAG is a lysosomal enzyme expressed at high levels in the proximal tubular cells (PTC). Increased protein processing or damage to the PTC may increase NAG release and activity in the urine. NAG activity may therefore be an early marker of kidney damage.

Geriatric (>9 years) cats with variable plasma creatinine concentration were recruited from 2 first opinion practices in central London. Cats received a physical examination, plasma biochemistry, measurement of systolic blood pressure, urinalysis and evaluation of urine protein to creatinine ratio (UP/C). Total T4 concentration was evaluated in all non-azotaemic cats. A colorimetric enzymatic assay involving the conversion of 3-Cresolsulfonphthaleinyl-N-acetyl-β-glucosaminide to 3-cresol-purple was used to assess NAG activity. Non-azotaemic cats were offered re-examinations at 12 months and were classified according to their renal status at this time (non-azotaemic or azotaemic (plasma creatinine > 2.0 mg/dL)).

NAG activity was standardised to urine creatinine concentration to give the NAG index. Results are reported as median [25th, 75th percentile]. NAG index and UP/C were logarithmically transformed for parametric statistical analysis. Cats were grouped according to their plasma creatinine concentration; I <1.6 mg/dL, IIa 1.6–2.0 mg/dL, IIb 2.0–2.8 mg/dL, III 2.8–5.0 mg/dL, IV >5.0 mg/dL and according to their magnitude of proteinuria; UP/C<0.2 non-proteinuric, UP/C 0.2–0.4 borderline proteinuric, UP/C>0.4 proteinuric. A one-way ANOVA was used to compare groups and where necessary a Bonferoni post-test was applied. Pearson's correlation was used to assess the relationship between plasma creatinine and log UP/C with log NAG index. A Student t-test was used to compare log NAG index between non-azotaemic cats that remained non-azotaemic at 12 months and those which developed azotaemia.

NAG index was evaluated in 197 cats of which 93 were non-azotaemic. No significant difference was found in log NAG index when cats were grouped according to plasma creatinine concentration ($p=0.06$). The correlation between plasma creatinine and log NAG index was poor ($r=0.154$, $p=0.031$). A significant difference was found in log NAG index when cats were grouped by magnitude of proteinuria ($p<0.001$) and a significant positive correlation was found between log UP/C and log NAG index ($r=0.499$, $p<0.001$). In non-azotaemic cats NAG index was significantly higher in those cats that developed azotaemia (n=19, 1.52 [0.82, 5.32] U/g) than in those that remained non-azotaemic at 12 months (n=56, 0.64 [0.21, 1.30] U/g; $p=0.0014$).

NAG index may indicate early tubular damage in the cat before the onset of azotaemia and correlates with the magnitude of proteinuria and potentially tubular processing of protein in cats with variable azotaemia.

ABSTRACT #101

N-ACETYL-β-D-GLUCOSAMINIDASE INDEX AS AN EARLY BIOMARKER FOR CHRONIC RENAL INSUFFICIENCY IN CATS WITH HYPERTHYROIDISM. C Lapointe, MC Bélanger, M Dunn, C Bédard, M Moreau. Faculté de médecine vétérinaire. Université de Montréal, St-Hyacinthe, Québec, Canada.

Hyperthyroid cats are at risk of developing chronic renal insufficiency (CRI) and diagnostic tools currently used to screen for renal insufficiency in hyperthyroid cats are either not reliable or impractical. The aim of this study was to determine whether n-acetyl-β-D-glucosaminidase index (NAG) was a good biomarker for chronic renal insufficiency (CRI) in these cats.

Twenty-four newly diagnosed hyperthyroid cats with a normal renal biochemical profile and ten healthy (H) cats were enrolled and evaluated for HT_4 at baseline. Hyperthyroid cats were started on methimazole and they were reevaluated once euthyroid. At the end of the study, three groups were discemed: H, normal euthyroid (NE) and euthyroid cats with CRI (ECRI). Baseline group characteristics were scrutinized to predict CRI. NAG_i over time was also evaluated.

Baseline NAG_i was significantly different between groups (P=.004). ECRI had a higher median value (13.12 U/g) when compared to H (1.38 U/g). NAG_i values decreased significantly over time in NE. Using a $NAG_i > 2.76$ U/g, negative and positive predictive values for CRI were 77.7% and 50%, while the combination of a urinary specific gravity (USG) ≤ 1.035 and a $T_4 > 7.80$ µg/dl enhance prediction level to 88.9% and 83.3%, respectively.

NAG_i did not differentiate NE from ECRI at baseline. However, it appears to be a biomarker for active renal damage during HT_4. The combination USG-T_4 could optimize the identification of appropriate candidates for definitive treatment of HT_4. NAG_i could also be used to adjust methimazole dosage during medical treatment.

ABSTRACT #102

RETINOL BINDING PROTEIN IN SERUM AND URINE OF HYPERTHYROID CATS BEFORE AND AFTER TREATMENT WITH RADIOIODINE. I van Hoek, E Meyer, L Duchateau, K Peremans, S Daminet. Ghent University, Ghent, Belgium.

Retinol binding protein (RBP) is a marker of renal tubular damage that is variably detected in urine of untreated hyperthyroid (HT) cats (van Hoek et al., JIM 2008;329:208–213). No data are available on serum RBP in cats or on the influence of treatment on serum or urinary RBP. In humans, serum RBP concentrations can be significantly lower in hyperthyroidism compared to eu- and hypothyroidism. The objectives of this study were to evaluate serum and urinary RBP levels in HT cats compared to healthy cats (H), and the influence hereon of radioiodine (^{131}I) treatment.

Inclusion criteria were clinical signs compatible with hyperthyroidism, increased total thyroxin (TT4) serum concentration (nmol/L) and increased thyroidal uptake of $^{99m}TcO_4^-$. Blood was taken by jugular venipuncture and urine by cystocenthesis 1 day before and 4, 12 and 24 weeks after ^{131}I treatment. After centrifugation, samples were aliquoted and stored frozen until assayed. A polyclonal rabbit anti-human RBP antibody was used in a commercial sandwich ELISA validated for RBP assessment in feline urine (van Hoek et al., JIM 2008;329:208–213). Parallelism of serial dilution curves of feline serum samples with trend lines from human RBP standards, indicated adequate recovery of feline RBP in serum. RBP concentrations were expressed as µg/L in serum and as RBP/urinary creatinine (RBP/c, 10^{-2}µg/mg creatinine) ratio in urine.

Ten HT cats and 8H cats were included. Mean ± SD of serum RBP, urinary RBP/c ratio and serum TT4 concentration in HT cats before and after treatment and H cats are described below. A linear mixed model was used for statistical analysis.

HT cats	0	+4 weeks	+12 weeks	+24 weeks	H cats
Serum RBP	199 ± 87	246 ± 196	224 ± 145	184 ± 123	174 ± 60
Urinary RBP/c	1.4 ± 1.5	0.2 ± 0.4	0.3 ± 0.5	0.6 ± 1.0	below Q. L.
Serum TT4	150 ± 52	27 ± 33	26 ± 31	24 ± 24	26 ± 4

There was a significant difference between H and HT cats before treatment in urinary RBP/c ratio (P=0.015) and in serum TT4 concentration (P<0.001). Serum RBP did not differ significantly between H cats and HT cats before (P=0.494) or 4 weeks (P=0.335), 12 weeks (P=0.383) or 24 weeks (P=0.835) after treatment. Serum TT4 concentration decreased significantly at all time points after treatment (P<0.001) and urinary RBP/c decreased significantly 4 and 12 weeks after treatment (P=0.003). There was no significant change in serum RBP concentration (P=0.796) before and after treatment. There was a significant correlation in HT cats between serum TT4 concentration and urinary RBP/c at all time points (0.42, P=0.007), but no significant correlation between serum RBP and TT4 (0.03, P=0.858) or urinary RBP/c (−0.16, P=0.333).

Serum RBP from HT cats does not differ statistically significantly from those of H cats and does not change after treatment, supporting the hypothesis that urinary RBP in HT cats is caused by a decreased renal function which is reversible upon treatment with ^{131}I.

ABSTRACT #103

RELATIONSHIP BETWEEN PROSTATOMEGALY WITH OR WITHOUT MINERALIZATION AND CYTOLOGIC DIAGNOSIS IN 55 DOGS. CA Bradbury[1], J Westropp[2], R Pollard[3]. [1]School of Veterinary Medicine, Colorado State University, Fort Collins, CO. [2]Department of Medicine and Epidemiology, and [3]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California at Davis, Davis, CA.

Prostatic neoplasia must be distinguished from other types of prostatic disease such as prostatitis, paraprostatic cysts, prostatic abscess and benign prostatic hypertrophy so as to direct therapy and allow prognostication. Mineralization has previously been associated with prostatic neoplasia in dogs. This studies purpose was to determine the connection between prostatic mineralization seen with imaging and cytologic assessment of the prostate in a large number of dogs.

Medical records of 55 dogs (22 neutered, 33 intact) with radiographic or ultrasonographic evidence of prostatomegaly and a cytologic diagnosis were retrospectively evaluated. Images were reviewed for mineralization and compared to results of fine needle aspiration (n=21), traumatic catheterization (n=2), biopsy (n=19) or necropsy (n=13).

22/55 (40%) of dogs had prostatic neoplasia. Mineralization in neutered dogs had a positive predictive value (PPV) of 100%, negative predictive value (NPV) of 50%, sensitivity and specificity of 84% and 100% respectively. Mineralization in intact dogs had a PPV of 22%, NPV of 96%, and sensitivity and specificity of 67% and 77% respectively. All neutered dogs with prostatomegaly but not prostatic neoplasia had bacterial prostatitis and were castrated within the previous 3 months due to signs of prostatic disease. Intact dogs with prostatomegaly and mineralization not diagnosed with neoplasia had paraprostatic cysts (n=3), cystic hyperplasia (n=2), benign prostatic hyperplasia (n=1) or E.coli prostatitis (n=1).

Prostatomegaly with mineralization detected by abdominal radiography or ultrasound is highly indicative of neoplasia in neutered dogs but less reliable in sexually intact dogs.

ABSTRACT #104

TREATMENT OF URETHRAL AND BLADDER STONES IN 28 DOGS WITH ELECTROHYDRAULIC LITHOTRIPSY. A. Defarges, M. Dunn. Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada.

Electrohydraulic lithotripsy (EHL) has been used as an alternative to cystotomy in human medicine to remove urinary tract stones. This prospective clinical study was performed to evaluate the efficacy and safety of EHL to fragment bladder and urethral stones in dogs.

Dogs presenting between January 1 2005 and June 1 2007 diagnosed with lower urinary stones by radiographs or ultrasound were included in the study. Physical exam, hematology, biochemistry, urinalysis and urine culture were performed at presentation. Under anesthesia, EHL and voiding urohydropulsion were performed. After 12 hours of fluids, patients were rechecked by ultrasound and discharged with antibiotics and anti-inflammatory drugs for 5 days. All patients were reevaluated 1 month, 3 and 6 months after presentation by physical exam, urinalysis and ultrasound.

Twenty-eight dogs (19 males, 9 females) presenting bladder and/or urethral stones were included in the study and 32 procedures were performed. Median weight was 8.3 kg. Only 2 dogs necessitated a

cystotomy because of risk of obstruction. 79% of patients presented calcium oxalate stones, 14% struvite and 7% mixed. Stone-free rate was 100% for urethral stones, 50% for bladder stones in females and 20% for bladder stones in males; 80% of cases presented no clinical signs 6 days after the lithotripsy. The recurrence rate at 6 months was 28%.

Results of this study support the use of lithotripsy as a minimally invasive treatment for urethral stones and bladder stones in female dogs.

ABSTRACT #105
EFFICACY AND SAFETY OF LASER LITHOTRIPSY TO MANAGE UROCYSTOLITHS AND URETHROLITHS IN DOGS: 100 CONSECUTIVE CASES. J Lulich, C Osborne, H Albasan, M Monga. University of Minnesota, Saint Paul, MN.

We hypothesized that laser lithotripsy was an effective and safe method for removal of canine uroliths. To test this hypothesis we prospectively studied 100 dogs with naturally occurring urocystoliths and/or urethroliths.

Lithotripsy was performed during anesthesia with a holmium-YAG laser via cystoscopy. Basket retrieval and voiding urohydropropulsion were used to remove urolith fragments. Post-procedural contrast cystography was used to assess efficacy and safety. Urine samples for analysis and culture were collected before and three times after urolith removal from the first 40 dogs.

Complete urolith removal was achieved in 82% of dogs; 52/66 with urocystoliths, 17/17 with urethroliths, and 13/17 with urocystoliths and urethroliths. Incomplete urolith removal was detected in 18 cases; 9 dogs had uroliths $\geq$3 mm in diameter; 9 dogs had uroliths $<$3 mm. Multivariate analysis revealed that gender (female) was the most significant predictor for success.

Two dogs developed post-procedural urinary tract obstruction. Hematuria ($>$5RBC's/hpf) was detected in 53% on day 0, 84% on day 1, 13% on day 4, and 3% on day 11. Leukocyturia ($>$5WBC's/hpf) was detected in 13% on day 0, 47% on day 1, 0% on day 4, and 3% on day 11. Clients perceived that their dog exhibited urinary discomfort ($>$10 on a 0 to 100 scale) in 62% on day 0, 60% on day 1, 38% on day 4, and 3% on day 11.

Although associated with immediate and reversible hematuria and noninfectious inflammation, lithotripsy is a safe and effective method of managing urocystoliths and urethroliths in dogs.

ABSTRACT #106
LASER LITHOTRIPSY AND CYSTOTOMY ARE EQUALLY EFFECTIVE FOR MANAGEMENT OF CANINE UROCYSTOLITHS AND URETHROLITHS. J Bevan, J Lulich, C Osborne, E Pluhar. University of Minnesota, Saint Paul, MN.

We performed a case-control study to compare effectiveness, resources and complications between lithotripsy and surgery. Between October 2004 and October 2006, urocystoliths and urethroliths affecting 39 male and 27 female dogs were managed via laser lithotripsy. Surgical records from 66 dogs of similar gender, weight, and similar urolith type, volume, and location were selected for comparison.

Incomplete urolith removal occurred in 15 dogs managed with lithotripsy. Seven had uroliths $\geq$3 mm in diameter; 5 had uroliths 1 to $<$3 mm, and 3 had uroliths $<$1 mm. Incomplete urolith removal occurred in 9 dogs managed with surgery. Seven had uroliths $\geq$ 3 mm in diameter; 1 had uroliths 1 to $<$ 3 mm, and 1 had uroliths $<$1 mm. Efficacy between lithotripsy and surgery was not different (p value = 0.18). In the lithotripsy group, seven ancillary procedures were performed to completely remove urolith fragments $>$3 mm. In the surgery group, 11 ancillary procedures were performed to completely remove uroliths $>$3 mm.

Laser lithotripsy required more (23 minutes) time (p value = 0.0024). Procedure time was not different when unsuccessful cases were culled. Neither procedure cost nor anesthetic time for lithotripsy was different from surgical urolith removal. Duration of hospitalization for dogs undergoing lithotripsy was significantly shorter than dogs undergoing surgery.

There were no major complications (i.e. death or dehiscence) in any dog in either group. One dog developed acute urinary obstruction 1 day post-lithotripsy.

Laser lithotripsy is a minimally invasive, comparable alternative to surgery for managing canine urocystoliths and urethroliths.

ABSTRACT #107
STRUVITE RELATIVE SUPERSATURATION: A GOOD PREDICTOR OF STRUVITE STONES DISSOLUTION IN VITRO. C Tournier, E Malandain, S Abouhafs, S Aladenise, C Venet, C Ecochard, R Sergheraert, V Biourge. Royal Canine Research Centre, Aimargues, France.

Relative supersaturation (RSS) is a method that allows to measure the potential for a urine to dissolve or form crystals and that has been validated in cats. The aim of this study was to assess if struvite RSS is a good predictor of *in vitro* struvite dissolution kinetic in cat urine.

Two different commercial complete dry expanded diets with 2 levels of Na content, designed to dissolve struvite uroliths, were selected: diet A (1.3% Na as fed) and diet B (0.4% Na). Those diets were fed successively to 7 Chartreux cats for 2 weeks. Urinary volume, pH, specific gravity and concentrations of 10 solutes (Ca, Mg, Na, K, NH_4^+, phosphate, citrate, sulfate, oxalate, uric acid) were measured on the pooled urine collected during the last 7 days of each study period. Based on those data, the urinary relative supersaturation (RSS) for struvite (MAP) was calculated using Supersat^{TM}. Each pooled urine was divided in aliquots based on mean urinary volume produced daily per cats. The aliquots were placed in bottles and stored at -20 °C pending the test. Two groups of 2 feline struvite stones (219 $\pm$ 1 mg) homogeneous in source, shape and weight were selected. On day 0, a bottle of each urine was defrosted, and struvite stones were added. The bottles were then placed in a water bath at 38 °C for 24 hours. After that, the urines were filtered to collect the stones. The stones were lightly dried on an absorbent paper and weighed, and the process restarted until complete dissolution of the stones.

During the study, cats remained healthy, maintained their body weight and consumed their diet adequately. Table 1 summarizes the results.

	Mean Urine Volume (mL/cat/day)	Urinary pH	Specific Gravity	MAP RSS	Number of days for complete dissolution	Dissolution kinetic (mg/day)
Diet A	107.9	6.1	1.052	0.2	16	14,6
Diet B	55.7	6.1	1.068	0.4	34	6,8

When RSS is $<$ 1 (undersaturation zone), urine dissolves struvite stones efficiently and the lower the RSS, the quicker the dissolution kinetic. This observation shows that RSS is thus a good predictor of urine potential to dissolve stuvite stones. Diet B has also been shown to induce struvite dissolution *in vivo*. This *in vitro* model might thus be a good way to assess efficacy *in vivo*.

ABSTRACT #108
QUANTIFICATION OF END-PRODUCTS OF PURINE CATABOLISM IN DOGS FED DIETS VARYING IN PROTEIN AND PURINE CONTENT. E Malandain[1], E Caussé[2], C Tournier[1], S Aladenise[1] A Rigolet[2], C Ecochard[1], V Biourge[1]. [1]Royal Canin, Aimargues, France. [2]Rangueil Hospital, Toulouse, France.

Urate and xanthine stones result from urine saturation of end-products of purine catabolism. In dogs urinary excretion of urate and xanthine is affected by breeds (i.e. Dalmatians) or drug treatment (allopurinol). To date, a purine and severely protein restricted diet has been recommended to reduce urinary xanthine and urate excretion in affected dogs. The purpose of this study was thus to study the influence of 3 dry-expanded diets with different purine and protein content on urinary purine end-product excretion in dogs.

Six healthy neutered-female medium Schnauzers (8.8 $\pm$ 0.44 y) were included in the study. They were successively fed 3 commercial diets with various purine and protein content: A: a poultry and corn-based diet (25% protein), B: an egg and rice diet (18% protein) and C: an egg and rice diet (9.8% protein as fed). Dogs were fed based on the equation 132 kcal/$kg^{0,75}$/d. Water was available ad libitum. After five days of adaptation, dogs were moved in metabolic cages and all urines were collected over 5 days. Purines (adenine,

guanine, hypoxanthine, xanthine) and uric acid were assayed in the diet by HPLC. Urine samples were pooled and conserved at −20 °C pending assays. End-products of purine catabolism (hypoxanthine, xanthine, uric acid and allantoin) were quantitatively assayed by capillary electrophoresis. Data were analyzed by repeated measured ANOVA. Results are expressed as the mean ± SEM.

Dogs maintained their weights over the duration of the study. Daily mean consumptions of purines were for diet A: 2727 ±63, for diet B: 686 ±19 and for diet C: 465 ±18 µmol/d. Mean daily urinary excretion of total purine end-products were significantly higher for diet A 6063 ±497 than both diet B: 3962 ±212 and diet C: 3309 ±353 µmol/24h. The ratio of uric acid/(uric acid+allantoin) representing the proportion of uric acid not converted into allantoin and excreted in urine did not vary depending on the diet (A: 11.8 ±3.9, B: 11.2 ±3.3, C: 9.8 ±2.5%) but significant individual variations were noted between dogs (mean 11%, range 4.1–21%).

This study confirms the great influence of purine intake on purine end-products excretion. It shows that severe protein restriction is not mandatory to decrease purine end-product urinary excretion. Measurements of total purine end-products excretion might be a more relevant method to assess a benefit of the diet on purine metabolite excretion in normal dogs than the excretion of uric acid. Evaluation of the ratio: uric acid/(uric acid+allantoin) in urine could be helpful in identifying dogs with increased risk of forming purine uroliths.

ABSTRACT #109

INFLUENCE OF A LOW CARBOHYDRATE, DRY FORMULATED DIET ON URINARY SATURATION FOR CALCIUM OXALATE AND STRUVITE IN HEALTHY ADULT FEMALE CATS. M Mustillo, J Bartges, C Kirk, S Cox, B Young, T Moyers, H Byrd. The University of Tennessee, Knoxville, TN.

Urolithiasis is common in cats and calcium oxalate is the second most common mineral occurring in uroliths. Obesity is associated with increased risk for feline calcium oxalate urolith formation, and is often managed by feeding a low carbohydrate diet. In some human beings, consumption of a low carbohydrate diet is associated with increased calcium oxalate urolith formation. We hypothesized that feeding a commercially available low carbohydrate diet to healthy adult female cats will increase urinary excretion of calcium and oxalate, decrease urinary excretion of magnesium and citrate, induce aciduria, and increase urinary saturation for calcium oxalate when compared with a commercially available adult maintenance diet.

Six healthy, spayed female cats, aged 4–7 years, and weighing 3.5–6.5 kg, were evaluated. Cats were randomly assigned to be fed a commercially available maintenance diet (Purine ONE adult chicken and rice dry formulation, Nestle Purina) or a low carbohydrate diet (CNM DM dry formulation, Nestle Purina) in a cross-over design. Diets were fed for approximately 6 weeks at which time 24-hour urine samples were collected using a modified litter box. Twenty-four hour urine samples were mixed, the volume recorded, and pH, sodium, potassium, chloride, calcium, magnesium, phosphorous, citrate, oxalate, and ammonia concentrations were determined. Molar concentrations of these analytes were entered into a computer program (EQUIL 89d, University of Florida) for determination of relative supersaturation for calcium oxalate monohydrate and dihydrate, and struvite. Data were analyzed using 1-tailed, paired t-tests; $p \leq 0.05$ was significant.

Body weight did not change between diet periods. Consumption of the low carbohydrate diet was associated with significantly higher urine pH (6.96 ± 0.26 vs 6.11 ± 0.15), ammonia excretion (0.50 ± 0.22 vs 0.11 ± 0.08 mM/kg/24 hr), citrate excretion (0.036 ± 0.032 vs 0.021 ± 0.016 mM/kg/24 hr), and sodium excretion (3.72 ± 1.56 vs 1.85 ± 0.67 mEq/kg/24 hr), and significantly lower magnesium excretion (0.33 ± 0.15 vs 0.98 ± 0.68 mg/kg/24 hr) and urinary relative supersaturation for calcium oxalate dihydrate (0.52 ± 0.29 vs 0.99 ± 0.49) when compared with the adult maintenance diet. No significant difference was found for urinary volume, urinary excretion of calcium, chloride, creatinine, oxalate, phosphorous, or potassium, or urinary relative supersaturation for calcium oxalate monohydrate or struvite.

Consumption of a low carbohydrate diet by healthy adult female cats did not increase risk for formation of calcium oxalate or struvite urolith formation, and was associated with lowering risk for calcium oxalate dihydrate formation.

ABSTRACT #110

THE EFFECT OF HETASTARCH ON SPECIFIC GRAVITY AND OSMOLALITY OF URINE IN THE DOG. L Smart, K Hopper, J Aldrich, J George, PH Kass. William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA.

Urine osmolality (UOSM) is a measure of the number of solutes within a given weight of urine, and signifies renal concentrating ability. Urine specific gravity (USG) is used clinically to estimate UOSM. Although USG has been shown to have a linear correlation with UOSM in dogs, the relationship is altered when there are significant numbers of high molecular weight (MW) molecules in the urine. The purpose of this investigation was to evaluate the effect of hetastarch (HES), with an average MW 600 and degree of substitution 0.7, on USG as compared to UOSM. Our hypothesis was that USG would no longer predict UOSM in dogs given intravenous HES.

