effectively treating the condition for which it was prescribed. Cyclosporine is a commonly used immunosuppressive agent in dogs, and acts by inhibiting T-cell function, specifically by decreasing production of the cytokines IL-2 and IFN-. We are developing a quantitative reverse transcriptase PCR-based test measuring activated T-cell production of these cytokines with the goal of being able to accurately measure the degree of immunosuppression in dogs treated with drugs such as cyclosporine. Our long-term goal is to develop a practical cytokine assay that can be performed on samples submitted by veterinarians. The specific objective of this current study was to assess the effect of sample storage conditions on our cytokine assays. Heparinized whole blood collected from 3 healthy adult hound dogs was stored for 0, 12, 24, and 48 hours at both room temperature and 4°C. After storage, T-cells were activated with a combination of PMA and ionomycin. RNA was collected from the samples using a commercial kit designed specifically for isolation from whole blood. Cytokine levels were then measured using qRT-PCR. RNA transcripts of IL-2, IFN- , and GAPDH (reference gene) were reverse-transcribed to their complementary DNA strands, which were then amplified via PCR. A sufficient amount of RNA was retrieved at all time points from both room temperature and refrigerated samples, and amplification of the three gene products was successful. Our study demonstrates that our qRT-PCR-based cytokine assays will work on samples submitted under standard veterinary practice handling conditions.

42

SERUM CONCENTRATIONS OF ACUTE PHASE PROTEINS IN HOOKWORM (*ANCYLOSTOMA* SP.) INFECTIONS IN DOGS. **E.M.S. Schmidt[1], A.F.M. Lima[1], G.J. Santos[1], C.P. Rubio[1], T.F.M. Santos[1], P.C. Silva[2], J.J. Fagliari[2].** [1]School of Veterinary Medicine and Animal Science and [2]School of Agrarian and Veterinary Sciences, Sao Paulo State University, Botucatu, SP, Brazil.

Hookworms are parasitic nematodes that cause anemia and intestinal infections in dogs, especially with large worm burdens. They are the main cause of cutaneous larva migrans and also eosinophilic pneumonitis in humans. Acute phase proteins are sensitive markers of inflammation. In this study 80 asymptomatic dogs (age: 8 months-2 years) were evaluated to detect the presence of the hookworm thin-shelled, morulated eggs in feces using fecal flotation. They were divided in two groups: 40 dogs with a high quantity of eggs in feces (+++) (PD) and 40 healthy dogs (HD). Blood samples were collected to evaluate the protein fractions (APPs) in serum in both groups by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This study was approved by the Animal Experimentation Ethics Committee. Infected dogs showed significant increases for IgG (PD 1.79 ± 0.8 g/dL and HD 1.44 ± 0.72 g/dL, P = .04), for ceruloplasmin concentrations (PD 19 ± 15 mg/dL and HD 5 ± 3.5 mg/dL, P = .0001), for alpha 1-acid glycoprotein concentrations (PD 31.4 ± 17.9 mg/dL and HD 13.5 ± 12.1 mg/dL, P = .0001) and for a non-nominal identified protein of 23 kD (PD 641.5 ± 194.9 mg/dL and HD 519.8 ± 197.9 mg/dL, P = .007). No statistically significant differences (P > .05) in serum total protein, albumin, IgA, transferrin and haptoglobin concentrations were identified between groups. Results of this study suggest that parasite infections caused by *Ancylostoma* sp. triggers an acute inflammatory response in dogs. This study was supported by PROPe/UNESP, Brazil.

43

EXERCISE AFFECTS CORTISOL CONCENTRATIONS OF HORSES SUBJECTED TO A HIGH-SPEED TREADMILL. **T.S. Barbosa, L.A. Yonezawa, C.L. Marinho, J.L. Knaut, M.J. Watanabe, A. Kohayagawa.** School of Veterinary and Animal Science, Sao Paulo State University (Unesp), Botucatu-SP, Brazil.