Eight healthy, employee-owned dogs were included in the treatment group. Four of these dogs also served as the control group. USG and UOSM were measured every 30 minutes from t=0 minutes to t=360 minutes. Dogs were administered 20 mL/kg of either NaCl 0.9% (control group, n=4) or HES (treatment group, n=8) IV over one hour starting at t=90 minutes. Washout period between experimental protocols for the control group was a minimum of 4 weeks. Two-way repeated measures analysis of variance was used to assess changes over time and differences between control and treatment groups. Time-specific group differences were evaluated using Student's t-test with a sequentially rejective method of multiple comparison adjustment.

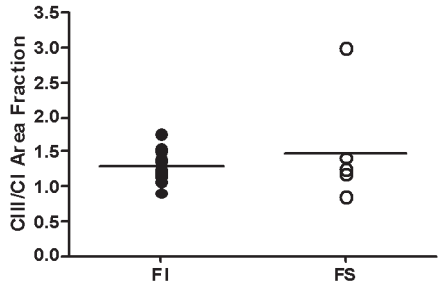
There was a decrease in UOSM in both groups starting at t=120 minutes and continuing for the study duration, and there was no significant difference in UOSM between treatment and control groups across all time points. There was an appropriate decrease in USG from t=120 minutes for the control group. In the treatment group, USG increased significantly at t=120 minutes (p=0.0006), t=150 minutes (p=0.0002) and t=180 minutes (p=0.0044). The most remarkable increase in USG occurred at t=150 minutes with a mean USG of 1.070 ± 0.021 (range 1.038–1.104).

Given the results of this study, USG does not correlate with UOSM, and therefore does not reflect renal concentrating ability, after a one-hour infusion of 20 mL/kg of HES. Interpretation of an increased USG after this therapy as an indicator of concentrated urine, rather than as an effect of hetastarch molecules within the urine, may lead to unfavorable treatment decisions.

ABSTRACT #111

RATIO OF PERIURETHRAL TISSUE COLLAGEN TYPE III/TYPE I CONTENT IN INTACT AND SPAYED FEMALE DOGS. JK Byron, TK Graves, JF Cosman, EM Long, M Becker. University of Illinois College of Veterinary Medicine, Urbana, IL.

Collagen types I (CI) and III (CIII) are responsible for tensile strength of tissue. It has been postulated in humans that a change in the ratio of CIII/CI in periurethral tissues may play a role in the development of stress urinary incontinence in post-menopausal women; however, it is not known whether the observed changes are more strongly influenced by age or hormonal status. Evaluation of the CIII/CI ratio in intact and spayed female dogs may help to answer this question and further describe those factors involved in canine urethral sphincter incompetence.



Frozen sections of periurethral tissues from 14 intact and 6 spayed female dogs were evaluated. All dogs were > 15 kg and

between 1 and 7 years old. One of the spayed females had urinary incontinence. Immunofluorescence was performed using anti-collagen I-FITC and anti-collagen III-Texas Red conjugated monoclonal antibodies. Three to 6 regions of tissue sections from each dog were imaged using a Leica TCS SP2 confocal microscope. Images were converted to binary and analyzed using Image J software (v1.37, NIH, USA) for pixel total area and area fraction. The CIII/CI ratio was calculated for each image and averaged within each dog. Comparisons were made between intact and spayed female dogs with a Welch's t test for unequal variances with and without the incontinent female included. No significant difference in CIII/CI total area or area fraction was found based on reproductive status. The incontinent dog was an outlier with a higher total area and area fraction CIII/CI ratio than all continent dogs. These results suggest that estrogen may not significantly influence the periurethral CIII/CI ratio in female dogs. Collagen content in urinary incontinent female dogs should be further evaluated to determine if incontinence is related to a higher CIII/CI ratio.

ABSTRACT #112
EFFECT OF THYROXINE SUPPLEMENTATION ON GLOMERULAR FILTRATION RATE IN HYPOTHYROID DOGS. K. Gommeren[1], H.P. Lefebvre[2], G. Benchekroun[3], S. Daminet[1]. [1]Department of Small Animal Medicine and Clinical Biology, Ghent University, Merelbeke, Belgium; [2]Department of Clinical Sciences, National Veterinary School of Toulouse, Toulouse, France; [3]Internal Medicine Unit, National Veterinary School of Alfort, Maisons-Alfort, France.

Glomerular filtration rate (GFR) is decreased in human hypothyroid patients, but information about kidney function in canine hypothyroidism is lacking. The objective of this study was to assess GFR in hypothyroid dogs, prior to substitutional therapy and after reestablishment of a euthyroid state.

Hypothyroid dogs (n=14) without gross abnormalities on renal ultrasonography and urinalysis were included. Blood pressure measurement and exogenous serum creatinine clearance (ECC) test were performed before treatment (t0, n=14), one month (t1, n=14) and 6 months (t6, n=11) after supplementing levothyroxine (20 µg/kg/day PO) therapy. At t1, response to therapy was monitored by measurement of serum total thyroxine and thyrotropin. If thyroid treatment needed to be adjusted, it was reassessed after one month.

Statistical analysis was performed using a general linear model, results were expressed as mean±SD.

Age at t0 was 6.25±1.4 years, body weight decreased ($P<0.05$) from 35±18 kg at t0 to 27±14 kg at t6. All dogs remained normotensive throughout the study. Basal serum creatinine also decreased ($P<0.05$) from 121±37 to 98±20 and 104±28 µmol/L at t0, t1 and t6, respectively. ECC conversely increased ($P<0.01$), the corresponding values were 1.6±0.4, 2.1±0.4 and 2.0±0.4 mL/min/kg, respectively.

Decreased GFR was observed in hypothyroid dogs. However, reestablishment of a euthyroid state increased GFR significantly.

Originally presented at the European College of Veterinary Internal Medicine – Companion Animals Congress, September 2007.

ABSTRACT #113
SEASONAL VARIATION IN DIAGNOSTIC TESTS FOR PITUITARY PARS INTERMEDIA DYSFUNCTION IN NORMAL AGED GELDINGS. CM Schreiber, AJ Stewart, EN Behrend, J Wright, R Kemppainen, KA Busch. Auburn University College of Veterinary Medicine, Auburn, AL.

The purpose of the study was to determine if seasonal variations exist in Alabama in the results of various tests used to diagnose pituitary pars intermedia dysfunction (PPID) in normal horses. In addition, we aimed to provide reference ranges for endogenous plasma adrenocorticotrophin (ACTH), α-melanocyte-stimulating hormone (α-MSH), and serum insulin and cortisol concentrations, and cortisol concentrations 15 and 19 hrs after a dexamethasone suppression test (DST) for each season of the year.

Fifteen healthy mixed breed geldings aged 12–25 years were used. Baseline blood samples were collected at 4 p.m. for determination of endogenous ACTH, α-MSH, cortisol and insulin concentrations. Dexamethasone (40 µg/kg) was then injected intramuscularly (T=0). Blood was collected at T=15 hrs (7am) and T=19 hrs (11am) for determination of cortisol concentrations. Sample collection was repeated monthly for 12 months. Radioimmunoassays were used to measure all hormones. Differences in hormone concentrations by season and month were assessed using repeated measures ANOVA; $P \leq 0.05$ was considered significant.

There was a significant effect of season on α-MSH ($P=0.0019$) concentrations and cortisol response to DST at T=19 hrs ($P=0.02$). There was a tendency toward a seasonal effect on ACTH ($P=0.07$), but no effect on insulin or cortisol at T=0 or T=15 hr. There was no significant time effect in any variable when analyzed by month.

The seasonal effect on tests for PPID appears to be less marked in Alabama compared to the northeastern United States, and insufficient to affect the validity of tests evaluating ACTH, insulin or cortisol concentrations in most horses. Seasonal reference ranges should be used for α-MSH.

ABSTRACT #114
PREVALENCE AND RISK FACTORS FOR HYPERINSULINEMIA IN PONIES. CM McGowan[1], R Geor[2], TW McGowan[3]. [1]The University of Helsinki, Finland, [2]Virginia Tech, Blacksburg, Virginia, USA, [3]The University of Queensland, Brisbane, Australia.

Insulin resistance (IR) is a major factor in the susceptibility of ponies to pasture-associated laminitis yet epidemiological research to date is limited. The aim of this study was to determine the prevalence and risk factors of hyperinsulinaemia as an indicator of IR in ponies.

Pony studs within a 100-km radius of Gatton, SE Queensland, were identified via internet listings and relevant publications, contacted and visited on consent. Blood samples were obtained and analysed for serum insulin (DSL RIA), plasma ACTH (Immulite 1000), serum triglyceride and plasma leptin concentrations. Ponies were evaluated for body condition score (BCS, 1–5 scale), increased fat deposition (cresty neck, increased supraorbital fat) and history or evidence of laminitis. Hyperinsulinaemia was defined as serum insulin $\geq$ 20 µIU/ml. Equine Cushing's Syndrome (ECS) was defined as ponies $\geq$ 15 years of age with ACTH $>$ 50 pg/ml and used as an exclusion criterion.

Of the 26 pony studs or traders identified, 23 were able to be contacted and 22 available for visit (response rate 96%). The study population consisted of 208 ponies; 70 Australian Ponies, 67 Welsh Mountain Ponies or Cobs (WMP), 51 Connemaras and 20 Shetlands. Mean BCS was 4.0 ± 0.5; mean age 10 ± 7.1 years (median 9; range 1–34 years). The majority of ponies were currently used for breeding (53%), followed by young and dry stock (36%) and showing or riding (11%). 81% (152/188) of ponies were kept entirely on pasture, the other 19% being supplementary fed.

Twenty ponies (9.6%) were excluded based on suspicion of ECS. Of the remaining 188 ponies, the prevalence of IR was 27.7% (52/188) (95% CI: 21.3–34.1%). Mean BCS was greater in ponies with IR (4.2 ± 0.6) vs. those without (3.8 ± 0.5) ($P<0.001$). There was a mild correlation between serum triglyceride and both serum insulin (Spearman's $r = 0.36$; $P<0.001$) and plasma leptin concentrations (Spearman's $r = 0.39$; $P<0.001$). There was a moderate correlation between plasma leptin concentration and body condition score (Spearman's $r = 0.56$; $P<0.001$). Univariate analysis showed an increased risk of IR in ponies provided supplementary feed (OR 3.68; 95% CI 1.6–8.5). There was a significantly increased risk of IR in WMP (OR 2.5; 95% CI 1.3–5.0) and decreased risk in Connemaras (OR 0.2; 95% CI 0.1–0.5). Neither gender nor current use affected risk of IR, but risk was increased if owners reported a history of laminitis (OR 7.4, 95% CI 2.6–21.0), or if laminitic rings were detected on physical examination (OR 3.1, 95% CI 1.5–6.3). There was also an increased risk of IR in ponies with a cresty neck detected on physical examination (OR 4.6; 95% CI 2.3–9.1).

In conclusion, hyperinsulinaemia is prevalent in ponies in this population and associated with both indicators of obesity and historical or current laminitis. The WMP breed appears to be at increased risk in this population.

ABSTRACT #115
COMPARISON OF 4 ASSAYS FOR SERUM INSULIN ANALYSIS IN THE HORSE. TW McGowan[1], R Geor[2], H Evans[3],

M Sillence[4], K Munn[4], CM McGowan[5]. [1]University of Queensland, Brisbane, Australia.[2]Virginia Tech, Blacksburg, VA, USA. [3]Cambridge Specialist Laboratories, UK.[4]Charles Sturt University, Wagga, Australia.[5]University of Helsinki, Finland.

Many different assays have been used to determine insulin concentration in horses, the results of which are important in screening for insulin resistance and determining the prognosis in equids with Equine Cushing's Syndrome. While these assays have generally been validated for use in horses, there are minimal data on comparisons between different assays currently in use around the world. The aim of this study was to compare 4 assays (3 radioimmunoassay kits and one ELISA) for measurement of equine serum insulin concentration.

Blood samples were collected as part of an epidemiological study of insulin resistance in 208 ponies. Samples were centrifuged within 3 hours of collection, aliquots of serum taken and frozen at −80 °C until analysis. All samples were analysed using the DSL-1600 Insulin RIA kit (n = 208). Aliquots from the same sample were also analysed using the DPC Insulin RIA (n = 137), DiaSorin Insulin RIA (n = 59) and/or the Mercodia ELISA (n = 22) kits. Serum and plasma aliquots from the same horse (n = 17) were also compared using the DPC insulin RIA. All kits had been validated for use in horses for their performance on sensitivity, parallelism, and precision (intra-assay variation). All kits were within the acceptable parameters for recovery (≥90%). All kits showed excellent dilutional parallelism except the DSL RIA which tended to overestimate the insulin concentration above a 1:4 dilution. Intra-assay variation was good for the DiaSorin RIA (<5%), Mercodia ELISA (5.3%), and DPC RIA (7.3%), and acceptable for the DSL 1600 RIA (10.9%). Insulin values ranged from 0.3–645 μIU/ml and were not normally distributed. A Spearman rank correlation coefficient (r) and coefficient of determination (R^2) were determined between pairs of tests using linear regression. The Wilcoxon signed rank test was used to determine any differences between data sets.

All tests were strongly significantly correlated. Serum and plasma insulin both measured using the DPC RIA had the highest correlation (r = 1.00, R^2 = 0.98) and were not significantly different. The different assay kits had a range of r from 0.92 to 0.96, and R^2 from 0.84 to 0.96. However, despite excellent linear correlation, serum insulin concentrations were not directly comparable between different assays, with significant differences detected between datasets ($P<0.01$). For example, at an insulin of 30 μIU/ml measured by the DPC RIA, values for the DSL, Mercodia and DiaSorin assays were, respectively, 20, 54 and 72 μIU/ml. Furthermore, at high serum insulin concentrations (>200 μIU/ml), there was a reduction in the linear correlation, especially with the DSL RIA kit. In conclusion, the 4 commonly used, validated assays for measurement of serum insulin concentration were highly correlated but data from different assays should not be directly compared.

ABSTRACT #116
INCREASED ADIPOSITY IN HORSES IS ASSOCIATED WITH DECREASED INSULIN SENSITIVITY BUT UNCHANGED INFLAMMATORY CYTOKINE EXPRESSION IN SUBCUTANEOUS ADIPOSE TISSUE. R Carter[1], J Mc Cutcheon[1], T Burns[2], J Belknap[2], N Frank[3], R Geor[1]. [1]Virginia Tech, Blacksburg, VA.[2]The Ohio State University, Columbus, OH.[3]The University of Tennessee, Knoxville, TN.

Obesity and insulin resistance (IR) in horses are of intense interest to the equine practitioner due to associated sequelae, including laminitis. In humans and in animal models of obesity, increased adiposity leads to a chronic inflammatory state which underlies the IR. This study investigated whether an increase in adiposity over time in horses is also associated with changes in insulin sensitivity and expression of inflammatory (circulating and subcutaneous adipose tissue) cytokines. To induce weight gain, 12 Arabian geldings were fed 200% of their digestible energy requirements for 4 months. Before and after weight gain, adiposity was measured, subcutaneous adipose tissue biopsies were obtained from the nuchal crest, and frequently sampled intravenous glucose tolerance tests were performed and assessed by the minimal model of glucose and insulin dynamics. Real time quantitative PCR was performed on the adipose tissue biopsy samples to assess mRNA concentrations of inflammatory genes (TNF-α, IL-1β, IL-6, IL-8, IL-10), and plasma concentrations of TNF-α were measured by ELISA (Endogen Inc.). Data were analyzed by the Mann-Whitney U test ($\alpha = 0.05$) and are reported as median (interquartile range). At the end of the trial, body weight was increased ($P = 0.001$) by 18%, percent body fat estimated from subcutaneous fat thickness (ultrasonic assessment) had increased ($P = 0.002$) from 14 (12 – 18)% to 23 (19 – 26)%, and resting insulin concentration was increased ($P < 0.001$) from 4.3 (3.5 – 6.3) to 28 (20 – 43) mU/L. Insulin sensitivity decreased ($P < 0.001$) to 1/4 its original value, accompanied by an over fourfold increase ($P < 0.001$) in acute insulin response to glucose. These changes resulted in a similar disposition index ($P = 1.0$), indicating that decreased insulin sensitivity was effectively compensated for by an increased insulin secretory response. In contrast to the majority of data in human obesity, the decrease in insulin sensitivity was not associated with increases in indicators of inflammation. Plasma concentrations of TNF-α and adipose tissue mRNA expression of TNF-α, IL-1β, IL-6, IL-8, and IL-10 did not differ ($P > 0.05$) between pre- and post-weight gain samples. Thus, the results of this study demonstrate that in horses an acute increase in adiposity is associated with decreased insulin sensitivity and a compensatory increase in insulin secretory response independent of changes in the currently measured inflammatory cytokine expression in a subcutaneous fat depot.

ABSTRACT #117
URINE GLUCOSE CONCENTRATIONS DURING INTRAVENOUS GLUCOSE TOLERANCE TESTS IN HORSES. F. Tóth[1], N Frank[1], K. Perdue[1], R Geor[2], SB Elliott[1], RC Boston[3]. [1]University of Tennessee College of Veterinary Medicine, Knoxville, TN.[2]Middleburg Agricultural Research and Extension Center, Virginia Polytechnic and State University, Middleburg, VA.[3]University of Pennsylvania, Kennett Square, PA.

When intravenous glucose tolerance tests are used, area under the blood glucose curve (AUCg) values are calculated, and it is assumed that blood glucose levels decline in response to insulin. However, it has not been determined whether blood glucose concentrations also decrease as a result of glucose loss into the urine. We hypothesized that urine glucose concentrations would increase significantly during the combined glucose-insulin tolerance test (CGIT) and frequently-sampled intravenous glucose tolerance (FSIGT) test in healthy adult horses. A new FSIGT test was also designed with the aim of eliminating glucose loss into the urine. Six horses were included in a crossover study. Treatments included the CGIT, which involves infusion of 150 mg/kg dextrose and 100 mU/kg insulin, and two different FSIGT tests: the established procedure using 300 mg/kg dextrose and 30 mU/kg insulin and a new test using 100 mg/kg dextrose and 20 mU/kg insulin dosages. Urine samples were collected every 10 minutes via a catheter inserted into the urinary bladder. Mean urine AUCg curve values were calculated for each procedure. Treatment effects were evaluated using ANOVA.

Glucosuria was detected and urine glucose concentrations increased significantly over time during each of the tests. Mean urine AUCg values differed significantly ($P < 0.001$) between groups, with the lowest value registered for the new FSIGT test. Results demonstrate that glucosuria develops during intravenous glucose tolerance tests in horses and this may affect AUCg. This problem was not resolved by selecting a lower dextrose dosage of 100 mg/kg.

ABSTRACT #118
SEASONAL CHANGE IN ENERGY METABOLISM OF PONIES COINCIDES WITH CHANGES IN PASTURE CARBOHYDRATES: IMPLICATIONS FOR LAMINITIS. KH Treiber[1], RA Carter[1], PA Harris[2], RJ Geor[1]. [1]Virginia Tech, Blacksburg, VA. [2]Equine Studies Group WALTHAM Centre for Pet Nutrition, Leics, UK.

Obesity and altered insulin dynamics are associated with laminitis in grazing ponies. These metabolic predispositions may interact with seasonal changes in pasture carbohydrates, increasing the risk for laminitis. To evaluate potential seasonal interactions, we sampled ~30 ponies and their pasture bimonthly on one farm in northern Virginia in 2007. Pasture was sampled at 1400 h on 4 consecutive days each month for measurement of water-soluble (WSC)

and ethanol-soluble (ESC) carbohydrates and starch. Blood was collected between 0800 and 1100 h the following week to measure plasma glucose, non-esterified fatty acids (NEFA), triglycerides (TG), insulin and ACTH. Nine ponies developed clinical laminitis in late April/May and were categorized as CL. Data are shown in the table below as means ± SEM.

			January	March	May	July	September
Pasture	WSC, %		1.8 ± 0.1^{c}	0.85 ± 0.1^{c}	12.4 ± 2.7^{a}	6.7 ± 0.2^{b}	5.9 ± 1.0^{b}
	Starch, %		0.4 ± 0.1^{c}	0.5 ± 0.1^{bc}	1.0 ± 0.1^{a}	0.9 ± 0.2^{ab}	1.0 ± 0.1^{a}
	ESC, %		2.5 ± 1.2^{b}	1.2 ± 0.1^{ab}	8.6 ± 1.6^{a}	6.7 ± 0.7^{ab}	3.8 ± 0.6^{ab}
	Glucose mg/dL		98 ± 1^{bcd}	100 ± 2^{abcd}	104 ± 4^{ab}	95 ± 1^{bcde}	91 ± 1^{de}
Ponies	ACTH pg/mL		23 ± 2^{b}	23 ± 2^{b}	23 ± 1^{b}	21 ± 1^{b}	47 ± 4^{a}
	NEFA mEq/L		0.22 ± 0.0^{ab}	0.23 ± 0.0^{ab}	0.13 ± 0.01^{bd}	0.11 ± 0.01^{cd}	0.17 ± 0.01^{ac}
	Insulin	NoCL	13.4 ± 1.1^{b}	16.1 ± 1.2^{b}	37.7 ± 3.2^{a}	15.2 ± 1.3^{b}	16.8 ± 0.7^{b}
	mIU/L	CL	43.1 ± 2.7^{ab}	52.6 ± 4.3^{ab}	140.6 ± 22^{a}	61.7 ± 4.2^{ab}	23.6 ± 2.1^{b}
	TG	NoCL	42.9 ± 1.1^{ab}	31.3 ± 0.8^{cd}	30.9 ± 1.4^{bcd}	40.5 ± 1.0^{abc}	42.2 ± 1.2^{ab}
	mg/dL	CL	53.8 ± 1.0^{a}	40.8 ± 1.0^{a}	56.8 ± 2.4^{a}	59.4 ± 1.1^{a}	50.5 ± 0.6^{a}

Means in the same row without the same subscript differ ($P < 0.05$) according to within-subject ANOVA with Fisher-Hayter pairwise comparisons. Over all months, ponies which developed laminitis in May (CL) had higher insulin ($P = 0.002$) and TG ($P = 0.027$) than ponies which did not display clinical laminitis in 2007 (NoCL), so data are reported separately.

In all ponies, plasma glucose and insulin were highest in spring (May) in association with the highest values for pasture WSC and ESC. Conversely, plasma TG and NEFA declined around May. Therefore carbohydrate metabolism appears to be up-regulated and fat metabolism down-regulated during spring when pasture starches and sugars are highest. These changes superimposed on already elevated insulin concentrations or insulin resistance in high-risk ponies may contribute to the development of laminitis in the spring.

ABSTRACT #119

ECHOCARDIOGRAPHIC REPEATABILITY OF EQUINE AORTIC VALVE PROLAPSE. GD Hallowell[1] and I.M. Bowen[2]. [1]14, Threadcutters Way, Shepshed, Leicestershire, UK. [2]School of Veterinary Medicine and Science, University of Nottingham, UK.

The aims of this study were to describe the most suitable views for identification of aortic valve prolapse (AVP) and to report how repeatable this finding was in the horse. Eight healthy Thoroughbred and Irish Draught cross horses (mean±SEM:600±26.7 Kg) were recruited. Echocardiography was performed daily for five days on each horse by two operators. Standard images of the aortic valve were obtained and valves were interrogated using continuous, pulsed wave and color-flow Doppler. Images were evaluated by one operator for the five separate examinations and by one operator for a single examination. Inter- and intra-operator repeatability were assessed. Only appropriate long-axis standard views of the aortic valve (AoV) were used for evaluation of the prevalence and repeatability of AVP as non-standard views can artificially create the appearance of valve prolapse. Data were compared using repeated measures ANOVA, Student's T, Friedman, and McNemar's tests as appropriate. Intra-class correlation coefficients (ICC's) were calculated. AVP was observed in 88% and physiological aortic regurgitation (PAR) in all horses on at least two days. Prolapse was observed in more than one cusp in 50% of horses and all three cusps in one horse. Prolapse was observed to affect the right coronary cusp most commonly (100% of horses with AVP), the non-coronary cusp the next most common (50%) and the left coronary cusp least commonly (17%). This was most reliably identified on a right parasternal long axis view of the left ventricular outflow tract and could only be seen on a right parasternal short axis view of the aortic valve if AVP was severe. There were no differences in any of the measurements, time indices, prevalence of AVP or PAR or dimensions of the regurgitant jet identified using color-flow Doppler over five days in the same horse except for free wall diameter in systole ($p=0.018$) and diastole ($p=0.019$). There were no differences between measurements performed on images obtained by the two different operators and agreement regarding AVP and PAR between echocardiograms performed by different operators was between 79 and 100%. There were no differences between measurements performed on different occasions or between operators. Intra-class correlation coefficients showed good (>0.85) agreement between echocardiograms obtained by different operators on the same day except for pre-ejection period (0.504) and inter-ventricular septum dimensions (0.304). There was extremely good (> 0.85) correlation between measures taken from the same horses on different days except for free wall diameter in systole and diastole (0.537), AoV thickness (0.666) and PAR jet width (0.390). In conclusion, this is a repeatable reliable technique for identification of AVP and PAR in the horse.