The term stress describes the state of the organism under the influence of external or internal forces. Exercise is a stress situation for which the body must find a equilibrium and this requires adaptative responses of the hormonal system. The effects of exercise on circulating hormones remain controversial and little is known about this kind of exercise in horses. This study aimed to evaluate the influence of short-term and high intensity exercise on cortisol concentrations of horses subjected to exercise on a high-speed treadmill. Ten untrained horses were used and they performed a high intensity test. The exercise protocol consisted of 5 min at 50% $VO_{2max,}$ 5 min at 1.5 m/s, 90 s at 105% VO_{2max} set at +6% and 5 min at 3.0 m/s (0% slope) on a high-speed treadmill. Total serum cortisol was determined by RIA using a commercially available kit Coat-a-count and the times analysed were at rest before exercise (M0), immediately after exercise (PE), and 15 min, 30 min, 6 h, 12 h, 24 h, 48 h, 72 h and 96 h after test end. Exercise resulted in an immediate significant increase (P<.05) at PE and 15 and 30 minutes after test end, but the values decreased at 6 h after test end. The study showed that exercise with this protocol elicits a classic physiological stress response in horses.

44

THE EFFECT OF THE DEGREE OF BLOOD CONTAMINATION OF URINE ON THE INTERPRETATION OF THE URINARY PROTEIN TO CREATININE RATIO OF DOGS. **E.K.P. Jillings[1], S. Azarpeykan[1], R.A. Squires[2], N. Lopez-Villalobos[1].** [1]Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand; [2]School of Veterinary & Biomedical Sciences, James Cook University, Townsville, Australia.

The interpretation of the urinary protein to creatinine ratio (UPC) in urine samples with concurrent hematuria can be confusing, as blood may increase the protein level measured in the urine. To mimic hematuria, blood from 18 dogs was added to their own urine sample in increasing levels (from 0 to 5%) to determine whether the urine color for varying degrees of blood contamination can be utilized to aid interpretation of the validity of the UPC results. For each urine sample, urinary protein and creatinine were measured biochemically, urine dipstick analysis, specific gravity by refractometry and microscopic sediment examination were performed, and the urine color was visually assessed. A complete blood count (CBC) and serum biochemistry panel were performed on each dog. Blood contamination of the urine that did not result in a visible change in color of the urine sample from yellow (i.e., microscopic hematuria) did not increase the UPC above the normal range of <0.5. As such, in the presence of microscopic hematuria, the UPC level in yellow urine (with no

evidence of concurrent urinary tract inflammation) should be considered valid. Thus, the practice of discouraging UPC assessment in animals with microscopic hematuria should be discontinued. However, hematuria that results in a visible color change from yellow may increase the UPC above 0.5. In this situation hematuria would need to be considered as a differential diagnosis for the proteinuria.

45

HYPERMAGNESEMIA IS ASSOCIATED WITH INCREASED MORTALITY IN CANINE INTENSIVE CARE UNIT PATIENTS. **U. Jeffery.** Department of Veterinary Pathology, College of Veterinary Medicine, Iowa State University, Ames, IA, USA.

The clinical significance and etiology of elevated total serum magnesium in dogs has received little attention. The study aims were to establish the relationship between mortality and hypermagnesemia for dogs admitted to an intensive care unit (ICU) and to determine the extent to which hypermagnesemia could be explained by reduced glomerular filtration rate (GFR). A retrospective case control design was used. One hundred dogs admitted to ICU that died or were euthanized and 100 dogs that survived to discharge were selected using a random number generator. Hemolyzed samples were excluded. The numbers of dogs with hypermagnesemia, normomagnesemia and hypomagnesemia were 31, 57 and 12, respectively, for dogs that died or were euthanized and 11, 79 and 10 for dogs that survived to discharge. The odds ratio for death or euthanasia in hypermagnesemic dogs compared with normomagnesemic dogs was 3.90 (95% confidence interval 1.81-8.42), which was significantly different from 1 (p=.00). The odds ratio for death or euthanasia in hypomagnesemic dogs compared to normomagnesemic dogs was 1.66 (95% confidence interval 0.67-4.11), which was not significantly different from 1 (p=.27). For the 42 hypermagnesemic dogs, creatinine was elevated in 23 and normal or low in 19. Thoracic lesions were present without elevations in creatinine in 10 hypermagnesemic dogs. A linear regression model using serum creatinine predicted 18.49% of the variation in total serum magnesium for normomagnesemic and hypermagnesemic dogs that died or were euthanized. The model was not significantly improved by addition of albumin. In conclusion, hypermagnesemia is associated with increased mortality in canine ICU patients and for some of these dogs elevated total serum magnesium could not be explained by reduced GFR.