ABSTRACT #120

CARDIAC TROPONIN I CONCENTRATIONS IN PONIES CHALLENGED WITH INFLUENZA VIRUS. MM Durando[1], EK Birks[1], VB Reef[1], SB Hussey[2], DP Lunn[2]. [1]University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA. [2]Colorado State University College of Veterinary Medicine, Fort Collins, CO.

This study was performed to determine the effect of influenza virus challenge on circulating cardiac troponin I (cTnI) concentrations in ponies. In people, viral infections are a common cause of myocarditis. Similarly, myocardial damage/myocarditis is thought to occur secondary to viral respiratory infections in horses. However there is a lack of published information in the equine literature confirming a causal relationship between viral infections and myocarditis. The hypothesis was that influenza virus infection in ponies would cause myocardial damage, detectable by elevations in plasma cTnI. cTnI is a sensitive and specific biochemical marker for myocardial damage with utility in horses, and has been used to monitor viral-associated myocarditis in people and in experimental models.

The study included 29 influenza-naïve yearling ponies: 23 were part of an influenza vaccine study, with 11 unvaccinated ponies (UN) and 12 that received a primary vaccination series 6 months previously with Recbombitek™ (Merial) (VAC). These 23 ponies were challenged with 10^8 EID_{50} influenza virus A/eq/Kentucky/91 using a nebulizer. An additional 6 ponies, housed in a similar manner but not exposed to influenza, were used as controls (CON). Physical examinations and body weights were recorded, and heparinized blood collected from all groups 1 day prior to challenge, and on days 1, 3, 5, 7, 10, 14, 21, 24 and 28 after challenge. Repeated measures ANOVA, chi–square, and/or clustered regression analyses were used to evaluate possible relationships among the various treatment groups and cTnI. Significance was set at $P<0.05$.

All virus challenged ponies developed typical clinical signs of infection. All CON ponies had normal cTnI concentrations at all time points (mean±SD, 0.00±0.01 ng/ml). One VAC pony (0.32 ng/ml on day 5) and two UN ponies (0.46 ng/ml on day 28, and 0.13 ng/ml on day 14) had cTnI concentrations greater than our upper normal limit of 0.08 ng/ml. At all other sample times, cTnI concentrations were < 0.05 ng/ml. There were no significant associations between body weight, temperature or clinical event score (an indicator of severity of clinical disease) and cTnI ($P = 0.34$, 0.93, and 0.13, respectively). There were no significant differences in cTnI between groups (mean±SD; CON, 0.00±0.01, VAC, 0.01±0.03 and UN, 0.01±0.05 ng/ml; $P = 0.16$). When separated into abnormal vs normal cTnI, there were 0/6 abnormal in CON, 1/12 in the VAC, and 2/11 in UN, with no significant differences between groups ($p = 0.49$).

This study shows that acute myocardial damage does not commonly occur secondary to influenza virus infection in sedentary ponies; however, transient elevations in cTnI suggesting mild myocardial damage do occur. Possible relationships in active performance horses, as opposed to sedentary ponies, as well as time points beyond 28 days should be examined.

ABSTRACT #121

TEMPORAL DETECTION OF *LAWSONIA INTRACELLULARIS* USING SEROLOGY AND REAL-TIME PCR IN THOROUGHBRED HORSES RESIDING ON A FARM ENDEMIC FOR EQUINE PROLIFERATIVE ENTEROPATHY. N. Pusterla[1], R. Jackson[2], R. Wilson[2], J. Collier[1], S. Mapes[1], C. Gebhart[3]. [1]School of Veterinary Medicine, University of California, Davis, CA. [2]California Polytechnic State University, San Luis Obispo, CA. [3]College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

Equine poliferative enteropathy (EPE) caused by *Lawsonia intracellularis* has recently been recognized as an emerging disease in foals. The goal of this study was to document exposure to *L. intracellularis* in a population of Thoroughbred horses residing on a farm endemic for EPE.

The study population included 68 resident mare and foal pairs which were sampled over a period of 11 months (January-November). Serum samples from mares at delivery and foals pre- and post-colostrum ingestion and monthly thereafter were collected and tested for the presence of *L. intracellularis* antibodies by immunoperoxidase monolayer assay (IPMA). Serum collected from foals was also used to determine the concentration of total solids. Additionally, feces from mares at delivery and foals post-partum and monthly thereafter were collected, processed for nucleic acid purification and assayed for *L. intracellularis* using real-time PCR.

Thirty-seven mares (54.4%) had positive titers ($\geq$60) against *L. intracellularis* by IPMA at the time of foaling with titers ranging from 60 to 240 (mean titer 133). The seroprevalence in mares increased with each foaling month (33% January, 47% February, 55% March, 71% April, 100% May). All tested foals had negative antibody titers ($<$60) against *L. intracellularis* prior to colostrum ingestion. Passive transfer of colostral antibodies against *L. intracellularis* was documented in 36 foals (53.7%) with titers ranging from 60 to 240 (mean titer 123). Colostral antibodies remained detectable in the serum of foals for 1 (31 foals), 2 (4) and 3 (1) months. Exposure rates in foals by month ranged from 0 to 10% and the antibodies remained detectable for 1 to 2 months. Overall, 22 foals (33.3%) showed evidence of natural exposure to *L. intracellularis* throughout the study period. None of the study foals developed signs compatible with EPE and the concentration of total solids in the serum of all foals remained within normal limits. Only one single fecal sample collected from one foal in May tested PCR positive for *L. intracellularis*.

In conclusion, the results showed that colostral antibodies against *L. intracellularis* were passively transferred to foals born on a farm endemic for EPE. Further, the exposure rates in mares and foals varied with the month of collection. The measurable immunological response following passive transfer of colostral antibodies or following primary exposure appeared to be short-lived in foals. Despite high exposure to *L. intracellularis* in foals, no clinical EPE cases were recorded. It is possible that the high rate of foals with evidence of passive transfer of colostral antibodies against *L. intracellularis* may have reduced the incidence of clinical cases. Healthy resident mares and foals did not appear to shed *L. intracellularis* in feces.

ABSTRACT #122

PREVALENCE OF *RHODOCOCCUS EQUI* ISOLATES RESISTANT TO MACROLIDES OR RIFAMPIN AND OUTCOME OF INFECTED FOALS. S Giguère[1], E Lee[1], ND Cohen[2], MK Chaffin[2], N Halbert[2], RJ Martens[2], RP Franklin[3], CC Clark[4]. [1]University of Florida, Gainesville, FL. [2]Texas A&M University, College Station, TX. [3]Equine Medical Center of Ocala, Ocala, FL. [4]Peterson & Smith Equine Hospital, Ocala, FL.

The objectives of this study were to establish the prevalence of *R. equi* isolates resistant to macrolides or rifampin and to determine the outcome of foals infected with resistant isolates. Thirty-four isolates classified as resistant to erythromycin, clarithromycin, azithromycin, or rifampin, were obtained from 6 laboratories between 1997 and 2007. For each isolate, minimum inhibitory concentration of the 4 antimicrobial agents was determined using both the E test and broth macrodilution. Each isolate confirmed to be resistant to at least one antimicrobial agent was also evaluated for presence of the virulence plasmid using PCR amplification of the *vapA* gene.

Only 19 of the 34 isolates (55.9%) submitted as resistant were *R. equi* isolates resistant to at least one drug. Two isolates were resistant to rifampin only whereas 17 isolates were resistant to all 3 macrolides and rifampin. Two of the 19 resistant isolates were avirulent. The prevalence of resistant isolates at the University of Florida was not significantly different from that of Texas A&M University. The overall prevalence of *R. equi* isolates resistant to macrolides or rifampin was 3.7%. The survival rate of foals infected with resistant isolates (25%) was significantly lower ($P < 0.004$) than that of foals from which susceptible isolates were cultured (69.6%).

In conclusion, approximately half the *R. equi* isolates identified as resistant to a macrolide or rifampin by diagnostic laboratories are either not *R. equi*, not resistant, or avirulent. Foals infected with resistant isolates are less likely to survive than foals infected with susceptible isolates.

ABSTRACT #123

EQUINE HERPES VIRUS-1 (EHV-1) RECRUDESCENCE AND VIREMIA IN HOSPITALIZED CRITICALLY ILL HORSES. EA Carr[1], N Pusterla[2], H Schott[1], J Dechant[2], S Holcombe[1]. [1]Michigan State University College of Veterinary Medicine, East Lansing, MI. [2]University of California, Davis, Davis, CA.

Outbreaks of EHV-1 appear to be occurring with increased frequency and reports of spread within a hospital population exist. Equine herpes virus-1 infection occurs through exposure to viral particles shed in nasal secretions or placenta and tissues of an aborted fetus. Once infected, latency is a hallmark of herpes viruses. Recrudescence and nasal shedding can occur in animals with or without clinical evidence of disease making identification of potentially infectious horses challenging. Risk factors for recrudescence and shedding are poorly documented but many types of stressors (e.g., weaning, castration, long-distance transport, movement to a new facility and immunosuppression) have been suggested. Horses presenting to referral hospitals with severe illnesses are typically under significant stress and elevated serum cortisol and catecholamine concentrations have been documented in such horses. Little information exists on the effect of acute critical illness on EHV-1 recrudescence and shedding. Knowledge about this potential risk would be important for development of effective biosecurity policies to limit the risk of EHV-1 transmission in a hospital setting.

Horses greater than 6 months of age presenting with acute abdominal disorders (surgical colic or colitis) were included in the study. For inclusion, horses with colic had to have had an exploratory celiotomy as part of their treatment course. Horses with colitis had to have evidence of systemic inflammatory response syndrome including at least 3 of the following criteria; heart rate $\geq$ 60 bpm, plasma lactate $\geq$ 3.0 mmol/liter, rectal temperature $\geq$ 102.5 F, or absolute neutropenia or neutrophilia. Whole blood and nasal secretions were collected on day 1, day 4–7 and day 10–12. All samples (uncoagulated blood, nasal secretions) were processed for nucleic acid purification and tested for EHV-1 using real-time PCR assays targeting the glycoprotein B (gB) gene and the polymerase (ORF 30) gene.

One hundred and twenty-two horses met the inclusion criteria. There were 89 surgical colics and 33 colitis cases. Age ranged from 6 months to 28 years with a median age of 12 years. There were 56 mares (46%) 61 geldings (50%) and 5 stallions (4%). Samples were collected from all horses on day 1, 75 horses (61%) on days 4–7 and 19 horses (16%) on days 10–12. One hundred and seven horses survived to discharge. None of the samples was positive for EHV-1 DNA.

These results suggest that nasal shedding and viremia of EHV-1 in hospitalized critically ill horses with acute abdominal disorder is extremely rare. These results may be useful in the implementation of biosecurity protocols for decreasing the risk of EHV-1 infection in the hospital setting.

ABSTRACT #124

CONTROL OF EHV-1 VIREMIA & NASAL SHEDDING BY CURRENT COMMERICAL VACCINES. L. Goehring[1], S.B. Hussey[1], S. Rao[1], R. Bigbie[2], D.P. Lunn[1]. [1]Colorado State University, Fort Collins, CO. [2]Fort Dodge Animal Health, Overland Park, KS.

Equine herpesvirus-1 is a cause of significant morbidity and mortality in horses, and its most important disease manifestations are abortion and myeloencephalopathy. The pathogenesis of both these diseases depends on establishment of viremia, and control of viremia is currently regarded as a likely critical goal for immunoprophylaxis. An experiment was performed to determine whether two current commercially available vaccines were capable of reducing EHV-1 viremia and nasal shedding.

The study design was a blinded, randomized challenge trial. Three groups of 8 yearling colt ponies, with no history of EHV-1 infection, were established. Each pony group received one of three

treatments: vaccination with a modified live vaccine (MLV) against EHV-1 (Rhinomune™, Pfizer); vaccination with an inactivated EHV-1 vaccine (Pneumabort-K™, Fort Dodge); or injection with a saline placebo. Each treatment was administered 3 times, at intervals of 1 month between the first two treatments, and 3 months between the second and third treatments. All ponies were challenged by nasal instillation of 5×10^7 pfu of EHV-1 (Findlay, Ohio 2003) 1 month after the third treatment. Clinical signs of disease, including rectal temperature, nasal discharge, anorexia, coughing, and depression, were recorded daily for 2 days prior to challenge infection, and 21 days post-challenge. Nasal shedding of virus and viremia were measured on the same days, using a real-time PCR test procedure. Differences in viremia and viral shedding between groups were analyzed using a generalized linear model with ANOVA (SAS™), and differences were declared significant when $P \leq 0.05$.

All ponies demonstrated clinical signs of disease consistent with EHV-1 infection post-challenge infection, including pyrexia, nasal discharge, inappetance and partial anorexia. The duration and severity of these signs appeared reduced in the vaccinated ponies but statistical analysis has not been completed. In the control group, 6/8 ponies were viremic on one or more days (total of 13 pony viremic days), in the Rhinomune group there were 4/8 viremic ponies (total of 5 pony viremic days), and in the Pneumabort-K group there were 2/8 viremic ponies (total of 3 viremic days). Statistical analysis demonstrated a significant effect of vaccination on viremia on Day 5 ($P = 0.0015$). For nasal shedding, there was a reduction in shedding in both groups, which was significant on Days 1–Day 11 post-challenge.

This study demonstrated that both a commercial MLV and an inactivated vaccine containing a high antigen titer were capable of significantly suppressing EHV-1 shedding after a highly pathogenic viral challenge. This is the first report of any suppression of viremia by commercial vaccines, which may be important in controlling the most important clinical manifestations of EHV-1 infection.

ABSTRACT #125

TREATMENT OF EHV-1 INFECTION USING RNA INTERFERENCE. A Fulton, GA Perkins, S Peters, N Osterrieder, GR Van de Walle. Cornell University, College of Veterinary Medicine, Ithaca, NY.

RNA interference mediated by small interfering RNAs (siRNAs) is an important defense mechanism against viral infections in plants, and synthetic siRNAs have been shown to inhibit viral replication of human immunodeficiency virus, herpes simplex virus and others. siRNAs bind to complementary target mRNA resulting in degradation and inhibition of protein expression. Equine herpesvirus type 1 (EHV-1) causes myeloencephalitis, abortions and respiratory disease and spreads rapidly by aerosolization of the virus and inhalation. Vaccines against the disease are marginally efficacious and metaphylactic and therapeutic agents are not available. siRNAs, delivered nasally, could prove to be a novel and efficacious method of EHV-1 control. Effective siRNAs could be given (i) prior to co-mingling of horses at competitions, sales, and show events and (ii) during an EHV-1 outbreak to decrease viral load in the herd and prevent new infections. We investigated whether siRNAs against EHV-1 genes responsible for viral replication and cell entry would decrease EHV-1 infection, which was studied first *in vitro* and then *in vivo*.

In vitro: siRNAs against two essential EHV-1 genes were synthesized and transfected at various concentrations into rabbit kidney-13 cells, followed by infection with the neurovirulent EHV-1 strain Ab4. Cell culture supernatant was collected at 24-h post infection (p.i.) and the number of infectious units determined by plaque assays. In addition, the monolayers were examined to measure plaque sizes and morphology by fluorescent microscopy. Both siRNAs significantly reduced viral replication as measured by the magnitude of virus production and plaque size ($p < 0.05$, Student's t-test), and a maximal effect was noted at 75 nM with an 80-fold reduction. Interestingly, combining both siRNAs proved to be as effective as use of a single siRNA at 75 nM, but at a much lower concentration, namely 12.5 nM of each siRNA.

In vivo: Four-week old BALB/c mice were given various concentrations of the siRNAs, alone or in combination, intranasally and were subsequently infected with 10^5 PFU Ab4 at 0.5, 6, 12 and 24-h later. Treatment with siRNAs not directed against EHV-1 genes, the carrier (lipofectamine) and media were control groups. All mice were weighed daily for two weeks and the lungs were harvested (3 mice per group) on days 2 and 4 p.i. for viral titers. Mice treated with siRNAs 30-min and 6-h prior to EHV-1 infection lost less weight compared to the controls, and viral titers in the lungs were significantly lower ($p < 0.05$, Student's t-test).

From these results, we conclude that siRNAs directed against two genes vital to EHV-1 replication are highly effective at down regulating viral replication *in vitro* and also *in vivo* in a mouse model of EHV-1 infection. Therefore, siRNAs show potential for preventing EHV-1 infection in horses. The efficacy of siRNAs in the definitive host, the horse, will be tested in the near future.

ABSTRACT #126

NITAZOXANIDE AND TIZOXANIDE INHIBIT EHV-1 AND INFLUENZA TYPE A VIRUS REPLICATION *IN VITRO*. RJ Callan, LV Ashton, and LS Goehring. Colorado State University College of Veterinary Medicine and Biological Sciences, Fort Collins, CO.

There is a need for development of specific antiviral treatment of equine viral diseases. Nitazoxanide (NTZ, Navigator) and its metabolite, tizoxanide (TIZ), are thiazolide compounds that demonstrate antimicrobial activity against a variety of protozoal, bacterial, and viral organisms. The hypothesis for this study is that NTZ and TIZ will decrease replication of equine herpesvirus type 1 (EHV-1) and influenza type A viruses in cell culture. The purpose of the study was to determine the antiviral effective concentrations of NTZ and TIZ that result in 50% (EC_{50}) and 90% (EC_{90}) reduction of equine herpesvirus type 1 (EHV-1) and equine influenza virus (EIV) production in culture supernatants. In addition, the cytotoxicity and selectivity index of the compounds on cell culture were determined.

Three EHV-1 and three EIV isolates were cultured on RK-13 or Madin-Darby Canine Kidney (MDCK) cells, respectively, in the presence of varying concentrations of NTZ and TIZ. *In vitro* cytotoxicity concentration (CC_{50}) of NTZ and TIZ was determined as the concentration resulting in a 50% decrease in viability of uninfected cells at 96 hours incubation using an alamar blue reduction assay. Cells were grown in 96 well plates and infected with 7 plaque forming units of virus in the presence of varying concentrations of compound. Viral titers of culture supernatants were determined at 96 hours by plaque assay or $TCID_{50}$. The EC_{50} and EC_{90} for the compounds were determined by linear regression of supernatant viral titers. The selectivity index was calculated as the EC_{50}/CC_{50}.

The CC_{50} of NTZ and TIZ for RK-13 cells was 15 μM and 25 μM, respectively. The CC_{50} of NTZ and TIZ for MDCK cells was 65 μM and 130 μM, respectively. The mean EC_{50} and EC_{90} and the SI for EHV-1 and EIV are listed in the table below.

	Nitazoxanide			Tizoxanide		
Viruses	EC_{50} (μM)	EC_{90} (μM)	SI_{50}	EC_{50} (μM)	EC_{90} (μM)	SI
EH V-1	0.90 ± 0.18	2.89 ± 1.06	17.42 ± 3.80	0.68 ± 0.12	2.25 ± 0.49	38.20 ± 6.91
EIV	0.14 ± 0.06	0.34 ± 0.11	0.0021 ± 0.001	0.14 ± 0.08	0.34 ± 0.14	0.0011 ± 0.001

NTZ and its metabolite TIZ show antiviral activity against EHV-1 and EIV. Both compounds were more effective at inhibiting EIV replication than EHV-1. The *in vitro* results indicate that NTZ may be an effective drug for the prevention or treatment of EHV-1 and EIV infection in horses. Based on these results, further *in vivo* testing of NTZ for the control or treatment of EHV-1 or EIV in horses is warranted.

ABSTRACT #127

EFFECT OF PREDNISONE ADMINISTRATION ON THROMBELASTOGRAPHY PARAMETERS IN HEALTHY BEAGLES. L Rose, C Bédard, M Dunn. University of Montreal Faculty of Veterinary Medicine, Saint-Hyacinthe, Quebec.

Long-term corticosteroid therapy has been associated with an increased risk of thromboembolic complications in dogs. The purpose

of this prospective study was to use thrombelastography (TEG) to detect the development of a hypercoagulable state in beagles receiving oral prednisone. We hypothesized that the administration of corticosteroids to healthy dogs would result in a hypercoagulable profile on TEG tracings. To our knowledge, the effect of corticosteroid therapy on TEG tracings has not been previously investigated in dogs.

Six healthy adult Beagles were included in the trial. Dogs received 1 mg/kg of prednisone once daily for two weeks, followed by a 6-week washout period and then 4 mg/kg of prednisone once daily for two weeks. TEG tracings were obtained before prednisone administration (baseline), at the end of the washout period and at the end of both corticosteroid trials. TEG analyses were performed in duplicate on citrated whole blood 30 minutes after collection using recombinant human tissue factor at a final concentration of 1:3600. The reaction time (R), the alpha angle (α), the kinetic time (K) and the maximum amplitude (MA) were recorded.

A repeated-measures linear model was used to test for the effect of treatment. Significant results were obtained for K ($P=0.0002$), α ($P=0.0013$) and MA ($P<0.0001$), with tracings compatible with a hypercoagulable profile after corticosteroid trials when compared to baseline. A significant difference between corticosteroid dosages was only detected for MA ($P=0.0037$).

Further studies are needed to determine the underlying mechanisms of this hypercoagulability.

ABSTRACT #128
UTILITY OF THROMBELASTOGRAPH (TEG®) PLATELET-MAPPING™ FOR EVALUATION OF CLOPIDOGREL IN DOGS. BM Brainard, SM Kleine, SC Budsberg. University of Georgia, Athens, GA.

Clopidogrel inhibits the action of ADP at the platelet P2Y12 receptor. Drug effects may be measured by evaluating ADP-induced platelet aggregation. We hypothesize that PlateletMapping using the thrombelastograph (TEG) is able to quantify the degree of platelet inhibition in dogs given clopidogrel. PlateletMapping uses heparinized blood, ADP, and a reptilase-based activator. Six dogs were given clopidogrel at doses of 0.5 and 1 mg/kg PO, and blood samples were analyzed every 20 minutes for 1 hour and hourly to 3 hours. Platelet function was assessed using PlateletMapping™, and compared with impedance aggregometry in three dogs at each dose. The maximum amplitude (MA) of the TEG tracing was compared to baseline to determine the degree of clopidogrel-induced platelet inhibition. Most dogs displayed partial (40–60%) inhibition by 1 hour, and by 3 hours after the initial dose, MA had decreased to a mean of 20% of baseline, regardless of dose ($p<0.001$ vs. baseline). Correcting for the effect of thrombin activation on the TEG tracing, the 3-hour MA values indicated near 100% inhibition of ADP-induced platelet aggregation. Impedance aggregometry showed a rapid decrease in ADP-induced platelet aggregation, decreasing to zero in most dogs by 2 hours ($p < 0.001$ vs. baseline). One dog at 0.5 mg/kg did not show a change in ADP-stimulated platelet aggregation measured by aggregometry, but did show a decrease when evaluated with PlateletMapping. PlateletMapping appears to be a viable test, and demonstrates clopidogrel to be an effective, rapidly acting drug for inhibition of ADP-induced platelet aggregation in the dog.

ABSTRACT #129
EVALUATION OF TISSUE FACTOR AND KAOLIN ACTIVATED THROMBOELASTOGRAPHY ON FELINE CITRATED WHOLE BLOOD FROM CLINICALLY HEALTHY CATS. CR Bjornvad, B Wiinberg, AL Jensen, AT Kristensen. Department of Small Animal Clinical Sciences, University of Copenhagen, Denmark.

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 is routinely used in the diagnostic workup and monitoring of humans and dogs with hemostatic disorders and it has shown to 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.

The objective of this study was to evaluate a human recombinant tissue factor (TF) and a Kaolin (K) activated TEG assay on citrated whole blood (WB) from clinically healthy cats, with the aim of estimating reference ranges for physiological hemostasis in healthy cats.

Citrated WB was collected from 17 clinically healthy cats and stored at RT for 30 min before analysis. Duplicate TEG analyses with TF (1:50,000) or Kaolin as activator were performed. 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 difference in R, K, α and MA between TF and K activation. Statistical significance was set at $p < 0.05$.

The observed TEG parameters (median (range)) for TF were R = 8.95 min (5.00–14.35), K = 5.85 min (3.33–15.05), $\alpha = 29.9^\circ$ (18.3–50.7) and MA = 39.9 mm (24.6–54.1). With kaolin activation the TEG parameters were R = 9.00 min (3.80–14.80), K = 4.85 min (1.85–7.65), $\alpha = 38.5^o$ (26.8–62.1) and MA = 47.1 mm (34.9–57.7). Using TF or K as activators, significant differences were observed for the TEG parameters K, α and MA ($P<0.05$) but not R, indicating similar activation time but enhanced clotting kinetics and clot strength with kaolin activation.

In conclusion, feline citrated WB can be used for TEG analysis with TF or K as activators. However, in this study, Kaolin seems to activate coagulation processes more than human recombinant tissue factor. Coagulation activation and kinetics of feline WB in the TEG assay differ from references in dogs, the coagulation reaction starts later and develops slower in cats. Further studies on coagulation kinetics in cats are in progress.

ABSTRACT #130
A CONTROLLED STUDY OF HUMAN INTRAVENOUS IMMUNOGLOBULIN IN THE TREATMENT OF CANINE PRIMARY IMMUNE-MEDIATED THROMBOCYTOPENIA. D Bianco, PJ Armstrong, RJ Washabau. University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

We have previously reported (JVIM 2007; 21: 694–699) that human intravenous immunoglobulin (hIVIG) was well tolerated and associated with rapid platelet count recovery in an uncontrolled study of canine primary immune-mediated thrombocytopenia (pIMT). In the present clinical trial, we used a prospective, randomized, double-masked, placebo-controlled study design to test the hypothesis that early adjunctive therapy with hIVIG in pIMT dogs treated with corticosteroids would be a safe therapeutic intervention to improve survival rate to discharge, accelerate platelet count recovery, and shorten hospitalization time without increasing the cost of treatment. Eighteen client-owned dogs with pIMT were enrolled beginning in August 2006. The population consisted of 16 purebreds and 2 mixed breed dogs with a mean age of 8.3 +/− 1.7 years and a mean body weight of 20.9 +/− 2.8 kg. There were 14 spayed female and 4 neutered males.

Dogs were randomized to receive either a single infusion of hIVIG (0.5 g/kg) or placebo on day 1 from initial admission. Groups did not differ with regard to age, body weight, platelet count, hematocrit, or albumin on presentation. The placebo group consisted of 9 dogs, and 2 dogs (22.2%) were lost from the study because of death due to pIMT. The hIVIG group consisted of 9 dogs and has not lost any dogs. Two dogs, one for each group, experienced an episode of relapse within six months from the initial diagnosis. The median lag time from the start of treatment until platelet count increased to >40,000/μL for placebo group dogs was 7.5 days (mean +/− SD, 7.8 +/− 3.9 days; range, 3–12 days) and 3.5 days (3.7 +/− 1.3 days; range, 2–7 days) for hIVIG group dogs. The median hospitalization time for placebo group dogs was 8 days (8.3 +/− 0.6 days; range, 4–12 days) and 4 days (4.2 +/− 0.4 days; range, 2–8 days) for hIVIG group dogs. There were no identifiable immediate or delayed adverse events associated with hIVIG administration.

Analysis of the data revealed a significant (<0.05) reduction in platelet count recovery time and length of hospitalization in hIVIG group dogs. There was no significant difference between groups with respect to costs of initial hospitalization. Although the population sample size is small, early adjunctive therapy with hIVIG appears to be safe and beneficial in dogs with pIMT.

ABSTRACT #131

THE EFFECT OF HETASTARCH *IN VIVO* ON PLATELET FUNCTION IN THE DOG. L Smart, KE Jandrey, JR Wierenga, F Tablin. William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA.

Hydroxyethyl starch, with an average molecular weight 600 kd and degree of substitution 0.7 (HES), is an artificial colloid solution commonly used in veterinary medicine. HES has been shown to decrease platelet function in humans *in vivo*, indicated by prolonged closure times (CT) as measured by the Platelet Function Analyzer-100® (PFA-100®). HES has also been shown to decrease canine platelet function *in vitro* by prolonging CT. Our hypothesis was that intravenous HES *in vivo* prolongs CT in dogs.

Eight healthy, employee-owned dogs were included in the treatment group. Four of these dogs also served as the control group. Washout period between experimental protocols for the control group was a minimum of 4 weeks. Baseline platelet count was greater than 100,000/μl in all dogs. CTs were measured using collagen and adenosine diphosphate platelet agonists. Dogs were given 20 mL/kg of either NaCl 0.9% (control group, n=4) or HES (treatment group, n=8) IV over one hour. Blood was drawn for CT before the infusion, and at 1, 3, 5, and 24 hours after the start of the infusion. Two-way repeated measures analysis of variance was used to assess changes over time, and differences between control and treatment groups. Time-specific group differences were evaluated using Student's t-test with a sequentially rejective method of multiple comparison adjustment.

There was a significant change over time from 0 to 24 hours ($p=0.0001$), a significant difference between groups across time ($p=0.0004$), and evidence of a significant group-by-time interaction ($p=0.0069$). At 3 hours, mean CT for the treatment group was 122.3 $\pm$ 18.1 seconds, which was significantly different ($p=0.0002$) from the control group (71.0 $\pm$ 3.5 seconds). At 5 hours, mean CT for the treatment group was 142.7 $\pm$ 33.9 seconds, which was significantly different ($p=0.0014$) from the control group (75.0 $\pm$ 8.6 seconds). Mean CT at 24 hours was within the reference interval for both the control and treatment group (66.0 $\pm$ 2.9 seconds and 81.8 $\pm$ 11.9 seconds respectively), however CT in three individual dogs in the treatment group at this time point remained prolonged.

Given these results, a clinically relevant dose of HES adversely affects platelet function, as assessed by closure time. Individual dogs may still have decreased platelet function 24 hours after a single 20 mL/kg dose of HES, and therefore, an increased risk of bleeding.

ABSTRACT #132

AN EVALUATION OF 9570 DOGS BY BREED AND DOG ERYTHROCYTE ANTIGEN TYPING. AS Hale[1], J Werfelmann[1], M Lemmons[2], B Smiler[2] and J Gerlach[2]. [1]Midwest Animal Blood Services, Inc., Stockbridge, MI. [2]Michigan State University, E. Lansing, MI.

Nine thousand five hundred and seventy dogs were evaluated by breed and dog erythrocyte antigen assay (DEA) for the purpose of predicting compatibility during routine transfusion service.

Retrospectively, data from these dogs was evaluated for DEA frequency in relation to breed type and total incidence within the submitted population. Sample testing occurred between 1995 and 2006. All dogs were evaluated for DEA by tube agglutination utilizing polyclonal antisera derived through canine alloimmunization recognizing DEA 1.1, 1.2, 3, 4, 5 and 7. Ninety breeds were represented. All recognized AKC breed groups were represented in the sampling. Data from mixed breed dogs was also correlated. Population incidence for the total sampling is represented in the table below.

DEA Antigen	Population Frequency
1.1	42%
1.2	12%
3	7%
4	98%
5	11%
7	20%

Twenty nine percent of all dogs tested positive for DEA 4 only (''Universal'' type). Breeds demonstrating an incidence of ''universal'' type greater than the average population frequency included Airedale, American Bulldog, Boxer, Bull Mastiff, English Bulldog, English Mastiff, German Shepherd, Greyhound, Irish Wolfhound, Old English Sheepdog, Pitbull, Saluki and Scottish Deerhound. Twenty-six percent of all dogs tested positive for DEA 1.1 and 4 only. Breeds demonstrating an incidence of DEA 1.1,4 greater than the average population frequency included Afghan, Bassett Hound, Bernese Mountain Dog, Border Collie, Bouvier des Flandres, Bullmastiff, Bull Terrier, Chesapeake Bay Retriever, Cocker Spaniel, Dalmatian, English Mastiff, English Setter, English Springer Spaniel, Great Dane, Golden Retriever, German Shorthair Pointer, Great Pyrenees, Irish Setter, Labrador Retriever, Malamute, Malinois, Newfoundland, Poodle, Rottweiler, Samoyed, St Bernard, Standard Poodle and Vizsla. Using the currently recommended field typing scheme, 55% of all dogs submitted for typing could be used as a red blood cell donor without DEA mismatch if the recipients are type matched for DEA 1.1.

ABSTRACT #133

A GEL COLUMN BASED ANTIGLOBULIN TEST TO DETECT ERYTHROCYTIC AUTO- AND ALLOANTIBODIES IN CATS. Mayank Seth, Karen V Jackson, Urs Giger. Section of Medical Genetics, University of Pennsylvania, Philadelphia, PA.

The antiglobulin (Coombs') test detects auto- and alloantibodies directed against erythrocytes and has been used to diagnose immune-mediated hemolytic anemia (IMHA) and to identify blood type incompatibilities. Many techniques, including tube and microplate agglutination, as well as flow cytometry and gel-column tests, using varied species-specific reagents, have been developed.

We report here on the use of a novel, commercially available, simple, feline antiglobulin containing gel column assay (DiaMed, Switzerland). Briefly, for the direct antiglobulin test (DAT) patient's ~2% red cell suspension was placed on top of one gel column with pre-loaded antiglobulin reagent and one without (saline control), and then the columns were centrifuged. In the indirect antiglobulin test (IAT) patient's serum or plasma was incubated with a red cell suspension on top of gel columns at 37 °C prior to centrifugation. With increasing red cell retention in the gel column the results were graded from 0 to 4+.

Of 64 anemic Coombs' tested cats, 13 were DAT positive ($\geq$1+; 12 $\geq$2+). Eight of these 13 DAT positive cats were clinically diagnosed with either primary or secondary IMHA. The other 5 DAT positive cats did not show clinical signs of hemolysis and were diagnosed with various underlying illnesses. Among the 51 DAT negative cats 8 were suspected to have a hemolytic anemia: 4 Abyssinians and 2 domestic shorthair cats had increased osmotic fragility of erythrocytes, 1 Somali had a pyruvate kinase deficiency and in 1 case the cause of hemolysis remained unknown. When screening samples from 62 healthy blood donors, all cats were found to be DAT negative.

Antiglobulin enhanced gel column crossmatches were performed on select samples from 42 anemic cats to 264 donor blood units. In addition, samples from 8 healthy non-anemic donor cats with suspected compatibility issues were screened against 65 additional units. The major crossmatch (patient plasma and donor red cells) test revealed 90 incompatibilities, including 2 A-B mismatches and 10 reactions involving the *Mik*-antigen. The minor crossmatch (donor plasma and patient/other donor red cells) test revealed 53 incompatibilities, of which 6 were related to the presence of *Mik*-alloantibodies. Among the 143 incompatibilities observed overall, 81 were appreciated only in antiglobulin-containing gel columns and not saline gel columns.

In addition, we used the gel column technique with anti-*Mik* serum for the *Mik* typing of 132 cats, of which 4 samples (3%) were found to be *Mik* negative. All *Mik* reactions were stronger in the antiglobulin-containing columns than in the saline columns.

In conclusion, the feline antiglobulin gel column test seems simple and standardized to use and helpful in identifying IMHA. Moreover, the use of the antiglobulin column in the major and minor crossmatch test facilitates the identification of transfusion incompatibilities (e.g. A, B, *Mik* and other antigens and their alloantibodies), although the clinical relevance of some of these incompatibilities is not yet determined.

ABSTRACT #134
COMPARISON OF GEL COLUMN, CARD, CARTRIDGE, SLIDE AND TUBE TECHNIQUES FOR AB BLOOD TYPING OF CATS. Mayank Seth, Karen V Jackson, Urs Giger. Section of Medical Genetics, University of Pennsylvania, Philadelphia, PA.

The clinical importance of the feline AB blood group system with its type A, type B and rare type AB has been well recognized. Indeed, A-B blood incompatibilities, responsible for life-threatening acute hemolytic transfusion reactions, as well as neonatal isoerythrolysis, can be avoided by prior blood typing, which may be performed by several methods.

This study directly compares a gel column diffusion assay (GEL, DiaMed, Switzerland with lectin as anti-B), a card based agglutination assay (CARD, DMS Laboratories, NJ), an immunochromatographic cartridge (CHROM, Alvedia, France) and laboratory slide (SLIDE) and tube (TUBE) agglutination techniques on samples from Ryan's Veterinary Hospital, the Penn Animal Blood Bank and external samples sent to the Transfusion Laboratory. A total of 38 EDTA blood samples from healthy cats and 20 from sick cats with an emphasis on the less common type B and rare type AB were typed using all of the above methods. In addition, samples from 432 cats were tested only with the GEL and TUBE methods; the latter being historically the gold standard. All plasma samples from cats, which express the type B antigen on the erythrocyte surface, were evaluated for the presence of anti-A alloantibodies (back-typing).

In the complete comparative typing study of 58 cats, 35, 14 and 9 samples were determined to have type A, type B and type AB, respectively. Fifty-two (90%) samples gave the same typing results with all techniques. The CHROM method misidentified 1 type A cat as type AB and 2 AB cats; 1 as type A and 1 as type B. The CARD test misidentified 1 type A cat as type AB and 4 AB cats; 1 as type A and 3 as type B. The SLIDE and GEL methods both mistyped 1 type A as an AB cat. Interestingly, samples from 2 FeLV positive domestic cats were the source of many A-B blood type discrepancies. All B cats had strong anti-A alloantibodies, while AB cats had no anti-A alloantibodies in their plasma. The reactions of the GEL and CHROM methods were generally clearest to assess and archive.

In the comparison study of GEL and TUBE typing methods, 372, 42 and 18 samples were determined to be type A, B and AB, respectively. GEL and TUBE tests gave the same results in 430 (99.5%) of cases. GEL test results were inconclusive for 2 FeLV positive type A cats.

We conclude that currently available commercial laboratory and in-clinic techniques generally provide accurate typing results. The GEL and CHROM methods made interpretation of results simple. The rare type AB posed the most discordant results and FeLV positive cats potentially represent another unique subset in terms of serological typing.

ABSTRACT #135
THE EFFECTS OF WEIGHT LOSS ON GENE EXPRESSION IN DOGS. Yamka RM, KG Friesen, X Gao, S Malladi, S Al-Murrani and L Bernal. Hill's Pet Nutrition Center, Topeka, KS.

Seven neutered/spayed obese Beagles (> 35% body fat; average age = 5.86 ± 3.34 years; average weight = 17.0 ± 3.7 kg) were identified for a weight loss study to test the efficacy of a dry low-fat, fiber-enhanced therapeutic food formulated for weight loss (33.2% crude protein, 8.7% crude fat and 26.7% total dietary fiber on dry matter basis). The dogs were fed for weight loss (1.0 × RER) for a period of 4 months. During the study all dogs underwent DEXA and chemistry screen analysis at 0, 1, 2, 3 and 4 months. In addition, blood samples were collected into PAXgene blood RNA tubes at the beginning and end of the study to characterize changes in gene expression differences resulting from weight loss on the food. RNA was extracted according to the procedures provided in the PAXgene Blood RNA Kit Handbook (Qiagen, Valencia, CA). RNA was hybridized to an Affymetrix Gene Chip Canine-2 Genome Array and signal normalized using Robust Multi-Array Average. Data was filtered using following a false discovery rate of 0.1 and > 1.3-fold change were considered different for day 0 and end of study. On average, dogs lost 2.8 ± 0.8 kg body fat (41.2% of initial fat mass) in 4 months. Gene expression profiles were modified in these dogs after 4 months of weight loss on the food. The nutrigenomic effect of the food can be seen in the shift from an obese to a lean profile. Of the genes identified, there was a down-regulation of genes associated with fat accumulation, including long chain fatty acid CoA ligase 1, growth factor receptor-bound protein 2 and hypoxia-inducible factor 1 alpha. In summary, obese dogs fed the weight loss food lost 2.8 ± 0.8 kg (41.2% of initial fat mass) body fat in 4 months, which was accompanied by a shift from an obese to lean genomic profile.

ABSTRACT #136
LECITHIN:CHOLESTEROL ACYLTRANSFERASE ACTIVITY AND ITS FATTY ACYL SPECIFICITY IN CATS FED DIETS OF VARYING FATTY ACID COMPOSITION. R. Angell[1], K. Bigley[1], M. McClure, Y. Mitsuhashi[1], D. Nagaoka[1], J. E. Bauer[1,2]. [1]Companion Animal Nutrition Laboratory, [2]Faculty of Nutrition, Texas A&M University, College Station, TX.

Beyond the fact that cats express low delta-6 desaturase activities, lipid metabolism in this species remains to be fully explored under varying dietary or other experimental conditions including other important metabolic enzymes such as lecithin:cholesterol acyltransferase (LCAT), which is involved in reverse cholesterol transport. To characterize LCAT activity and demonstrate its fatty acyl specificity in cats fed varying types of dietary fat, intact young females were fed diets enriched with high-oleic sunflower (n = 9), menhaden fish (n = 10), or safflower (n = 10) oil (8 g oil/100 g kibble) for 4 wk. Fasted blood samples were drawn at d0, d14, and d28 for determination of plasma total (TC), unesterified (UC), and esterified cholesterol (EC) concentrations, LCAT activity, and fatty acid (FA) composition of the EC fraction. UC decreased at d28 compared to d0 and d14, while EC increased at d28 compared to d0 and d14 (all $p < 0.05$). LCAT and TC showed no time or diet effects. Plasma EC FA profiles reflected the specificity of LCAT for linoleic acid (LA) and numerous diet and time effects were observed. Even though similar amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were supplied by the fish oil diet and significant amounts of EPA were detected in the plasma EC fraction, no DHA was identified in this fraction, indicating very little or no affinity of feline LCAT for DHA. The fish oil diet supplied docosapentaenoic acid (DPA) as well, but this FA was also absent in the plasma EC fraction, suggesting little or no affinity of feline LCAT for DPA. We conclude that feline LCAT has no measurable affinity for DHA, but that feline LCAT demonstrated specificity for LA regardless of diet fed. Finally, it was noteworthy that some changes in feline plasma EC FA composition were statistically significant after only 14 days of feeding the experimental diets. This observation indicates rapid modification of feline FA metabolism by dietary fatty acid composition. By d28, further significant differences due to time were seen, indicating that while initial differences can be seen in as little as 14 days, a longer study period may be necessary to establish a metabolic steady state. Whether 28 days is enough time to achieve this effect cannot be determined by the present study. However, our studies in dogs and other species indicate that 28 days is suitable to achieve steady state effects of plasma fatty acids. Nonetheless, we did demonstrate that 28 days feeding is a long enough study period to observe alterations in feline FA metabolism. Finally, because no diet or time effects were seen on LCAT activity, the values for all cats at all time points were averaged to obtain a basal LCAT activity for adolescent cats of 92.4 ± 4.5 nmol of UC esterified/ml of plasma/hr (mean ± SEM). This is lower than an earlier reported value for LCAT activity in adolescent cats, and is likely due to differences in methodology. The present work utilized an endogenous substrate method while the earlier study employed an artificial proteoliposome substrate technique.

ABSTRACT #137
THE IMPACT OF SIGNALMENT AND BODY CONFORMATION ON ACTIVITY MONITORING IN COMPANION DOGS. DC Brown, KE Michel, M Love, C Dow. University of Pennsylvania, Philadelphia, PA.

An objective method for recording activity could be useful for assessing the behavior of companion dogs in their everyday environment as many of these behaviors have to do with the dogs' level of activity. The Actical Activity Monitor (AAM) is a sensitive,

watch-sized, accelerometer that continuously measures the intensity, frequency and duration of movement for extended periods. Our objective was to describe the impact of signalment and body conformation on activity monitoring using the AAM in companion dogs.

At least 20 dogs were recruited in each of 5 weight ranges. Signalment and 7 body conformation measurements were recorded. While wearing an AAM, each dog was led through a series of standardized activities: lying down, walking laps and trotting laps. In addition, our hypothesis was that a dog's age and body conformation could influence the outcome of activity monitoring during an activity that is less controlled; therefore all dogs were trotted up and down stairs, permitting each dog to adjust its stride between steps. Linear regression analysis was used to determine which signalment or body conformation factors were associated with the AAM activity counts.

Data was collected on 104 dogs. There was no significant impact of signalment or body conformation on activity counts recorded during lying down, walking laps, and trotting laps. However, when trotting up and down stairs, there was a significant impact of age and bodyweight such that, for every 1 kg increase in bodyweight, there is a 1.9% decrease in average activity counts and for every 1 year increase in age, there is a 4.1% decrease in average activity counts.

When the activity is well controlled, there is no significant impact of signalment or body conformation on the average activity counts recorded by the AAM. However, when dogs are allowed leeway in the extent of effort exerted within an activity, older dogs and larger dogs will deliver lower average activity counts than younger and smaller dogs and so these factors will need to be considered in studies using these monitors as an outcome.

ABSTRACT #138
ACTIVITY MONITORING CAN DIFFERENTIATE INTENSITY OF EXERCISE IN COMPANION DOGS. KE Michel, M Love, C Dow, DC Brown. University of Pennsylvania, Philadelphia, PA.

Extent of activity directly affects an individual dog's energy balance and factors into that dog's caloric requirement. An objective method that captures the activity level of dogs in their everyday environment could aid in tailoring feeding recommendations to better reflect an individual companion dog's opportunity and inclination to exercise. The Actical Activity Monitor (AAM) is a sensitive, watch-sized, accelerometer that continuously measures the intensity, frequency and duration of movement for extended periods. Our objective was to investigate whether the AAM could be used to differentiate the intensity of activity in companion dogs.

While wearing an AAM, each dog was led through a series of standardized activities: lying down, walking laps, and trotting laps. At least 20 dogs were recruited in each of 5 weight ranges. The Wilcoxon signed-rank test was used to compare sedentary activity counts to walking activity counts and walking activity counts to trotting activity counts. Receiver operating characteristic curves were generated to determine the optimal activity counts for predicting whether an animal was sedentary, walking, or trotting.

Data was collected on 104 dogs. The median counts (range) for sedentary activity was 10 (0–64), for the walking activity was 290 (91–622), and for the trotting activity was 785 (359–1517). Both sedentary and walking activity counts and walking and trotting activity counts were significantly different from one another ($p<0.001$). At an activity count of 65 there is 100% specificity and 100% sensitivity in distinguishing the sedentary activity from the walking activity. At an activity count of 441 there is 92% specificity and 92% sensitivity in distinguishing the trotting activity from the walking activity.

In conclusion, activity counts recorded by the AAM were highly accurate in differentiating among standardized activities in companion dogs.

ABSTRACT #139
ANTIOXIDANT STATUS AND BIOMARKERS OF OXIDATIVE STRESS IN DOGS WITH DIABETES MELLITUS. JR Smith[1], SK Cox[1], SD Lauten[1], RC Hill[2], JW Bartges[1], CA Kirk[1]. [1]College of Veterinary Medicine, The University of Tennessee, Knoxville, TN. [2]College of Veterinary Medicine, University of Florida, Gainesville, FL.

Increasing evidence implicates oxidative damage in the progression and pathologic complications of human diabetics. This study assessed antioxidant status and oxidative stress in dogs with diabetes mellitus (DM). Antioxidant status was measured in diabetic (n=10) and control (n=10) dogs by HPLC of vitamin E isomers, reduced (GSH) and oxidized glutathione (GSSG), and calculation of the GSH:GSSG ratio. Biomarkers of protein, lipid and DNA peroxidation (fructosamine, isoprostanes, and Comet assay, respectively), and neutrophil function were used to evaluate oxidative stress. Correlation between glycemic control and antioxidant status/ oxidative stress was also investigated. A diabetic index was generated using clinical signs, body condition score, insulin dose, fructosamine, fasted blood glucose and urinary glucose and ketones. Diabetic dogs were separated into those with good (n=4) or poor (n=6) control.

Serum fructosamine concentration was significantly higher (DM = 472.9 mmol/l, controls 281.3 mmol/l; $p<0.0001$), while specific vitamin E isomers, serum GSH (DM = 885.8 ng/ml, controls = 1188.1 ng/ml, $p=0.01$) and 8-$F_2\alpha$-isoprostanes (DM = 0.31 ng/ml, controls = 3.74 ng/ml; $p<0.0001$) were significantly lower in diabetic dogs versus controls. Antioxidant status/ oxidative stress was not associated with glycemic control in diabetic dogs.

Despite strong association of DM with oxidative stress in humans, this simple relationship is not found in diabetic dogs. They have both increased and decreased parameters of systemic oxidative stress compared with control dogs. This may be due to higher levels of antioxidants in canine therapeutic diets, the relatively short duration of disease in dogs compared to humans, or other factors.

ABSTRACT #140
TRIGLYCERIDE REFERENCE VALUES FOR A MEAL CHALLENGE TEST TO ASSIST DIAGNOSIS AND MANAGEMENT OF CANINE HYPERLIPIDEMIA. KF Elliott, JS Rand, LM Fleeman, JM Morton. Centre for Companion Animal Health, Uni. of Queensland, Australia.

Hyperlipidemia in dogs is associated with clinical disorders, including recurrent seizures and acute pancreatitis. Dietary therapy is typically recommended for management of hyperlipidemia and a standardized method for evaluating efficacy of dietary management in controlling postprandial triglyceride concentrations is needed. This study aimed to determine reference values using 95% tolerance intervals for 24 hour fasting and postprandial peak triglyceride concentrations for healthy dogs after a standard meal, to assist in diagnosis and management of hyperlipidemia.

Twelve (6 male, 6 female) lean, healthy, neutered, mixed-breed dogs were studied. Dogs were fed a dry commercial maintenance diet (fat 31%ME, CHO 45%ME, protein 24%ME, fiber 2 g/100 kcal) for 3 weeks. After a 24 hour fast, plasma triglyceride concentrations were measured at −1, −0.08, 1, 2, 3, 4, 5, 6, 9, and 12 hours after a meal (median amount eaten: 127 kcal/$kg^{0.75}$). Raw and log transformed data were tested for normal distribution using Kolmogorov-Smirnov test and for outliers using Grubb's test. To determine the most appropriate time for blood sampling (the time most closely associated with highest postprandial triglyceride concentration), Spearman's correlation coefficients, associated 95% confidence intervals (CI) and p values were calculated to assess correlations in raw data between postprandial peak triglyceride and triglyceride concentrations at each time point. Tolerance intervals were calculated with log-transformed data using a parametric method.

One dog had a postprandial peak triglyceride concentration 44% higher than the next highest dog; however its fasting value was toward the low end of the tolerance interval. It was found to be an outlier using Grubb's test and was not included in calculation of tolerance intervals. Triglyceride concentration at 6 hours after ingestion of the standard meal had the closest association with postprandial peak triglyceride concentration with and without this dog ($r=0.93$ and 0.91, respectively; $p<0.01$; 95% CI: 0.77 to 0.98 and 0.68 to 0.98, respectively). The tolerance interval for postprandial peak triglyceride concentration was 49 to 191 mg/dL (0.54 to 2.11 mmol/L). The tolerance interval for triglyceride concentration 6 hours after eating was 35 to 176 mg/dL (0.38 to 1.94 mmol/L). The tolerance interval for 24 hour fasted triglyceride concentrations

sampled 5 minutes prior to a meal was 14 to 91 mg/dL (0.15 to 1.00 mmol/L). In 50% of the dogs, triglyceride concentrations did not return to baseline by 12 hours after eating.

In conclusion, measurement of both fasting and postprandial peak triglyceride concentrations after a standard meal are important for detecting hyperlipidemia and for evaluating responses to therapeutic diets, because fasting concentrations alone are not likely to be predictive of postprandial peak concentrations. The reference values determined in this study can be applied in a meal challenge test to dogs which have been fasted 24 hours, fed the same standard meal, and sampled 5 minutes prior to the meal and 6 hours after eating.

ABSTRACT #141

LIPID METABOLISM EFFECTS IN CATS FED DIETS VARYING IN MEDIUM CHAIN FATTY ACIDS AND LINOLEIC ACID CONCENTRATIONS. L. Trevizan[1,2], K. Bigley[2], W. Anderson[3], M.K.Waldron[3], J.E. Bauer[2,4]. [1]LEZO, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, RS, Brazil, [2]Comp. An. Nutr. Lab., [3]Nestlé Purina Pet Care, St. Louis, MO, USA, [4]Faculty of Nutrition, Texas A&M Univ., Col Stn, TX.

Medium chain triglycerides (MCT) containing fatty acids of 6 to 12 carbons are found in ingredients such as coconut oil and milk byproducts. They are readily absorbed and efficiently metabolized in mitochondria. However, in dogs and cats, some studies show that ca. 22% Metabolic Energy (ME) caused diet aversion, especially when enriched in caprylic acid (C8:0). In dogs, 11%ME MCT showed no effects on food intake, but cats have not been evaluated at this concentration. Thus, we hypothesized feeding cats with 11%ME MCT does not cause diet aversion. The objective of this study was to evaluate a diet containing increased MCT by substituting a portion of the safflower oil containing dietary linoleic acid (LA) with coconut oil. The high MCT diet (HMCT) contained 11.4%ME MCT and 4.3%ME LA (29.9%ME protein, 32.3%ME NFE, 37.8%ME fat) while the low MCT diet (LMCT) contained 4.3%ME MCT and 14.5%ME LA (29.8%ME protein, 29.4%ME NFE, 40.7%ME fat). Nineteen adult female cats were separated into two groups (LMCT, n=9; HMCT, n=10). The cats had been fed a pre-experimental diet (PED) containing no MCT, 5.3%ME LA , and 27% ME total fat for 30 days. Test diets were fed for 9 weeks using week 1 as a transition period. Cats were initially fed according to their metabolic body weights ($100^{*}W^{0.67}$) and water offered ad libitum. Daily consumption records, weekly body weights (BW) and body condition scores (BCS, 1 to 9) were used to adjust amounts fed and to calculate the daily metabolic energy factors for each cat so that an ideal BCS of 5 was maintained. Blood samples (7 ml in EDTA) were taken after overnight fasting via saphenous vein at d 0, 14, 28 and 56 to measure plasma triglycerides (TG), total cholesterol (TC), lipoprotein distribution (LP) and lecithin cholesterol acyl transferase (LCAT). Repeated measures analyses (SAS, Mixed Models Procedure) and Tukey (α=0.05) multiple comparisons were performed. A significant time effect was observed on food consumption, BW, BCS and metabolic factor reflecting the previous PED diet. A diet effect on plasma TG revealed a 28% increase (p=0.0234) with the HMCT diet. However, no diet effects were seen in TC, LP or LCAT activity. Time effects were observed in which TC increased on d 14 and again d 28 but returned back to the d 14 level at d 56 (p=0.0001), LCAT increased at d 14, only. All LP (β, pre-β and α) in both diets reached peak values at d 28 (p=0,0001) consistent with elevation of TC. No diet differences were seen in food intake, metabolic factor, and BW, demonstrating that 11%ME MCT does not produce aversion in cats. The TC increase was likely the result of increased dietary fat content of the test diets. A modest increase of plasma TG occurred with HMCT but it was within normal limits. Thus, it is feasible to formulate feline diets containing MCT without diet refusal. Such diets may be useful in both normal cats and those with fat malabsorption syndromes.

ABSTRACT #142

POST-FEEDING SATIETY AND WEIGHT LOSS EFFECT OF A VEGETABLE-BASED FIBER SUPPLEMENT IN BEAGLES. Y. Mitsuhashi[1,2], K. Bigley[1], J.E. Bauer[1,2]. [1]Companion Animal Nutrition Laboratory, [2]Intercollegiate Faculty of Nutrition, Texas A&M University, College Station, TX.

Dietary insoluble fiber is believed to support weight loss by increasing satiety and allowing increased amounts of food consumption without adding calories, whereas dietary soluble fiber has potential to reduce cholesterol. Therefore, in this study, we evaluated a vegetable-based fiber supplement containing both soluble (SF) and insoluble fiber (IF) types on plasma lipid profiles, satiety, and weight loss in Beagles.

Two diets were fed differing in fiber content. Purina ONE® healthy weight formula (ONE) was compared to a diet consisting of ONE plus a vegetable-based fiber supplement (FIB). Total dietary fiber contents were 3.83 g/100 kcal (ONE) vs 6.89 g/100 kcal (FIB). The SF content of the FIB diet was 83.3% more than that of ONE (1.10 g/100 kcal vs 0.60 g/100 kcal in ONE) and IF content of the FIB diet was 79.6% more than that of ONE (5.80 g/100 kcal vs 3.23 g/100 kcal in ONE). For the satiety studies, female adult Beagles with average body fat (BF) of 37.5±1.4 (SEM) %, and body weight (BW) of 13.4±0.6 (SEM) kg were randomly divided into 2 diet groups. Diets were fed at 8am and 3pm (7h interval, n=14) or, during a second trial, at 8am and 11am (3h interval, n=12) for 15 min each over a 3 or 2 day period, respectively. Amounts offered at each feeding were 1.2 times MER using a cross-over design with 3 days washout. Blood samples were collected after the 8am feeding at 45 min and 120 min and food intakes were recorded. For the weight loss study, 7 obese Beagles were selected (average BF 45.1±1.6%, BW 15.2±1.0 kg, BCS 8.0±0.3 [9 point scale]) and divided into 2 groups. The diets were fed once daily (ca. 60% of obese MER) for 42 days. Postprandial blood samples were collected at 60 min (days 1, 28 and 42) for lipid and lipoprotein analysis. Food intakes and BW were recorded daily and weekly, respectively. Repeated measures ANOVA was performed under α=0.05. Where there was significance, paired t or one-way AOV / Kruskal-Wallis tests with Tukey multiple comparisons were performed after checking normality.

In the satiety trials, both the 3 and 7h interval food intakes were not different from the control group indicating similar satiety of both diets. However, significantly fewer total calories were consumed with the FIB diet during the 3 h interval study (p=0.017). Plasma triacylglycerol (TG) concentration was significantly increased at 120 min post feeding independent of diet compared to 45 min (p<0.01). In the weight loss trial, the FIB group significantly reduced % BW (p=0.002) and % BF (p=0.008) over time. Plasma TG, total cholesterol (TC) and lipoprotein (LP) did not change. Thus, the increased percentage of IF in the FIB diet provided fewer calories with the same degree of satiety as the higher calorie intake of the control diet. In addition, this increase supported more efficient weight loss. However, the modestly increased amount of SF with the FIB diet did not affect plasma lipid profiles. In conclusion, the FIB diet appeared to provide similar satiety at lower calorie intake thereby promoting weight loss without modifying TG, TC and LP responses.

ABSTRACT #143

ALTERATIONS OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE ACTIVITY AND CHOLESTEROL METABOLISM IN OBESE BEAGLES DURING WEIGHT LOSS. R. Angell[1], D. Bandy[1], K. Bigley[1], Y. Mitsuhashi[1], D. Nagaoka[1], T. Umeda[2], K. Otsuji[2], J. E. Bauer[1,3]. [1]Companion Animal Nutrition Laboratory, [3]Faculty of Nutrition, Texas A&M University, College Station, TX, USA, [2]Kao Corp., Tokyo, Japan.

Little research has focused on the relationship between lecithin:cholesterol acyltransferase (LCAT) activity and cholesterol metabolism in dogs even though it is the major lipid metabolic enzyme involved in reverse cholesterol transport. To study alterations in LCAT activity and lipid metabolism during weight loss in this species, four experimental weight-loss diets were fed to 12 obese female Beagles for 8 wk in a partial crossover design (n = 6). High- (HGI) or low-glycemic index (LGI) starch (waxy corn or high amylose corn, respectively) and diacylglycerol (DAG) or triacylglycerol (TAG) oil were combined to compose diets with similar fatty acid (FA) profiles. Both starches were gelatinized prior to formulation. Experimental diets were fed as a water-based gruel after acclimating the dogs to a similar gruel type basal diet. Dogs were fed the amount of kcal required to maintain their obese body weights. Consumption records were kept daily and body weights were measured weekly. Fasted blood samples were obtained at baseline, wk4, and wk8 to

measure plasma LCAT activity using a radiolabeled endogenous substrate technique. Total (TC), unesterified (UC), and esterified cholesterol (EC) concentrations were determined via enzymatic methods. Fatty acid composition of the plasma phospholipid (PL) and EC fractions were determined after total lipid extraction, fractionation via thin-layer chromatography, and capillary gas chromatography. All groups lost weight as a result of voluntary reduction of food intake of the gruel-based type of diet. Plasma UC concentrations increased in all groups from baseline to wk4 ($p < 0.05$). LCAT activities increased from baseline to wk4 in all groups and remained elevated at wk8 ($p < 0.05$). Plasma PL FA profiles reflected the diets fed with few diet or time effects. Plasma EC FA profiles reflected the previously observed specificity of canine LCAT for linoleic acid (LA) again with minimal diet or time effects. We conclude that an increase in LCAT activity and plasma UC concentration is observed in conjunction with weight loss in dogs. Diet effects on these two parameters were not observed, indicating that the changes in LCAT activity and UC concentration were indeed due to weight reduction because all diet groups experienced weight loss. Dietary oil type (TAG vs. DAG) did not affect the plasma EC or PL fatty acid composition. These findings are likely due to the similar fatty acid composition of the experimental oils and diets and indicates that positional isomers of oil do not affect resultant FA profiles under the conditions employed.

ABSTRACT #144

INFLUENCE OF GENDER AND SEXUAL ALTERATION STATUS ON FELINE ADIPONECTIN. AL Lusby, CA Kirk, JW Bartges. The University of Tennessee College of Veterinary Medicine, Knoxville, TN.

Adiponectin is a hormone secreted almost exclusively from adipocytes that correlates closely with insulin sensitivity in human beings and rodents. In contrast to other adipokines, adiponectin levels decrease as fat mass increases. This hormone holds potential diagnostic and therapeutic applications for feline obesity and diabetes. Although previous studies have identified strong associations between obesity and gender and/or reproductive status in cats, the influence of adiponectin in these associations has not been evaluated. The purpose of this study was to identify the impact of gender and reproductive status on adiponectin and insulin resistance. These findings may help to better understand the role of adiponectin in obesity and insulin resistance in the cat.

This study compared four groups of lean (BCS 4–6/9), healthy, young adult cats (1–6 years; n=40) to determine the influence of gender and gonadectomy on total serum adiponectin levels. Each group consisted of ten cats in each of the following categories: intact female (IF), intact male (IM), neutered female (NF), neutered male (NM). General health status was assessed through physical exam, complete blood count, and chemistry panel with electrolytes. Body weight and body condition scores (BCS) were also recorded. Serum samples for adiponectin were collected from fasted cats at one time point and stored at −80 °C. A commercial ELISA kit was used to measure adiponectin (B-Bridge International, Mountain View, CA). A linear ANOVA model was used for statistical analysis (SAS v.9.1) and compared gender, reproductive status, body weight with total adiponectin.

Body weight differed between all groups except for IM and NF ($P < .05$) while BCS did not differ. There was no significant difference between adiponectin levels among groups ($P < .05$). However, NF tended to have greater adiponectin levels compared to IM ($P = 0.07$) and neutered animals tended to have higher adiponectin levels compared to intact animals ($P = 0.06$). While this study indicates total adiponectin levels are not influenced by gender or gonadectomy in domestic cats, there are strong trends that may be influenced by sample size.

ABSTRACT #145

INFLUENCE OF DIETARY PROTEIN CONTENT AND SOURCE ON FECAL QUALITY AND PROTEIN-DERIVED FERMENTATION PRODUCTS IN DOGS DIFFERING IN BODY SIZE. J Nery[1], C Tournier[2], V Biourge[2], L. Martin[1], H Dumon[1] and P Nguyen[1]. [1]Ecole Nationale Vétérinaire de Nantes, France. [2]Royal Canin, Aimargues, France.

When given the same diet, large breed dogs produce feces of poorer quality than smaller ones due partially to higher fermentative activity in the hindgut. Undigested proteins are important substrates for fermentation by the colonic microflora. They can be metabolized to several bioactive products, which could have deleterious effects on the colonic mucosa. The aim of this study was to evaluate the effect of dietary protein source and amount on fecal quality and concentration of some key protein fermentation products in dogs differing in body size.

Twenty-seven female dogs of 6 different breeds (from 3.35 to 34.65 kg BW) were divided in 4 groups according to BW (miniature: MI, medium: ME and maxi: MA) and propensity to have softer feces (maxi tolerant: MT and maxi sensitive: MS). Five diets varying in protein source and level were tested in a 2-phase protocol consisting of a crossover and Latin square designs respectively. Diets were formulated to be isoenergetic and to have similar content of fat, TDF and ash. Main dietary protein sources and levels were as follows: wheat gluten meal in diets WGLP (CP=19.9%) and WGHP (CP=34.3%), mix of poultry and wheat gluten meal in diet WPMP (CP=26.2%), and poultry meal in diets PMLP (CP=19.3%) and PMHP (CP=35.2%). Feces were scored daily and fecal moisture was determined from a 7-day collection pool after a 7-day adaptation period. Fresh stools were collected (1–2 samples/dog and diet) and analyzed for ammonia and branched-chain fatty acids (BCFA). Data were statistically analyzed using ANOVA followed by Fisher's PLSD post hoc test.

Fecal quality was lower both with poultry meal diets ($p < 0.0001$) and with high CP levels ($p < 0.01$) whereas fecal moisture varied only with protein source ($p < 0.0001$). Compared to MI dogs, MS dogs had a higher fecal moisture and a lower fecal quality on all diets ($p < 0.0001$). Ammonia fecal concentration varied with dietary protein source ($p < 0.0001$) and level ($p < 0.0001$). Specially diet PMHP induced a significantly higher ammonia fecal concentration. MS dogs were particularly prone to have high ammonia concentrations ($p < 0.0001$). BCFA varied both with protein source ($p < 0.001$) and level ($p < 0.0001$). An effect of dog size ($p < 0.01$) was only observed on diets WGHP and WPMP.

Decreasing CP levels and introducing a source of highly digestible protein such as wheat gluten improves fecal score. Moreover it reduces protein fermentation in the hindgut. This is of interest to the overall hindgut health as ammonia production induces a faster cell turnover and modified intestinal cell's morphology and metabolism, affecting colonic absorption functions. According to our results these deleterious effects seem to be of higher importance in MS dogs.

ABSTRACT #146

BODY CONDITION SCORING AND DEXA MEASUREMENTS IN PHYSICALLY INACTIVE DOMESTIC SHORTHAIRED CATS. CR Bjornvad[1], PJ Armstrong[2], DH Nielsen[1], E Svalastoga[1], AT Kristensen[1]. [1]Department of Small Animal Clinical Sciences, The Royal Veterinary and Agricultural University, Copenhagen, Denmark. [2]College of Veterinary Medicine, University of Minnesota, Twin Cities, MN.

In cats and humans, obesity is predisposing to development of type 2 diabetes. Further, physical inactivity and indoor confinement predispose for development of type 2 diabetes mellitus in cats. The purpose of this study was to determine the percentage of body fat in indoor confined physically inactive normal and overweight domestic shorthaired cats.

Twenty-five physically inactive and indoor confined domestic shorthaired cats were recruited through the Companion Animal Veterinary Teaching Hospital, University of Copenhagen, Denmark. Informed consents were obtained from all owners. At admission, the cats underwent a thorough health examination including physical exam, body condition scoring (9-point scale), urine analysis, haematological and biochemical blood profile including T4-measurement and FIV/FeLV-testing. Two cats were excluded following the health examination. For the remaining 23 cats, body composition and whole body bone mineral density was assessed under anaesthesia using dual-energy X-ray absorptiometry (DEXA).

All 23 cats had been neutered (nine females and 14 males). Median age was 6 years (range 3–11). Following body condition scoring, the cats were divided into 4 groups BCS5 (n=5), BCS6 (n=6), BCS7 (n=7) and BCS8 (n=5). No cats had a BCS less than 5 or above 8. Females and males were equally distributed in all groups

except BCS8 that only included male cats. The cats in BCS5 were younger (3 years (range 3–5)) than cats in the other groups (7 years (range 3–11)). Percent body fat for the groups were BCS5: 31.3% (range 27.4–33.7), BCS6: 37.4% (range 32.5–40.7), BCS7: 43.6% (range 34.4–49.0) and BCS8 47.9% (range 44.1–52.2). Percent body fat was significantly different between all groups. There was no difference in Bone Mineral Density (g/cm^2) between groups or between males and females.

According to the 9 integer scale BCS system a BCS of five is considered normal weight. On the other hand, normal weight cats should have a body fat content of less than 30%. The relatively high body fat content in the normal weight cats in this study could be attributable to the sedentary lifestyle following indoor confinement. The physical inactivity may result in less lean body mass compared with kennel cats measured in earlier studies. Concurring with other studies, cats start to gain weight at three to five years of age and male neutered cats tend to become fatter than female neutered cats.

ABSTRACT #147

THE DISPOSITION AND PHARMACOKINETICS OF THE OXIDATIVE METABOLISM SUBSTRATES MIDAZOLAM, PHENYTOIN, AND THEOPHYLLINE IN GREYHOUND DOGS.

Butch KuKanich, Michelle Hubin, and Jon Nauss. Kansas State University, Department of Anatomy and Physiology, Manhattan, KS.

The purpose of the study was to assess the disposition and pharmacokinetics of midazolam, phenytoin, and theophylline in healthy Greyhound dogs. Midazolam is a CYP3A12 substrate in dogs, whereas the definitive mechanisms of metabolism for phenytoin and theophylline have not been identified in dogs. Phenytoin is a CYP2C9 and CYP2C19 substrate in humans and theophylline is primarily a CYP1A2 substrate in humans. There is evidence supporting the role of CYP1A in the metabolism of theophylline in dogs.

Six healthy Greyhound dogs, 3 male and 3 female, were used in the study which was approved by the Kansas State University Institutional Animal Care and Use Committee. On separate occasions, with at least a 7 day washout period, midazolam (0.25 mg/kg), phenytoin (11 mg/kg), and theophylline (7.88 mg/kg) administered as aminophylline, were administered IV. Blood samples were obtained at predetermined intervals for the determination of plasma drug concentrations with mass spectrometry (midazolam) or fluorescence polarization immunoassay (phenytoin, theophylline). The pharmacokinetics were estimated with noncompartmental analyses.

The half-life (t 1/2), clearance (Cl), and volume of distribution (Vd) for phenytoin, and theophylline were similar to values reported in dogs (see Table I). The Cl and Vd for midazolam appear to be different in Greyhounds. The results are suggestive that the disposition and pharmacokinetics phenytoin and theophylline are similar in Greyhound and non-Greyhound, non-Beagle dogs, but midazolam may be different. Further studies are indicated.

Table 1. Pharmacokinetic parameters of midazolam, phenytoin, and theophylline in Greyhound dogs. Pharmacokinetic parameters from previous studies in non-Greyhound dogs are included for comparison.

Parameter	*Units*	*Breed*	*Midazolam*	*Phenytoin*	*Theophylline*
Half-life	**hr**	**Greyhound**	1.2	**5**	**9.3**
		non-Greyhound	*1.3*[1]	*4.5*[2]	*8.4*[3]
Clearance	**mL/min/kg**	**Greyhound**	10.2	**2.4**	**1**
		non-Greyhound	*27*[1]	*2.8*[2]	*0.8*[3]
Volume of Distribution	**L/kg**	**Greyhound**	**1.0**	**1**	**0.75**
		non-Greyhound	*3.0*[1]	*1*[2]	*0.55*[3]

ABSTRACT #148

RONIDAZOLE PHARMACOKINETICS IN CATS AFTER IV ADMINISTRATION AND ORAL ADMINISTRATION OF AN IMMEDIATE RELEASE CAPSULE AND A COLON-TARGETED DELAYED RELEASE TABLET.

DN LeVine[1], MG Papich[1], JL Gookin[1], GS Davidson[1], JL Davis[1], WC Stagner[2], R Goldman[1], L Williamson[2]. [1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC. [2]Campbell University Pharmaceutical Sciences Institute, Buies Creek, NC.

Tritrichomonas foetus, a parasite of feline colonic mucosa, causes unrelenting diarrhea for which ronidazole (RDZ) has been identified as the only effective treatment. RDZ kills *T.foetus in vitro* (RDZ $\geq$ 0.1 µg/ml) and has eliminated *T.foetus* in cats (30–50 mg/kg PO q12h for 14 days). RDZ produces neurotoxicity in some cats and disposition of the drug in felines is unknown. The objective of this study was to characterize the pharmacokinetics of RDZ in cats after intravenous (IV) and oral administration and to assess a novel formulation for colon-targeted delivery of RDZ.

RDZ was compounded into immediate-release capsules (95 mg), with immediate-release confirmed by *in vitro* dissolution studies. An IV solution was prepared by dissolving RDZ in 5% dextrose in water (3.2 mg/ml). RDZ was administered orally (mean dose 28.2 mg/kg) and IV (~9.2 mg/kg) to 6 healthy adult cats in a crossover design with at least one-week washout between crossovers. Plasma was collected from catheters over 48 hours and analyzed for RDZ using high pressure liquid chromatography. RDZ was well-tolerated by the cats after each administration.

Following IV administration of RDZ, the terminal half-life ($t_{1/2}$) was 9.72±0.38 hrs and initial concentration (C_0) was 14.94± 2.77 µg/ml. Volume of distribution at steady state was 0.66± 0.06 L/kg and the systemic clearance was 0.80±0.07 mL/kg/min. Following oral administration, RDZ was rapidly and completely absorbed with detection in plasma of all 6 cats by 10 min after dosing and systemic availability of 99.3±17.2%. The maximum plasma concentration (C_{MAX}) of RDZ was 36.20±2.63 µg/mL, time to maximum plasma concentration (T_{MAX}) was 1.39±1.29 hr, and terminal $t_{1/2}$ was 10.44±0.83 hr. After recognizing that RDZ was rapidly absorbed from immediate-release capsules we formulated delayed release RDZ by coating tablets with guar gum. Bacterial digestion of guar gum and subsequent drug release are theoretically restricted to the colon where *T.foetus* resides. *In vitro* dissolution studies confirmed that RDZ was not released from guar gum coated tablets at either gastric or intestinal pH. These tablets were administered to four cats orally (~32.9 mg/kg) and absorption studies repeated. The delayed release formulation extended the T_{MAX} to 16 hrs and produced a C_{MAX} of 28.90±9.77 µg/ml. Drug absorption was complete (bioavailability 117.0 ± 31.8%) and corresponded to time of projected arrival of RDZ in the colon.

In conclusion, RDZ is rapidly and completely absorbed from immediate-release capsules in the proximal GI tract and persists in plasma for over 48 hrs. These attributes may predispose cats to neurotoxicity with twice-daily administration. By targeting release of RDZ to the colon, the site of *T. foetus* infection, delayed release tablets may provide improved efficacy at lower doses or less frequent intervals that reduce risk of systemic toxicity.

ABSTRACT #149

RELATIONSHIP BETWEEN MUTATIONS OF THE PANCREATIC SECRETORY TRYPSIN INHIBITOR GENE AND PANCREATITIS IN MINIATURE SCHNAUZERS AND DOGS OF OTHER BREEDS.

MA Bishop, PG Xenoulis, MD Levinski, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Anecdotal evidence suggests that Miniature Schnauzers (MS) are predisposed to pancreatitis. We have previously reported the identification of 3 mutations (2 exonic mutations: N20K, N25T and 1 intronic mutation: IVS3+26-27ins(T)33-39,15_61dup11) of the pancreatic secretory trypsin inhibitor gene (PSTI) in MS. In humans, multiple mutations of the PSTI gene have been linked to hereditary pancreatitis. Therefore, the aim of this study was to evaluate the prevalence of mutations in the PSTI gene in a population of MS and dogs of other breeds, and to determine whether the presence of these mutations is associated with pancreatitis.

A total of 4 groups of dogs were enrolled in this study: 55 MS with pancreatitis, 13 dogs of other breeds with pancreatitis, 40 healthy MS, and 17 healthy dogs of other breeds. Diagnosis of pancreatitis was based on a combination of clinical signs suggestive of pancreatitis and a serum cPLI concentration above the cut-off value for pancreatitis. A complete history and clinical findings were recorded for each dog. Serum and whole blood were obtained from each dog. DNA was extracted from whole blood and PCR was performed to amplify the 3 regions of the gene with the previously reported mutations, using specific primers. PCR products were purified and sequenced directly by automated cycle sequencing.

Sequences were compared with the published canine genome project sequence for this gene and also among the 4 groups of dogs. Proportions of dogs with mutations of the PSTI gene were compared among groups using a Fisher's exact test and odds ratios (OR) with their 95% confidence intervals (CI) were calculated.

The 2 exonic mutations were always linked and were commonly present in all 4 groups of dogs studied. The intronic mutation was found only in MS (46 of 55 MS with pancreatitis and 25 of 40 healthy MS). Additionally, this mutation was always linked to the first 2 mutations. Of the MS with pancreatitis, 35/55 (63.6%) were homozygous for the intronic mutation compared to 16/40 (40.0%) of healthy MS (OR = 2.625; p = 0.0366; 95% CI: 1.135–6.069). However, being heterozygous for the intronic mutation was not significantly associated with pancreatitis (p = 0.4765).

Results of this study suggest that this intronic mutation is unique to MS. The 2 exonic mutations were found in both MS and dogs of other breeds and probably represent single nucleotide polymorphisms (SNPs). MS that were homozygous for the intronic mutation were significantly more likely to have pancreatitis than MS that were heterozygous or did not have the mutation. Due to the fact that the intronic mutation was present in both healthy and diseased MS, we hypothesize that pancreatitis in MS is likely multifactorial. Further studies are in progress to determine the role of this mutation and other risk factors in the development of pancreatitis in MS.

ABSTRACT #150

SPECIFICITY OF CANINE PANCREAS-SPECIFIC LIPASE (SPEC CPL™) IN DOGS WITH A HISTOLOGICALLY NORMAL PANCREAS. S Carley[1], JE Robertson[2], SJ Newman[3], JM Steiner[4], D Kutchmark[2], RL Relford[2]. [1]Santa Barbara, CA. [2]IDEXX Laboratories, Westbrook, ME. [3]University of Tennessee, Knoxville, TN. [4]Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Recently, an enzyme-linked immunosorbent assay (ELISA) for the measurement of canine pancreas-specific lipase (Spec cPL) has become commercially available. The purpose of this study was to evaluate the specificity of the Spec cPL in dogs with a histologically normal pancreas.

Forty-four dogs euthanized for reasons unrelated to this study were enrolled. CBC, biochemistry panel, and Spec cPL were analyzed prior to euthanasia and the entire pancreas removed post euthanasia. The pancreata were sectioned every 1 cm and reviewed by the same pathologist (SJN) using a previously published classification system (Newman, et al. Veterinary Pathology 18:115–118, 2006). A mean cumulative score (MCS) for neutrophilic inflammation, lymphocytic inflammation, pancreatic necrosis, peripancreatic necrosis, edema, atrophy, fibrosis and nodules and disease activity index (AI) and disease chronicity index (CI) were determined for each pancreas.

Seventeen client-owned dogs from a private veterinary practice were entered into the study. Dogs were euthanized for medical reasons: trauma (6), dystocia (2), nonpancreatic neoplasia (3), osteoarthritis (1), anemia (1), diabetic ketoacidosis (1), pancreatic neoplasia (1), and pancreatitis (2). Twenty-seven dogs from an animal shelter were entered in the study. Each dog was examined and bloodwork was reviewed. One dog was diagnosed with diabetes mellitus, and one dog was underweight and anemic. The remaining 25 dogs appeared clinically healthy with 6 dogs having mild changes on bloodwork.

Dogs were classified into 2 groups for statistical comparison. One group contained dogs with a disease involving the pancreas (pancreatitis, diabetes mellitus and pancreatic neoplasia). The other group contained healthy dogs and dogs with a disease not involving the pancreas. A Student's t-test comparing the MCS for each lesion, AI and CI of the 2 groups revealed a statistical difference between the pancreata for all parameters. Population distribution data was established for the dogs that were healthy or had diseases not involving the pancreas. A pancreas with a MCS for each histologic category, AI and CI which included 90% of this population was considered normal. Based on these criteria, 31 dogs had a histologically normal pancreas. The Spec cPL was below the cut off for pancreatitis in 30 of these dogs resulting in a specificity of 96.8%.

In conclusion, the Spec cPL test is highly specific and is not elevated in dogs with a histologically normal pancreas.

ABSTRACT #151

FELINE EXOCRINE PANCREATIC INSUFFICIENCY: 15 CASES (1992–2007). K.A. Thompson[1], N.K. Parnell[1], A.E. Hohenhaus[2], G.E. Moore[1], M.P. Rondeau[3]. [1]Purdue University, Lafayette, IN. [2]The Animal Medical Center, New York, NY. [3]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Exocrine pancreatic insufficiency occurs uncommonly in cats and is poorly described in the veterinary literature. The purpose of this retrospective study is to describe a population of cats with feline exocrine pancreatic insufficiency (EPI). Databases from 1992–2007 of four referral practices were searched for cases of feline exocrine pancreatic insufficiency. Inclusion criteria included at least one clinical sign compatible with exocrine pancreatic insufficiency and a feline trypsin-like immunoreactivity (fTLI) of < 12 g/L or three consecutive day fecal proteolytic activity results < 6 mm.

Fifteen cats met the enrollment criteria and their medical records were reviewed. The median age of cases was 7 years (range: 0.3–15 years). The majority of cats were castrated males (9). The most common breed was the domestic short hair (9).

Clinical signs and physical examination findings were recorded for all 15 cats. Weight loss was the most common clinical manifestation and found in 14/15 cats (93%), followed by diarrhea/loose stools (11), vomiting (5), polyphagia (4), anorexia (4), lethargy (4), and fecal incontinence (3). Stool was described as voluminous (7), loose (6), hematochezia (4), increased frequency (4), malodorous (4), discolored (4), steathorrea (3) and greasy (3). The most common physical examination findings included thin/emaciated body condition in 9/15 cats (60%), muscle wasting (6), unthrifty hair coat (5), and thickened intestinal loops (5). Concurrent disease was present in 11 of 15 cats (73%). The most common concurrent diseases were urinary tract disease (5), enteritis (3), gastritis (2), Helicobacter infection (2), and hepatic disease (2).

The most common complete blood cell count abnormalities included normocytic, normochromic anemia 7/14 cats (50%), lymphopenia (6/13) and neutrophilic leukocytosis (4/13). The most common serum chemistry abnormalities included hyperglycemia (5/13), increased ALT (5/13) and total bilirubin (4/13). Serum cobalamin concentration was measured in 10 cats and was abnormal in all 10. The median serum cobalamin concentration was 99 ng/L (range: $<$27–176 ng/L [reference range 290–1499 ng/L]). Serum folate concentrations were increased in 4/10 cats with a median value of 21.1 µg/L (range 12.2–42.5 µg/L [reference range 9.7–21.6 µg/L]).

Fourteen of 15 cats received treatment including pancreatic enzyme supplementation (13/14), parenteral cobalamin supplementation (7/14) and metronidazole (5/14). Data regarding response to treatment was available in 12/15 cats. Of these 12 cats, 11 responded (92%).

This study shows cats with EPI exhibit many signs typical of EPI in the dog; however the majority of cats with EPI will have a concurrent disease and are likely to be cobalamin deficient.

ABSTRACT #152

RELATIONSHIP BETWEEN SERUM fPLI AND TRIGLYCERIDE CONCENTRATIONS IN CATS. JM Steiner, KM Aicher, JS Suchodolski, PG Xenoulis. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Hypertriglyceridemia has been reported as a risk factor for pancreatitis in both humans and dogs. Serum feline pancreatic lipase immunoreactivity (fPLI) concentration has been reported to be a sensitive and specific marker for pancreatitis in cats. The objective of this study was to investigate a possible relationship between serum fPLI and triglyceride concentrations in cats.

Serum samples from 279 cats were used for this study. Serum fPLI concentration (reference range: 2.9–6.0 µg/L) was measured in all serum samples by an in-house radioimmunoassay. Serum triglyceride concentration (reference range: 25–133 mg/dL) was measured using an automated serum chemistry analyzer. Possible correlation between serum fPLI and triglyceride concentrations was evaluated using a Spearman test. The median serum fPLI concentration was compared between cats with a normal serum triglyceride concentration, those with an increased serum triglyceride concentration, and also those with severe hypertriglyceridemia (> 500 mg/dL). The proportion of cats with a serum fPLI concentration above

the reference range or the cut-off value for pancreatitis (12 μg/L) was compared between cats with normal serum triglyceride concentrations and those with increased serum triglyceride concentrations.

Forty-six cats (16.5%) had an increased serum triglyceride concentration (median: 209 mg/dL; range: 134–2,147 mg/dL), but only 6 cats had severe hypertriglyceridemia. There was no significant correlation between serum fPLI and triglyceride concentrations (Spearman r = 0.1085; p-value = 0.0703). The median serum fPLI concentration was not significantly different between cats with an increased serum triglyceride concentration (68.3 μg/L) and cats with a normal serum triglyceride concentration (56.8 μg/L; p-value = 0.0557). Median serum fPLI concentration in the 6 cats with severe hypertriglyceridemia (164.6 μg/L) was not significantly higher than in cats with a normal serum triglyceride concentration (56.8 μg/L; p-value = 0.090). Also, the proportion of cats with a serum fPLI concentration above the upper limit of the reference range or the cut-off value for pancreatitis was not significantly higher in hypertriglyceridemic cats than in cats with normal serum triglyceride concentrations (p-values = 0.4368 and 0.1078, respectively).

Several cats with hypertriglyceridemia were identified in this study, but only 6 cats had a severely increased serum triglyceride concentration. In this study none of the parameters assessing the relationship of serum fPLI and triglyceride concentrations reached statistical significance. However, the median serum fPLI concentration was higher, albeit not significantly so, in cats with hypertriglyceridemia or severe hypertriglyceridemia than in cats with normal serum triglyceride concentrations. While this study did not show hypertriglyceridemia to be a risk factor for pancreatitis in cats, further studies are needed to confirm these results.

ABSTRACT #153

ESOPHAGEAL AND GASTRIC ENDOSCOPIC FOREIGN BODY REMOVAL: COMPLICATIONS AND LONG-TERM FOLLOW-UP OF 102 DOGS. P Gianella, NS Pfammatter, IA Burgener. Division of Small Animal Internal Medicine from the Vetsuisse Faculty of the University of Bern, Switzerland.

Esophageal and gastrointestinal foreign bodies (FB) are a relatively common problem in dogs. The purpose of this study was to investigate complications and long term follow up of endoscopic FB removal in a large case number.

A total of 114 FB were endoscopically removed between 03/2001 and 11/2006. Six dogs were excluded due to FB in the nose (N=4) or lung (N=2). In the remaining 108 dogs, the FB was located in the esophagus (N=60), the stomach (N=38), or both (N=10). Thoracic radiographs were diagnostic for esophageal FB in all cases, whereas abdominal radiographs were diagnostic for gastric FB in 46/48 cases. The duration of clinical signs before presentation ranged from 2 hours to 40 days (51 dogs < 1 day, 20 1–3 days, 31 > 3 days, 6 not known). Only 18 dogs were known to have ingested foreign material at the time of presentation.

West Highland White Terrier (11/108), Yorkshire Terrier (9/108) and Bernese Mountain Dogs (9/108) were overrepresented compared to the hospital population. The most frequent clinical signs reported were vomiting (38%), retching/gagging (29%), cough and dyspnea (18%), anorexia (14%), and regurgitation (10%). Bony material accounted for 47% of the FB, followed by plastic (15%), metal (10%) and greenies (7%). Six dogs with esophageal FB underwent fluoroscopy due to contrast medium in the esophagus and were excluded. In the remaining 102 cases, endoscopy alone was successful in 92 dogs (90%) and a gastrotomy, but no esophagotomy, was required in 10 dogs.

Eight dogs (7.8%) had perforations (5 esophagus, 3 stomach), most commonly associated with bony FB (6/8). Four dogs died (3.9%) due to pneumothorax and cardiac arrest (2), respiratory arrest (1), or esophageal diverticle with sudden death (1), whereas 2 dogs were euthanized with pleural effusion and pneumothorax (1) or mediastinitis (1). Two dogs had aspiration pneumonia, whereof one required thoracotomy due to a periesophageal abscess. Only 1 dog developed an esophageal stricture and underwent successful endoscopic balloon dilations. Long-term outcome was available for 75/96 patients, whereof only 4 (5.3%) showed some coughing and retching a few days after discharge. Eight dogs died due to unrelated problems and 2 had another FB later on.

In conclusion, this study further supports the use of endoscopic FB removal. Even though the overall complication rate is low, potentially life-threatening complications may develop. Clinical signs for more than 1 day prior to endoscopy is associated with increased morbidity.

ABSTRACT #154

THE EFFECTS OF BODY POSITIONING ON QUANTITATIVE ASSESSMENT OF SWALLOWING USING CONTRAST VIDEOFLUOROSCOPY IN NORMAL DOGS. Cecily Bonadio[1], Rachel Pollard[2], Paul Dayton[4], Caroline Leonard[4], Stanley Marks[3]. [1]Veterinary Medical Teaching Hospital, School of Veterinary Medicine, [2]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, [3]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis, Davis, CA. [4]Department of Biomedical Engineering, UNC-NCSU, Chapel Hill, NC.

Contrast videofluoroscopy is the imaging technique of choice for evaluating dysphagic patients because it allows for assessment of anatomic and functional components of swallowing. In people, body position significantly alters the outcome of videofluoroscopic assessment of swallowing. The purpose of this study was to determine if quantitative videofluoroscopic measures of swallowing were significantly affected by body position in dogs.

Healthy dogs (n=15) with no history of dysphagia were recruited for this study. Dogs had normal physical examinations, CBC's, and serum biochemistry panels. A restraint device was built to facilitate imaging of dogs in sternal recumbancy. Each dog underwent videofluoroscopy of swallowing of liquid barium and barium soaked kibble in sternal and lateral recumbancy. Quantitative measures of liquid and kibble swallowing (timing of swallowing, pharyngeal constriction ratio, esophageal transit time, and number of esophageal peristaltic waves) were compared between body positions. The number of radiation safety violations per second was determined.

The time to opening of the upper esophageal sphincter was significantly longer for lateral versus sternal kibble swallows (p=0.043). In lateral recumbency, 54% of liquid and 71% of kibble swallows stimulated esophageal peristalsis. In sternal recumbency, 81% of liquid and 90% of kibble swallows stimulated esophageal peristalsis. Significantly fewer radiation safety violations occurred in sternal versus lateral esophagrams (p=0.003). Other variables were not significantly different.

Body positioning affects the number of swallows that result in esophageal peristalsis. Radiation exposure to technical staff is significantly reduced for esophagrams performed in sternal recumbency.

ABSTRACT #155

SERUM MAGNESIM AND ZINC CONCENTRATIONS IN DOGS WITH INFLAMMATORY BOWEL DISEASE. KK Gingerich, NK Parnell, GE Moore. Purdue University College of Veterinary Medicine, West Lafayette, IN.

Magnesium and zinc are essential trace elements absorbed in the proximal small intestine. Reduced magnesium and zinc concentrations have been documented in human malabsorptive conditions such as Crohn's disease. Initial clinical signs of magnesium deficiency include vomiting and diarrhea, with progression to cardiac and neuromuscular abnormalities. Clinically relevant signs of zinc deficiency include acrodermatitis, poor wound healing and diarrhea. Currently, the effect of malabsorptive disease on magnesium and zinc concentrations in dogs is unknown.

The purpose of the study was to compare serum magnesium and zinc concentrations in normal dogs and dogs with inflammatory bowel disease. We hypothesized that, compared to healthy dogs, dogs with inflammatory bowel disease would have decreased serum magnesium and zinc concentrations. In a prospective clinical study, 16 client-owned dogs were evaluated for chronic gastrointestinal signs. Inflammatory bowel disease was diagnosed through histological evaluation of endoscopically obtained gastrointestinal biopsies. At the time of endoscopy, serum was collected for commercial magnesium and zinc analysis. Serum was also obtained from 16 age-(within six months) and sex-matched clinically healthy dogs. The median age of cases was 6.3 years (range:1.5–15.4 years), and the median age of controls was 6.2 years (range: 1.5–14.9 years).

In dogs with inflammatory bowel disease, median serum albumin concentrations [1.3 gm/dl (range: 0.9–3.8 gm/dl)] were significantly lower than for control dogs [3.4 gm/dl (range: 2.6–4.2 gm/dl)] ($p=0.003$). Albumin concentrations were significantly correlated with total magnesium ($r=0.636$; $p=0.008$) and zinc ($r=0.702$; $p=0.003$) concentrations in dogs with inflammatory bowel disease. There was no correlation between albumin concentrations and total magnesium ($r=-0.195$; $p=0.469$) or zinc ($r=0.139$; $p=0.608$) concentrations in control dogs. Median total magnesium concentrations for dogs with inflammatory bowel disease [1.6 mg/dl (range: 0.8–2.4 mg/dl)] were significantly lower than for control dogs [2.1 mg/dl (range: 1.8–2.5 mg/dl)] ($p<0.001$). Median zinc concentrations for dogs with inflammatory bowel disease [0.66 ppm (range: 0.25–2.12 ppm)] were significantly lower compared to control dogs [1.00 ppm (range: 0.58–1.65 ppm)] ($p=0.044$).

This study suggests that magnesium and zinc deficiency should be considered in dogs with inflammatory bowel disease and secondary hypoalbuminemia. The clinical significance of these deficiencies in dogs with inflammatory bowel disease is currently unknown and should be further evaluated.

ABSTRACT #156

FECAL N-METHYLHISTAMINE CONCENTRATIONS IN NORWEGIAN LUNDEHUNDS WITH GASTROINTESTINAL DISEASE. N Berghoff, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

It has previously been suggested that mast cells play a role in the pathogenesis of inflammatory bowel disease (IBD) and other gastroenteropathies. Mast cells release inflammatory mediators, including histamine. Histamine is metabolized by two major enzymatic pathways, one of which yields N-methylhistamine (NMH). The purpose of this study was to compare fecal NMH concentrations in a group of Norwegian Lundehunds with chronic gastroenteropathy to those in a group of healthy control dogs. Norwegian Lundehunds are known to have a high prevalence of chronic gastroenteropathies, and were thus chosen as an initial study group.

Three fecal samples each were collected from 10 healthy control dogs and 21 Norwegian Lundehunds with chronic gastrointestinal (GI) disease. Histopathology was not available, but all Lundehunds had clinical signs of GI disease and/or showed abnormal results of GI function tests, including decreased serum cobalamin, folate, total protein, and/or albumin concentrations and/or increased fecal α_1-proteinase inhibitor concentrations. Samples for NMH analysis were extracted and analyzed as previously described (Tredget, EE. Journal of Chromatography B, 1997), using stable isotope dilution gas chromatography/mass spectrometry. Data were analyzed for normality and the medians of the maximum NMH concentration for the 3-day collection period and of the 3-day average NMH concentration were compared between the two groups (Mann-Whitney U test). Statistical significance was set at $p<0.05$. Maximum and 3-day average NMH concentrations in the group of Lundehunds were also evaluated for correlation with fecal α_1-proteinase inhibitor concentrations and serum cobalamin, folate, total protein, albumin, and C-reactive protein concentrations.

The triplicate samples from control dogs had maximum NMH concentrations between 13.8 and 321.7 ng/g feces (median: 57.3 ng/g), whereas those for the Lundehund samples ranged from 85.9 to 14,300 ng/g (median: 374.4 ng/g; $p=0.0004$). Median 3-day average NMH concentrations in the Lundehunds were significantly higher (median: 284.7 ng/g; range: 66.9–9,319 ng/g) than in the control dogs (median: 44.5 ng/g; range: 8.9–196.9 ng/g; $p=0.0005$). Fecal NMH concentrations did not correlate with fecal α_1-proteinase inhibitor concentrations or serum cobalamin, folate, total protein, albumin, or C-reactive protein concentrations.

These data show that fecal NMH was significantly increased in a group of Lundehunds with chronic GI disease. A potential shortcoming of this study is the lack of histopathology in the affected Lundehunds. However, all dogs examined had clinical signs and/or abnormal GI function tests. Intestinal mast cell degranulation and histamine release may also play a role in other dogs with gastroenteropathies. Thus, further studies are needed and ongoing in order to assess fecal NMH concentrations in dogs of other breeds with gastroenteropathies.

ABSTRACT #157

CHARACTERIZATION OF THE BACTERIAL MICROFLORA AND INNATE IMMUNITY RESPONSE IN GERMAN SHEPHERD DOGS WITH ANTIBIOTIC-RESPONSIVE DIARRHEA. K Allenspach[1], JS Suchodolski[2], FM McNeill[1], A House[1], A Hendricks[1], PG Xenoulis[2], JM Steiner[2], D Werling[3]. [1]Department of Veterinary Clinical Sciences and [3]Department of Pathology and Infectious Diseases, [2]Royal Veterinary College, University of London, UK, and the Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

The interaction of receptors mediating innate immunity and the intestinal bacterial microflora is believed to play an important role in the etiology of antibiotic-responsive diarrhea (ARD) in German Shepherd Dogs (GSDs). The aim of this study was to compare Toll-like receptor (TLR) 2 and TLR4 mRNA expression in duodenal biopsies from GSDs with ARD and healthy controls and to simultaneously assess the mucosa-adherent bacterial microflora in the same dogs.

Seven GSDs with ARD and 11 healthy retired racing Greyhounds were evaluated for this study. TLR2 and TLR4 mRNA expression was assessed using quantitative real-time PCR (qRT-PCR). The microflora were evaluated by obtaining cytology brush samples during gastroduodenoscopy followed by DNA extraction and PCR amplification using universal 16S rDNA primers. Constructed clone libraries were compared between groups using the UniFrac distance metric and the RDP Classifier. Indices for bacterial diversity and species richness were calculated and compared using *t*-tests.

TLR2 and TLR4 mRNA expression was significantly higher in the GSDs compared to healthy controls (TLR2 in GSDs vs controls: $p=0.03$, TLR4 in GSDs vs controls: $p=0.01$). In addition, dogs with ARD showed a 2–4 times higher expression of TLR2 mRNA compared to TLR4 ($p=0.02$). Principal component analysis of the bacterial intestinal clone libraries revealed clustering of individual dogs within each group, indicating that the small intestinal microflora of GSDs and control dogs are composed of distinct microbial communities. The abundance of several bacterial groups was altered in GSDs compared to control dogs. There was a trend for GSDs to be enriched in sequences belonging to *Proteobacteria* ($p=0.08$). Healthy dogs were more likely to harbor members of *Bacteroidetes* (including *Bacteroides* spp. and *Prevotella* spp.) and *Clostridiaceae* ($p<0.001$).

In conclusion, TLR2 and TLR4 mRNA expression is upregulated in the duodenum of GSDs with ARD, which is accompanied by an imbalance of the intestinal microflora compared to healthy control dogs. The increased TLR mRNA expression could contribute to an abnormal immune response, resulting in the development of ARD in GSDs.

ABSTRACT #158

MAST CELL STABILIZATION WITH BETA-ADRENERGIC RECEPTOR BLOCKADE ATTENUATES DYSFUNCTIONAL CARDIAC REMODELING IN CANINE MITRAL VALVE REGURGITATION. AR Dillon[1], DM Tillson[1], J Hathcock[1], T Denney[1], C Killingsworth[2], P Betty[2], LJ Dell'Italia[2]. [1]Auburn University, Auburn, AL. [2]University of Alabama-Birmingham, Birmingham, AL.

Dogs with induced mitral valve regurgitation (MR) have been shown to lose myocardial collagen, develop mast cell infiltrates in the LV wall, undergo cardiomyocyte lengthening and use an inherent renin-angiotensin system (RAS) unaffected by systemic ACE inhibitors. The mechanism of this dysfunctional cardiac remodeling secondary to volume overload appears distinct from changes resulting from cardiogenic or pressure overload remodeling. The goal was to characterize this cardiac remodeling (structurally and molecularly) in order to compare these findings with hemodynamic studies, and the geometric changes and LV wall stress obtained through cardiac magnetic resonance imaging (c-MRI). Study groups included normal dogs ($n=14$), dogs with MR ($n=9$) without medication, dogs with MR ($n=6$) receiving a β_1-receptor blockade (β_1-RB), and dogs with MR ($n=6$) receiving β_1-RB and a mast cell stabilizer (MCS).

Mild mitral regurgitation (MR) was induced by chordae tendonae rupture. Sufficient regurgitation was achieved when dogs had 1) a

6–12% decrease in forward stroke volume (SV), 2) increased LV EDP or decreased LV ESP, 3) increased pulmonary artery wedge (Paw), and an auscultable mitral murmur. Hemodynamic parameters were collected before and immediately after MR induction and again at 4 months. C-MRI images (Picker Vista 1.0T magnet; DICOM images; 8 mm slices) were collected during the cardiac cycle before MR induction and again at 4 months.

Treatment with β_1-RB improved isolated cardiomyocyte fractional shortening and B-receptor responsiveness, but did not attenuate increases in cardiomyocyte length or LV-EDV or affect eccentric LV remodeling. Improved cardiomyocyte function did not translate to benefits in LV wall stress, load dependent LV fractional shortening, or peak LV dP/dt. Treatment with β_1-RB (with or without MCS) attenuated epicardial extracellular collagen loss but not endocardial collagen loss, by picric acid-Sirius red assay (Type I&III). Treatment with β_1-RB+MCS normalized cardiomyocyte length and restored Ca^{++} transients. LV peak +dP/dt was decreased in β_1-RB treated dogs compared to baseline but was normalized in dogs receiving β_1-RB+MCS.

All groups had increased LV mass and total SV compared to baseline. Forward SV compared to baseline was decreased in MR and β_1-RB dogs, but was preserved in β_1-RB+MSC dogs. 3-D MRI images showed an increase in LV-EDV and LV 3-D radius/wall thickness in MR and β_1-RB dogs, but these and LV-ED wall stress were decreased in β_1-RB+MCS dogs compared to MR.

These data suggest use of β_1-RB and MCS in dogs with MR induced volume overload does attenuate dysfunctional LV and cardiomyocyte remodeling and improves LV function and geometry, without attenuating the previously described interstitial collagen loss. The effect of MCS may be directly on cardiomyocytes or through preservation of cardiomyocyte-collagen scaffolding proteins affecting outside-in signaling and eccentric remodeling.

ABSTRACT #159

AUTOLOGOUS MESENCHYMAL STEM CELLS IN EXPERIMENTAL MITRAL VALVE REGURGITATION OF DOGS ATTENUATED COLLAGEN LOSS AND DYSFUNCTIONAL MYOCARDIAL REMODELING. RH Presley[1], AR Dillon[1] DM Tillson[1] Niemeyer[1], J Hathcock[1], TS Denney[2], LJ Dell'Italia[3]. [1]Auburn University College of Veterinary Medicine, Auburn, AL. [2]Auburn University College of Engineering, Auburn, AL. [3]University of Alabama at Birmingham Center for Heart Failure Research, Birmingham, AL.

In early mitral valve regurgitation (MR) of dogs, increases in left ventricular (LV) diastolic wall stress induces neurohormonal and interstitial activity, including mast cell recruitment, the dissolution of collagen in the cardiac extracellular matrix (ECM), and elongation of cardiomyocytes. In ischemic cardiomyopathy, mesenchymal stem cells (MSCs) were capable of transforming into myofibroblasts and attenuating additional myocyte loss. The purpose of this study was to determine if implantated MSCs in dogs with MR and volume overload would 1) remain at the site of the injection in the absence of inflammation, 2) alter the collagen matrix in the ECM, 3) vary the activity of interstitial mast cells, and 4) alter the mRNA LV profile. Seven intact mongrel dogs without known heart disease had MSCs isolated from bone marrow samples using an accepted plastic adherence technique and each sample cultured. The MSCs were labeled with 0.9 ím polystyrene spheres impregnated with contrast agents of iron oxide (62.4%) microparticles and a fluorescein-5 isothiocyanate analog (Dragon Green). Viability of the cells (> 70%) was determined by Trypan Blue exclusion; and fluorescein label was documented inside the MSCs.

On Day 1, MR was induced via chordal rupture using a fluoroscopic-guided catheterization method. Immediately after MR, a 1 mL aliquot containing $2.18 \pm 0.82 \times 10^7$ (range $1.0–3.5 \times 10^7$) of autologous labeled MSCs was injected into the LV free wall in a single location via LV needle catheter. On Day -7, Day 1 post-op, and Day 38, cardiac MRIs were recorded, wall stress and geometry calculated, and the MSC in LV located via the iron oxide. On Day 1 Pre and Post MR, and Day 40 hemodynamics were collected. After humane euthanasia, LV was collected from the MSC injection site, adjacent to the site, and distant from the site. Samples were evaluated for mRNA pathways, collagen density, mast cell counts, and histopathology.

In the area of MSC implantation, determined by Picric Acid Sirius Red stain, a significantly higher collagen volume percent was noted, suggesting fibroblast transformation by the MSCs. Hemodynamic decreases in forward cardiac output from baseline were significantly lower($p < 0.05$) in dogs treated with MSCs (19.7% ±7.5) than MR placebo dogs (n=4) (39.4%±9.2). Increases in SVR were also higher ($p < 0.05$) in MR placebo dogs (84.6%±53.8) than MSC treated dogs (32.9%±21.0). By cardiac MRI, on Day 1 post MR and Day 38, MSCs were noted with no loss in signal. These data suggest autologous MSCs injected into the LV of dogs with early MR remained in the myocardium, promoted stabilization of the ECM and attenuated the initial dysfunctional cardiac remodeling. Global administration of MSCs may have clinical application in MR of dogs.

ABSTRACT #160

ECHOCARDIOGRAPHIC ASSESSMENT OF LEFT VENTRICULAR AND MITRAL VALVE GEOMETRY IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY. KE Schober, A Todd. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in the domestic cat. Obstruction of the left ventricular outflow tract (LVOT) is frequently observed in HCM (referred to as HOCM) and is usually due to systolic anterior motion and mid-to-late systolic contact of the mitral valve with the interventricular septum (IVS). Altered LV geometry secondary to abnormal myocardial growth as well as intrinsic mitral valve abnormalities secondary to congenital mitral valve malformations may both favor obstruction of the LVOT but may both be associated with a different outcome and a different response to treatment. This study addresses the hypothesis that abnormalities of the LVOT and the mitral valve are common in cats with HOCM and can be detected by two-dimensional (2D) transthoracic echocardiography.

A total of 102 cats (30 normal control cats, 43 cats with HCM without obstruction, and 29 cats with HOCM) underwent a comprehensive transthoracic 2D echocardiographic examination including the assessment of chamber size, wall thickness, and anatomy of the mitral valve apparatus and the LVOT using an ultrasonographic unit (Vivid 7, GE, Milwaukee, WI) and a transducer array of 7 to 10 MHz nominal frequency. A total of 20 variables were assessed, including the size of the left atrium (2 variables), anatomy of the left ventricle (8 variables) and the LVOT (2 variables), and morphology of the mitral valve (8 variables). All cats were imaged in lateral recumbency with the transducer from underneath. Normal control cats, cats with HCM, and cats with HOCM were compared using analysis of variance on ranks for unpaired observations and the Holm-Sidak test or Dunn's test for post-hoc analyses.

Cats with HOCM had increased ($P \leq 0.05$) LV wall thickness, LV myocardial area, number of papillary muscles and papillary muscle area, and increased length of the anterior mitral valve leaflet compared to cats without obstruction. There was no difference ($P > 0.05$) between groups with regard to age, body weight, sex, IVS thickness, chordae tendinae length, mitral annulus dimension, distance of the mitral valve leaflet coaptation point to the LV free wall, inter-papillary muscle distance, position of the papillary muscles in the LV cavity, and the IVS-to-aortic root angle.

Abnormalities of LV geometry, anatomy of the papillary muscles, and length of the anterior mitral valve leaflet are common in cats with HOCM, suggesting a possible role of such abnormalities in the genesis of the obstruction. Additional imaging and post-mortem studies are needed to validate our echocardiographic findings.

ABSTRACT #161

COMPARISON OF ECHOCARDIOGRAPHIC INDICES OF MYOCARDIAL STRAIN TO INVASIVE MEASUREMENTS OF LEFT VENTRICULAR SYSTOLIC FUNCTION. NM Ponzio, JD Bonagura, KE Schober. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Strain and strain rate (SR) are recently developed echocardiographic indices of regional myocardial systolic function that are relatively unaffected by passive motion, cardiac translation, and

tethering effects. Unlike Doppler-derived strain and SR, two-dimensional (2D) techniques are angle-independent.

This prospective study tested the hypothesis that 2D strain analysis of longitudinal and radial strain and SR can predict an invasive, gold-standard measure of global left ventricular (LV) systolic function ($+dP/dt_{max}$) over a range of hemodynamic and inotropic states. Simultaneous cardiac catheterization and Doppler echocardiography were performed in seven healthy, anesthetized dogs. Measurements of 2D-derived strain variables, conventional echocardiographic indices of LV systolic function, and LV $+dP/dt_{max}$ were compared over six treatment periods that altered loading and inotropic conditions. Statistical methods included linear regression and calculation of Pearson's or Spearman's correlation coefficients.

All myocardial segments could be analyzed. Linear relationships were identified between 2D-derived strain variables and LV $+dP/dt_{max}$ with longitudinal SR and global strain demonstrating the strongest correlations ($r > 0.75$, $P < 0.0001$). Correlation of traditional echocardiographic indices of LV systolic function with LV $+dP/dt_{max}$ were lower ($r < 0.74$, $P < 0.0001$). Correlations between global longitudinal strain and traditional echocardiographic indices of LV systolic function were relatively low (all $r < 0.70$, $P < 0.0001$) except for LV ejection fraction ($r = -0.876$).

These results indicate that 2D strain and SR may represent useful noninvasive indices of LV systolic function in dogs. Further studies are needed to develop reference ranges and assess repeatability in healthy canine populations and to assess this methodology in dogs with cardiac disease.

ABSTRACT #162

PHYSIOLOGIC PERIPHERAL PULMONARY ARTERY STENOSIS IN NEONATAL CAMELIDS. BA Scansen,[1] KE Schober,[1] JD Bonagura,[1] A Varga,[1] KA Scansen[2]. [1]Department of Veterinary Clinical Sciences and [2]Nationwide Children's Hospital, The Ohio State University, Columbus, Ohio.

Heart murmurs are common in neonatal camelids and are often related to cardiac malformation. In many crias, ejection murmurs are detected in the setting of an echocardiographically-normal heart. We sought to characterize the origin for some of these innocent murmus and identified a condition that appears similar to peripheral pulmonic stenosis (PPS) of infancy. PPS is a recognized cause of innocent murmurs in children and arises from flow turbulence due to physiologic narrowing and acute angulation of the branch pulmonary arteries (PA). This narrowing resolves within the first 3–6 months of life as pulmonary vascular resistance falls.

We performed an observational study of 13 camelids (10 alpacas, 3 llamas) with a mean age of 4.8 days (range 1–14 days). Each cria was evaluated for a systolic murmur (grade I to III/VI) by Doppler echocardiography (DE). No cardiac malformations were evident. However, in each case flow acceleration and turbulence were identified in the distal main and branch PAs. The maximal velocity (mean +/− SD) at the pulmonic valve measured by pulsed-wave DE was 0.86 m/s (+/− 0.14 m/s) and flow velocity increased significantly to 1.93 m/s (+/− 0.4 m/s) at the branch PAs ($p < 0.001$; paired t-test). Mean aortic outflow velocities were 1.04 +/− 0.21 m/s in these crias.

Three cases were available for follow-up DE at a mean age of 63 +/− 9 days and revealed a maximal velocity at the pulmonic valve of 0.88 +/− 0.05 m/s and no significant difference in velocity at the branch PAs (mean 0.96 +/− 0.2 m/s; $p = 0.443$). One alpaca underwent cardiac catheterization revealing a systolic pressure gradient of 25 mmHg between the main and branch PAs on the 8th day of life with no measurable gradient during repeated catheterization at 78 days of life.

Although observational in nature, these data suggest physiologic narrowing of the branch PAs may result in a systolic murmur in immature camelids. Physiologic PPS may therefore be a clinically important differential in newborn camelids with systolic heart murmurs. As in children, this narrowing may resolve during the first months of life. Further studies are warranted to more completely characterize this flow disturbance and related morphology in camelids as well as other domesticated species.

ABSTRACT #163

THE EFFECTS OF DEXTROSE AND MANNITOL ON TNF-α PRODUCTION FROM LPS-STIMULATED FELINE PBMC. CE Haak, AE DeClue. University of Missouri College of Veterinary Medicine, Columbia, MO.

High glucose concentration and osmolality have been shown to alter tumor necrosis factor (TNF)-α production from human peripheral blood mononuclear cells (PBMC). The objective of this study was to evaluate the effect of dextrose and mannitol on lipopolysaccharide (LPS)-induced TNF-α production from feline PBMC. Blood was collected from 4 adult cats and PBMC were harvested immediately using a histopaque technique. Cellular viability was assessed using trypan blue exclusion. Cell counts were normalized to 2×10^6 cells/well in RPMI, horse serum, penicillin and streptomycin. The cells were incubated at 37° C in either culture media alone (control), dextrose 600 mg/dl (HD), dextrose 400 mg/dl (MD), mannitol 600 mg/dl (HM) or mannitol 400 mg/dl (MM) for 24 hours. Then, LPS (25 ng/ml or 50 ng/ml) was added to the media. At 48 hours, cell culture supernatant was collected and TNF-α activity quantified using a cell killing bioassay. Data were analyzed using an ANOVA and post-hoc Fisher LSD method with a p-value of < 0.05 considered significant. LPS induced significantly increased cell culture supernatant mean±SD TNF-α activity (50 ng/ml, 844±936; 25 ng/ml, 458±379; 0 ng/ml, 164±258 pg/ml; $p < 0.001$) in a dose dependent manner regardless of treatment. TNF-α activity was significantly greater in the cell culture supernatant from the PBMC incubated with HM (717±62 pg/ml) than supernatant from PBMC incubated with HD (396±62 pg/ml, $p = 0.005$), MD (382±73 pg/ml; $p = 0.007$) or control (331±214 pg/ml, $p < 0.001$). In this study, mannitol had a greater pro-inflammatory effect on LPS stimulated feline PBMC than dextrose, ex vivo.

ABSTRACT #164

MEASUREMENT OF FELINE CYTOKINE GENE EXPRESSION IN A MODEL OF FELINE RENAL TRANSPLANTATION. LR Aronson, J Stumhofer, C Hunter. University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA.

Renal transplantation is currently an accepted treatment option for cats with acute and chronic renal failure. Following successful transplantation and immunosuppression consisting of cyclosporine (CyA) and corticosteroids (Dex), the incidence of acute rejection in the cat can be as high as 26%. Recent studies in the human literature have found that cytokines produced by T cells play a critical role in transplantation immunology particularly in the area of kidney rejection.

The purpose of this study was to evaluate the effects of current immunosuppressive therapy including CyA and Dex as well as a novel immunosuppressive agent, (hu)CTLA4-Ig, on cytokine production in an in vitro feline model.

Peripheral blood (3–4 ml) was collected from 16 healthy cats and the peripheral blood mononuclear cells (PBMC) isolated by density gradient centrifugation. Peripheral blood mononuclear cells were plated in triplicate at a concentration of 2×10^6 cells/mL and stimulated with either the mitogen Con A (10 μg/ml) alone or Con A in the presence of CyA (0.05 μg/ml), Dex (1x10−7 M), a combination of CyA and Dex or (hu)CTLA4-Ig (10 μg/ml). An ELISA was performed from the supernatant for INFγ, GM-CSF, IL-4 and IL-10. Pairwise comparisons were performed using the Wilcoxan signrank test with significance set at $p < 0.05$.

Compared to mitogen alone, CyA, Dex, the combination of CyA and Dex and (hu)CTLA4-Ig all caused a significant decrease in INF-γ and GM-CSF production. CyA and the combination of CyA and Dex caused a significant decrease in IL10 production. No significant difference in IL4 production in the presence of any drug compared to stimulus alone was identified.

The percutaneous needle biopsy remains the gold standard for diagnosing acute rejection in both human and veterinary patients, but it is an invasive procedure and complications can occur. In human transplantation, the evaluation of cytokine production from blood or urine can be useful in detecting acute rejection at a stage sufficiently early to allow therapeutic intervention. Results from this study may be useful in developing a similar noninvasive test that would be invaluable for the evaluation of the feline renal transplant patient.

Originally presented at the ACVS Symposium, Chicago, IL, October 2007.

ABSTRACT #165
EVALUATION OF SMART PILL CAPSULE FOR ASSESSMENT OF GASTRIC EMPTYING TIME, AND SMALL BOWEL, COLONIC, AND WHOLE GUT TRANSIT TIMES IN DOGS. Christopher Mole, Frédéric Gaschen, Lorrie Gaschen. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

The SmartPill pH.p capsule is equipped with pH, pressure, and temperature sensors, and transmits information to a receiver placed on the patient's back. Gastric emptying time (GET), intestinal transit time including small (SBTT) and large bowel transit times (LBTT), as well as total transit time are calculated from these data. The goal of this study was to evaluate the performance of the SmartPill pH.p capsule for assessment of changes in canine GI motility.

Six healthy dogs were used. After a baseline was determined, modification of the transit times was attempted with administration of prokinetics (metoclopramide and cisapride), acepromazine, and change to a high fat and a canned diet. Additionally, GET was assessed using ultrasound in parallel to SmartPill pH.p. Results were analyzed using the Wilcoxon matched pairs test for difference in medians or linear regression as appropriate.

Baseline times are shown as median and range: GET 476 min. (400–875), SBTT 203 min. (171–374), and LBTT 1882 min. (1396–2558). SmartPill pH.p was able to detect significant prolongation of GET following administration of acepromazine (702 min., 620–925) ($p < 0.02$), consumption of a high fat diet (900 min., 440–1130) ($p < 0.05$) and consumption of a canned diet (795 min., 615–1049) ($p < 0.05$). SBTT was significantly shortened in dogs receiving cisapride (138 min., 83–263) ($p < 0.05$). Finally, GET as measured with SmartPill correlated ($r = 0.70$) with t50% as measured with ultrasound.

SmartPill pH.p is able to detect changes in gastric and intestinal transit times due to various medications, and the method correlates with ultrasound evaluation of GET in dogs.

ABSTRACT #166
THE EVALUATION OF THE WIRELESS CAPSULE (SMARTPILL™) FOR MEASURING GASTRIC EMPTYING AND GI TRANSIT IN NORMAL DOGS. F. Andrews[1], R. Denovo[1], R. Reese[1], S. Elliott[1], T. Moyers[1], D. Barthel[2], M. Lyman[2], G. Daniel[1]. [1]University of Tennessee, College of Veterinary Medicine, Knoxville, TN and [2]SmartPill Corporation, Buffalo, NY.

Gastric emptying scintigraphy (GES) is used to measure gastric emptying in dogs, but the technique is non-ambulatory and requires ingestion of a radioactive meal. Also, GES does not provide information regarding small and large bowel transit time (SLBTT) and total bowel transit time (TTT). Recently, wireless ambulatory capsule technology, the SmartPill™ GI Monitoring System (SP) was introduced to measure gastrointestinal (GI) pH, pressure and temperature, and provide GE and transit time in humans. The purpose of this study was to compare GES to GE measured by the SP and to evaluate the SP for measurement of SLBTT, and TTT in normal healthy dogs.

Six healthy adult (age: 5 to 8 yrs) dogs (4 F and 2 MC), weighing 26.4 ± 1.7 kg were used in the study. Two weeks prior to starting the study, dogs were acclimated to a standard canned food diet (Beef and Chicken Entrée, Science Diet). To assess GE, SLBTT and TTT, food was withheld at 8:00 PM the evening prior to performing the study. On the morning of the study, each dog consumed the standard meal mixed with 99m TcDisofenn (6 mCi). Once the meal was consumed, the SP was administered orally and an additional small meal of the standard diet (55 gm) was given to facilitate passage of the SP into the stomach. Dogs were place in a cage and the SP wireless data receiver mounted on the cage. Gastrointestinal pH was collected every 5 seconds for 24 hours, along with luminal pressure and temperature until the pill was passed in the feces. After administration of the SP, GES was performed in a standard manner for 4 hours. GE measured by the SP was defined as the time from ingestion to a sudden rise in $pH > 4$ of at least 3 pH units from baseline. The TTT was measured from the time of ingestion of the wireless capsule until there was a drop in temperature or abrupt loss in signal associated with a bowel movement. By subtracting GE time from TTT we calculated SLBTT. Data was presented as mean (SEM). GE measured by SP and GES were analyzed using PROC CORR in SAS.

Over 90% of the data packets were received from the SP during the study period. The pill was recovered from each dog in the study. Mean (SEM) for GE with the SP was 6.34 h (0.94) and GES($T_{10\%}$) was 4.0 h (0.4). There was a strong correlation ($CC = 0.7587$; $P = 0.08$). Mean SLBTT was 31.74 h (7.40) and mean TTT was 38.35 h (7.78).

The SP represents a novel non-scintigraphic method for assessing GE in dogs and correlates well with GES. The GE measured by the SP suggests that it empties near the end of emptying of a solid meal. Also, the SP provides information regarding SLBTT and TTT. The SmartPill™ represents a novel, ambulatory, non-radioactive method to measure GE, SLBTT, and TTT and may be helpful in determining delayed GE and alterations in bowel motility.

ABSTRACT #167
INITIAL EXPERIENCE WITH ENDOSCOPIC RETROGRADE CHOLANGIOGRAPHY AND BILIARY STENT PLACEMENT IN NORMAL DOGS. A Berent[1], C Weisse[1], M Kochman[2]. [1]Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA.[2]Hospital of the University of Pennsylvania, Philadelphia, PA.

Endoscopic retrograde cholangiography (ERC) is a minimally invasive imaging modality used in humans for the diagnosis and treatment of extrahepatic bile duct obstruction (EBDO). Traditional therapy in veterinary patients has been surgical. The purpose of the present study was to describe a minimally invasive technique using ERC and endoscopic biliary stenting of the common bile duct (CBD) in normal dogs.

Six purpose-bred dogs with normal biliary tracts had duodenoscopy performed using a side-viewing duodenoscope. Under endoscopic guidance a sphincterotome was used to cannulate the CBD and pancreatic duct (PD) and contrast was used to opacify the duct using fluoroscopy. A guidewire was advanced through the catheter and up the CBD. A 5 or 7 French polyurethane stent was advanced over the guidewire into the CBD and patency was documented.

ERC was successful in 5/6 dogs ranging in weight from 10.5 to 26 kg. Biliary stenting was possible in 4/6 dogs. No major complications occurred during the procedure. Two minor complications included the submucosal injection of contrast at the major duodenal papilla making canulation and stent placement impossible (1) or difficult (1). No dog had evidence of GI or CBD perforation. All stents were able to be removed endoscopically with traction after placement.

ERC and endoscopic biliary stenting is possible in normal dogs but difficult for those not trained in these techniques. Further investigation of this minimally invasive technique for the relief of EBDO as a future alternative to surgery is recommended.

ABSTRACT #168
EVIDENCE OF INSULIN RESISTANCE IN HEALTHY MINIATURE SCHNAUZERS WITH IDIOPATHIC HYPERTRIGLYCERIDEMIA. PG Xenoulis,[1] Y Mitsuhashi,[2] JE Bauer,[2] MD Levinski,[1] JS Suchodolski,[1] and JM Steiner[1]. [1]Gastrointestinal Laboratory, and [2]Companion Animal Nutrition Laboratory, Texas A&M University, College Station, TX.

Miniature Schnauzers have been reported to have a high prevalence of idiopathic hypertriglyceridemia, which has been suspected to be familial in this breed. In humans, familial forms of hypertriglyceridemia have been associated with insulin resistance. The effects of familial hypertriglyceridemia on insulin function have not yet been studied in dogs. Thus, the aim of this study was to determine whether idiopathic hypertriglyceridemia in Miniature Schnauzers is associated with insulin resistance as determined by the homeostasis model assessment (HOMA).

Blood samples from 18 healthy Miniature Schnauzers with idiopathic hypertriglyceridemia and 23 healthy Miniature Schnauzers with normal serum triglyceride concentrations were used in this study. Samples were collected after food had been withheld for at least 12 hours. All dogs were free of clinical signs of any disease for at least 3 months prior to blood collection, had no history of a chronic disease, and were not receiving any medications that are known to affect lipid metabolism. The effect of lipemia was evaluated by comparison of each parameter before and after centrifugation of lipemic serum samples. Mean age and body weight

were calculated and compared between groups. Serum insulin and glucose concentrations were measured and the HOMA index (HOMA index = fasting insulin (mU/L) X fasting glucose (mmol/L)/22.5) was calculated, and medians were compared between groups using a Mann-Whitney U test. Proportions of dogs with serum insulin concentrations above the reference range were compared between groups using a Fisher's exact test, and odds ratios (OR) with 95% confidence intervals (CI) were calculated. Non-esterified fatty acid (NEFA) concentrations, which might be increased in patients with insulin resistance, were also measured in 14 control and 12 hypertriglyceridemic dogs.

The effect of lipemia on insulin concentrations was not significant ($p=0.16$). There was no significant age ($p=0.14$) or body weight ($p=0.078$) difference between the 2 groups. Median serum insulin concentration was significantly higher in hypertriglyceridemic Miniature Schnauzers (28.4 mU/L) than in controls (12.2 mU/L; $p<0.001$). Also, the proportion of dogs with serum insulin concentrations above the reference range was significantly higher in hypertriglyceridemic Miniature Schnauzers (44.4%) than in controls (13.0%; $p=0.036$; OR=5.3; 95% CI=1.2–24.6). The median HOMA index score was significantly higher in hypertriglyceridemic Miniature Schnauzers (5.9) than in controls (2.9; $p=0.003$). There was no significant difference in mean serum concentrations of NEFA between hypertriglyceridemic dogs and controls ($p=0.528$).

The results of the present study suggest that idiopathic hypertriglyceridemia in Miniature Schnauzers is associated with insulin resistance. Further studies are in progress to determine the prevalence and significance of insulin resistance in hypertriglyceridemic Miniature Schnauzers.

ABSTRACT #169

APPLICATION OF THE ^{13}C-GALACTOSE BREATH TEST FOR ASSESSMENT OF CANINE LIVER FUNCTION. Silva S,[1] Wyse C,[2] Goodfellow M,[1] Yam P,[3] Preston T,[4] Papasouliotis K,[1] and Hall E[1]. [1]Department of Clinical Veterinary Science, University of Bristol, Bristol, UK. [2]Department of Anatomy, University of Bristol, Bristol, UK. [3]Division of Companion Animal Studies, Institute of Comparative Medicine, University of Glasgow, Glasgow, UK. [4]Scottish Universities Environmental Research Centre, Glasgow, UK.

The ^{13}C-galactose breath test (^{13}C-GBT) has been validated for non-invasive, quantitative assessment of liver metabolic function in human medicine, but the use of this test in veterinary medicine has not been investigated.

The aim of this study was to evaluate the application of the ^{13}C-GBT for assessment of canine liver function through evaluation of a group of healthy dogs (n = 23) and a group of dogs with liver dysfunction (n=16). All dogs in the liver disease group displayed clinical signs of, and results of clinical investigations consistent with, hepatic dysfunction. Furthermore these individuals were subsequently diagnosed with parenchymal liver disease (n = 8) or primary hepatic vascular abnormalities (n = 8) on the basis of hepatic biopsy histopathology and diagnostic imaging.

After withholding food for 12 hours, each dog ingested a test meal consisting ^{13}C-galactose (5 mg/kg) and unlabelled galactose (25 g/m^2) dissolved in skimmed milk. Exhaled breath samples were collected using a face mask, 20 minutes before ingestion of the test meal and then at regular intervals thereafter for 6 hours; samples were stored at room temperature. No adverse effects were observed either during or after the collection period. The proportion of $^{13}CO_2/^{12}CO_2$ in the exhaled breath samples was measured by isotope ratio mass spectrometry. Non-linear regression analysis was used to calculate two mathematical indices ($t_{1/2}$ and t_{max}) to describe the rate of recovery of $^{13}CO_2$ in breath.

There was no significant difference in values of t_{max} and $t_{1/2}$ in the diseased group compared to the healthy controls, but there was considerable inter-subject variation in both groups. This variation may be due to differences in the rate of gastric emptying among animals, which could preclude detection of alterations in hepatic metabolism of galactose. The results of this study do not support the application of the ^{13}C-GBT for assessment of canine liver function, although the application of this test following intravenous substrate administration may warrant further investigation.

This study was funded by a Comparative Gastroenterology Society research grant.

ABSTRACT #170

MUSCLE ENZYME CONCENTRATIONS IN ENDURANCE HORSES ELIMINATED DUE TO LAMENESS. JK Kingston[1], A Barnes[2], S Beetson[2], C Kuiper[3]. [1]School of Veterinary Science, University of Queensland, Brisbane, Australia. [2]School of Veterinary and Biomedical Sciences, Murdoch University, Perth Australia. [3]Kentucky Equine Research, Brighton, Victoria, Australia.

Lameness is the most common reason for elimination of horses from endurance competitions. Due to the nature of these competitions, the causes of lameness are not quantified or determined. Exercise associated increases in serum muscle enzyme concentrations are commonly reported in endurance horses during competition. It is unclear what role, if any, muscle damage may play in lameness in endurance horses. The aim of the present study was to investigate changes in muscle enzyme concentrations in horses eliminated for lameness from a 160 km endurance ride.

Horses in the study were competitors in Tom Quilty Gold Cup National Championship 160 km ride held in Western Australia in September 2007. Blood samples were collected before the ride and at the completion of the ride (either after the final veterinary examination at 160 km or after the horse was eliminated at one of the during-ride veterinary check points). Veterinary examinations were performed on horses before, during, and at the finish of the ride as per standard endurance ride protocol.

Of the 48 horses participating in the study, 18 (38%) completed the ride, 16 (33%) were eliminated for lameness, 11 (23%) were eliminated for metabolic concerns (insufficient recovery to continue) and 3 (6%) were withdrawn at the riders' discretion. Mean speed of the finishers was 8.7 ± 0.6 km/hr. There was no clinical evidence of muscle damage or cramping in any of the pre-ride or post-ride veterinary examinations. All horses except for one had creatine kinase (CK) and aspartate aminotransferase (AST) concentrations within normal reference range in pre-ride blood samples. Seven (15%) of the 48 horses had post-ride CK activities greater than 10,000 iu/l, of these 4 were eliminated due to lameness, 1 was eliminated for metabolic concerns and 2 successfully finished the ride. The CK and AST concentrations for finishers, lameness eliminations and metabolic eliminations were 3722 ± 6886 and 1028 ± 1040 iu/l; 11926 ± 24788 and 1272 ± 1259 iu/l; and 2288 ± 2296 and 735 ± 796 iu/l, respectively. Although there was no significant difference in CK and AST activity at the end of the ride between finishers and non-finishers, 2 horses eliminated for lameness had the greatest increases in CK and AST activity.

Similar to the results of previous studies, the majority of horses eliminated from endurance competition had clinical signs of lameness. While a number of the lame horses also showed significant increases in muscle enzyme activities, a direct association between lameness and muscle enzyme activity could not be demonstrated. Despite being the major cause of elimination from endurance competition, there is limited information on causes of lameness during endurance events. Further studies exploring reasons for lameness in endurance competitions are needed.

ABSTRACT #171

LAMINAR OXIDANT STRESS AND ANTIOXIDANT GENE EXPRESSION IN THE BLACK WALNUT EXTRACT MODEL OF EQUINE LAMINITIS. TL Westerman, A Pettigrew, JK Belknap. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Due to the central role of oxidant stress in organ injury in human sepsis, we investigated both the presence of laminar oxidative injury, and the protective "antioxidant response" of the laminar tissue in the black walnut extract (BWE) model of equine laminitis. Laminar oxidative injury was assessed in the forms of lipid peroxidation and protein carbonylation. Lipid peroxidation of tissue was evaluated via 4-hydroxy-2-nonenal (4-HNE) immunochemistry (slot blot), whereas protein carbonylation was assessed via ELISA. Laminar tissue antioxidant response was assessed by analyzing mRNA concentrations of critical isoforms of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx). Archived snap frozen tissue samples (laminae, skin, lung and liver) were used from the BWE model at two time points post-BWE administration (3H [onset of leucopenia], n = 5, and 12H [onset of lameness], n = 5); these samples were com-

pared to control group samples (3HC [3H post water administration], n = 5, and 12HC [12H post water administration], n = 5). Real time quantitative PCR (RT-qPCR) was performed to assess mRNA concentrations of SOD isoforms (SOD 1-3), cytosolic glutathione peroxidase (cGPx) and phospholipid hydroperoxide GPx (phGPx, important in protection from membrane lipid peroxidation). Significant increases in 4-HNE concentrations were evident in laminar tissue at both 3H and 12H time points post-BWE (compared to control groups), whereas no increase in 4-HNE was present in the lung, liver or skin samples. No changes in laminar protein carbonylation were present between principal and control groups. Marked increases in the expression of laminar SOD-2 (mitochondrial SOD) was present at both the 3H and 12H time points ($p<0.05$). Laminar cGPX mRNA concentration did not change significantly, but laminar phGPx mRNA concentration increased at the 12H time point ($p<0.05$). Increased laminar 4-HNE concentration with no increase in protein carbonylation possibly indicate acute oxidant stress in the form of lipid peroxidation to the laminar cells, but not the end stage oxidative injury characterized by protein carbonylation. phGPx, an enzyme which blocks membrane lipid peroxidation, undergoes slow induction in the laminae. This late induction in the presence of a rapid onset of oxidant stress may contribute to the marked lipid peroxidation in affected laminae. The increased laminar SOD-2 (mitochondrial SOD) mRNA concentrations indicate a similar pattern of SOD-2 expression as previously reported in organ injury in rodent models of sepsis and human sepsis patients. Increased SOD-2 mRNA in light of a recently reported lack of laminar SOD activity at the 3H time point in the BWE model suggests either a post-transcriptional inhibition, or possibly a delayed increase in laminar SOD concentration and activity. These results indicate that oxidant stress may contribute to the development of acute laminitis and should be further studied to determine the potential of oxidant stress as a therapeutic target in equine laminitis.

ABSTRACT #172
THE POLYSACCHARIDE STORAGE MYOPATHY PHENOTYPE IN QUARTER HORSE-RELATED BREEDS IS MODIFIED BY AN *RYR1* MUTATION. ME McCue, SJ Valberg, M Jackson, M Lucio, L Borgia and JR Mickelson. University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

A dominant mutation in the *GYS1* gene causes one form of Polysaccharide Storage Myopathy (PSSM) in Quarter Horses. Horses with the *GYS1* mutation have a variable clinical phenotype ranging from subclinical disease to exertional rhabdomyolysis, to acute recumbency and death. Variable phenotypic expression may be attributed to environmental factors such as diet and exercise as well as modifying genes. We have identified one family of *GYS1* PSSM horses with a severe and occasionally fatal PSSM phenotype. The purpose of this study was to determine if a modifying gene(s) contributes to phenotypic variability of severely affected PSSM Quarter Horses and related breeds.

A severely affected *GYS1* PSSM family was selected for study from 3 families used in a whole genome association study to identify the *GYS1* mutation. The genotypes from these families and additional *GYS1* PSSM horses were re-evaluated after identification of the *GYS1* mutation. Our goal was to identify loci that were highly associated with the PSSM phenotype in the severely affected family, but not associated with the PSSM phenotype in the other 2 PSSM families. A microsatellite marker was found on ECA10 that met this criteria ($p = 0.000072$ severely affected family; $p = 0.3$ horses outside this family). The microsatellite marker was approximately 2 Mb from the *RYR1* gene leading to the hypothesis that a mutation in *RYR1*, previously reported to cause malignant hyperthermia (MH) in Quarter Horses, was modifying the *GYS1* phenotype. Horses in the severely affected family, 203 additional Quarter Horses heterozygous for the *GYS1* mutation and hair root samples from 330 randomly selected Quarter Horses were genotyped for the *RYR1* mutation by restriction fragment length polymorphism assay. Of the 44 horses with the *GYS1* mutation within the severely affected family, 26 had the MH mutation (59%). In contrast, the prevalence of the MH mutation in horses heterozygous for the *GYS1* mutation outside the family was 1.5% and in randomly selected horses 0.6%. From this data we hypothesized that the MH mutation in combination with the *GYS1* mutation was responsible for a more severe phenotype. To test this hypothesis, serum creatine kinase (CK) data were evaluated from 9 horses with the *GYS1* mutation, 4 of which also had the MH mutation. CK activity was determined 4 h after exercise for 15 days while horses consumed a high starch diet. Mean CK activity was significantly higher (t test $p<0.0001$) in horses with both MH and *GYS1* mutations (3591 ± 720 U/L compared to 1584 ± 220.5 U/L).

In conclusion, although the phenotype of horses with the *GYS1* mutation is influenced by diet and exercise, it may also be modified by the presence of the *RYR1* MH mutation. Therefore, in Quarter Horses and related breeds it may be prudent to test for both these mutations.

ABSTRACT #173
PRESENCE OF THE *GYS1* MUTATION IN DIVERSE BREEDS OF HORSES WITH POLYSACCHARIDE STORAGE MYOPATHY. ME McCue, SJ Valberg, M Jackson, M Lucio and JR Mickelson. University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

Polysaccharide Storage Myopathy (PSSM) is a common cause of neuromuscular disease in Quarter Horse related breeds, Draft and Warmblood horses when diagnosed by the presence of abnormal, amylase-resistant polysaccharide in skeletal muscle specimens. Using less stringent diagnostic criteria of increased amylase-sensitive glycogen, PSSM has also been diagnosed in a variety of other light breeds. Recently, a genome wide association study was used to identify a dominant mutation in the *GYS1* gene of Quarter Horses with PSSM. This *GYS1* mutation was present in 80% of PSSM Quarter Horses that were evaluated in the genome wide association study. In 20% of PSSM Quarter Horses in that study, PSSM was not linked to the *GYS1* gene, suggesting that there may be a separate, non-*GYS1* (type 2) form of PSSM.

The first objective of this study was to determine the prevalence of the *GYS1* mutation in PSSM horses (biopsy diagnosis) from diverse breeds. The second objective was to determine if the prevalence of the *GYS1* mutation differed between horses diagnosed with PSSM based on grade 1 (amylase-sensitive) or grade 2 (amylase-resistant) polysaccharide. Nine hundred and one PSSM horses from 36 different breeds, as well as horses of mixed breed origin, were identified from the Neuromuscular Disease Laboratory database: 831 cases had whole blood or tissue that was available for DNA isolation and genotyping for the *GYS1* mutation. Cases were genotyped using a restriction fragment polymorphism assay.

The PSSM mutation was identified in horses from 17 different breeds including Quarter Horses, Paints, Appaloosas, 5 Draft horse breeds, Haflingers, 3 Warmblood breeds, Morgans, Mustangs, Rocky Mountain Horses and Tennessee Walking Horses, as well as mixed breed horses. The prevalence of the *GYS1* mutation in PSSM horses was high in Draft (87%) and Quarter Horse related breeds (72%) and lower in Warmbloods and other light horse breeds, based on grade 2 diagnostic criteria. Overall, the PSSM mutation was present in 16% of grade 1 and 70% of grade 2 PSSM cases.

In conclusion, the *GYS1* mutation causes PSSM in at least 17 different horse breeds and is the predominant form of PSSM in Draft-related and Quarter Horse-related breeds. Muscle biopsies from horses with the *GYS1* mutation are most often characterized by the presence of abnormal, amylase-resistant polysaccharide inclusions. False positive diagnosis, as well as the possibility of a second glycogenosis in horses with neuromuscular disease (type 2 PSSM), may explain the absence of the *GYS1* mutation in horses diagnosed with excessive glycogen accumulation in muscle.

ABSTRACT #174
DOSE-DEPENDENT EFFECT OF CAFFEINE ON INTRACELLULAR CALCIUM CONCENTRATION OF CULTURED SKELETAL MYOTUBES DERIVED FROM EQUINE SKIN. Fernandez-Fuente M[1], Terracciano CMN[2], Piercy RJ[1,3]. [1]Comparative Neuromuscular Diseases Laboratory, Department of Veterinary Clinical Sciences, Royal Veterinary College, UK. [2]Harefield Heart Science Centre, Imperial College London, UK. [3]Dubowitz Neuromuscular Centre, Imperial College London, UK.

Muscle from Thoroughbreds with the genetic disorder Recurrent Exertional Rhabdomyolysis (RER) displays altered sensitivity to caffeine in comparison with muscle from normal horses, suggesting

that affected animals may have an inherited defect in the regulation of skeletal muscle intracellular calcium. At present, definitive diagnosis of the disorder requires an *in vitro* muscle contraction test that is not widely available to practitioners. With a view to investigating alternative and less invasive diagnostic methods, we hypothesised that fibroblasts derived from equine skin biopsy samples, when converted to muscle cells through forced expression of an equine muscle-specific transcription factor (MyoD), would display a dose-dependent increase in intracellular calcium concentration in response to caffeine.

Fibroblasts cultured from 4 mm punch skin biopsy samples, aseptically obtained from ponies, were subsequently transduced with a lentivirus (pCMV-eqMyoD-IRES-EGFP) that would force the co-expression of equine MyoD and green fluorescent protein. Unlike untransduced cells, virus treated cells were transformed to myotubes as verified by the expression of muscle-specific proteins over 7–15 days by immunocytochemistry and western blot. Myotubes were subsequently loaded with Indo-1 (10 ug/ml) for 30 minutes and intracellular calcium responses to increasing doses of caffeine (0–10 mM) in the bathing solution (37 °C) were measured by fluorescence. Caffeine was washed out between each test dose.

A dose-dependent and reproducible effect of caffeine on intracellular calcium concentration was detected. Specifically, a rise in intracellular calcium concentration was first detected with 1 mM caffeine and a maximum response was detected with 10 mM caffeine. On washout, intracellular calcium concentration returned to baseline.

These preliminary findings suggest that observation of responses of cultured skin cells that have been converted to muscle cells through forced expression of MyoD, may provide a less invasive method than muscle biopsy for the study of muscle intracellular calcium regulation and the pathophysiology of RER. Further study is required to determine whether skin-derived myotubes can be used in a practical diagnostic assay for RER in Thoroughbreds.

ABSTRACT #175
EFFECTS OF PRETREATMENT WITH DEXAMETHASONE OR LEVOTHYROXINE SODIUM ON ENDOTOXİN-INDUCED INSULIN RESISTANCE İN HORSES. F. Tóth[1], N Frank[1], R Geor[2], SB Elliott[1], RC Boston[3]. [1]University of Tennessee College of Veterinary Medicine, Knoxville, TN. [2]Middleburg Agricultural Research and Extension Center, Virginia Polytechnic and State University, Middleburg, VA. [3]University of Pennsylvania, Kennett Square, PA.

Endotoxemia has been associated with laminitis, and transient insulin resistance (IR) develops after administration of exogenous lipopolysaccharide (LPS) to horses. We hypothesized that resting insulin sensitivity would affect the magnitude of IR induced by LPS. Horses were pretreated with dexamethasone (20 mg/day PO) to induce IR or levothyroxine sodium (LT4; 48 mg/day PO) to increase insulin sensitivity. Twenty adult mares were randomly assigned to control (no pretreatment; n = 8), dexamethasone (n = 4), and LT4 (n = 8) groups. After the 14-day pretreatment period, horses were challenged by intravenous administration of 20 ng/kg body weight *Escherichia coli* O55:B5 LPS. Frequently-sampled intravenous glucose tolerance test procedures were performed at −14 days, −3 h, and 20 h relative to LPS administration. Areas under the plasma glucose (AUCg) and serum insulin (AUCi) curves were calculated.

Significant treatment × time effects were detected for AUCg ($P = 0.018$) and AUCi ($P < 0.001$) for the 14-day pretreatment period. Treatment with dexamethasone for 14 days significantly ($P < 0.001$) increased pre-LPS mean AUCg and mean AUCi values by 24% and 364%, respectively, suggesting a significant decrease in insulin sensitivity over time. Furthermore, pretreatment with dexamethasone exacerbated IR induced by LPS. Mean AUCg and AUCi values did not change significantly over 14 days in the LT4 group and this drug prevented LPS-induced IR. Results suggest that horses already suffering from IR are likely to show greater disturbances in insulin sensitivity when endotoxemia develops, and LT4 pretreatment ameliorates these responses to LPS.

ABSTRACT #176
ADMISSION AND SEQUENTIALLY MEASURED PLASMA LACTATE CONCENTRATIONS AS PROGNOSTIC INDICATORS IN ADULT EQUINE EMERGENCIES. BS Tennent-Brown, RC Boston, PA Wilkins. University of Pennsylvania (New Bolton Center), Kennett Square, PA.

Blood lactate concentration [LAC] may be a useful indicator of disease severity and outcome in equine patients. We hypothesized that prognosis for survival decreases in the face of persistently increased [LAC].

Adult emergency admissions (excepting ophthalmologic, superficial wound, and septic synovitis) were included. Admission [LAC] was measured on a commercial blood gas analyzer. Subsequent [LAC] were determined at 6, 12, 24, 48, and 72 hours after admission with a lactate monitor validated for use in the horse. Animals euthanized for financial reasons were not included. Logistic regression was used to evaluate risk, as captured in the odds ratio of death with increased [LAC].

Two hundred forty-nine horses were enrolled. All 6 samples were obtained in 108 cases. Overall survival was ∼82%. All colic patients treated medically survived, survival in surgically-managed colic patients was ∼83%. All gastrointestinal ruptures were non-survivors. Overall, risk of non-survival was significantly ($P \leq 0.05$) increased 7.3-fold for each mmol/L increase in [LAC] at admission, ∼4-fold at 12 hours, ∼5-fold at 24 hours, ∼4 fold at 48 hours and ∼50-fold at 72 hours. A diagnosis of 'Small Intestinal Strangulating' colic had an ∼37-fold increased risk of death for each 1 mmol/l increase in [LAC] at admission, followed by 'Colitis' (∼20-fold), 'Other'' (∼19-fold), and 'Reproductive' (∼11-fold).

Increased [LAC] appears to have utility as a prognostic indicator at admission and subsequent time periods. Further evaluation of its use, particularly by specific diagnosis category, and utilizing survival analysis techniques, is forthcoming.