46

SERUM ALDOLASE: A USEFUL BIOCHEMICAL MARKER FOR DETECTION OF MUSCLE DISEASE IN DOGS. **R. Bell[1], A. Chen-Allen[1], A. Kiszonas[2], T. Wills[3].** [1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, USA; [2]Department of Crop & Soil Sciences Western Wheat Quality Laboratory, Washington State University, Pullman, WA, USA; [3]IDEXX Laboratories, Inc, Westbrook, ME, USA.

Currently, laboratory diagnosis of muscle disease is suggested by an increase in serum creatine kinase (CK). The human literature shows that up to 10% of patients with active muscle disease have low or normal CK concentrations, and serum aldolase concentrations are often assessed to aid in diagnosis. Aldolase is present in all cells, but is found in particularly high concentrations in liver, muscle, brain, and erythrocytes. Goals for this study included (1) identification of assay interference by hemolysis, icterus, and lipemia (HIL), (2) determination of serum aldolase concentrations in healthy dogs, (3) determination of serum aldolase concentrations in dogs with muscle disease identified by increased CK concentrations, and (4) determination of serum aldolase concentrations in dogs with liver disease identified by increased serum alanine aminotransferase (ALT) concentrations. ANOVA was employed to test for differences. No statistical difference was found in aldolase concentrations with a HIL score up to a level 3 in each category (p=.5809, p=.5337, and p=.7662, respectively). The difference between control and liver-disease subjects was not significant (p=.4830). The difference between control and muscle-disease subjects was significant (p<.0001). The difference between liver-disease and muscle-disease subjects was significant (p<.0001). This study supports the hypothesis that serum aldolase concentrations are significantly increased in dogs with muscle disease as compared with healthy dogs and dogs with liver disease, and aldolase assessment may improve diagnosis and monitoring of myopathy in dogs in the future.

47

VALIDATION OF A METHOD FOR FREE GLYCEROL AND USE AS A CORRECTION OF TRIGLYCERIDE CONCENTRATIONS IN MULTIPLE SPECIES. **S. McGrath, N. Gregorich, S. Young, F. Clemo, J. Gass.** Baxter Healthcare Corporation, Round Lake, IL, USA.

Intravenous administration of nutritional lipid emulsions in preclinical efficacy and safety studies requires accurate measurement of glycerol and triglycerides for proper characterization of lipid metabolism and clearance. Automated triglyceride methods typically measure glycerol following the hydrolysis of triglycerides into glycerol and fatty acids. Free glycerol is not differentiated from triglyceride-derived glycerol, resulting in overestimation of triglyceride concentration. Therefore, a method to measure free glycerol was incorporated to correct the measured triglyceride concentration. The Free Glycerol Determination Kit (Sigma FG0100) was adapted for use with the Beckman Coulter AU400e chemistry analyzer. The assay results were used to calculate the "triglyceride equivalent" of the free glycerol, which in turn was used to correct the measured triglyceride result (Beckman Coulter OSR6X118). These methods were validated for accuracy, linearity, within and between run precision, ruggedness, stability, and specificity in serum from multiple species: dog, guinea pig, mouse, and rat. The acceptance criteria were a coefficient of variation of less than or equal to 3.8 percent for within run precision and plus or minus 15 percent for all other validation parameters. The free glycerol assay was found to be linear from 0 to 173 mg/dL and accurate from 1.4 to 173 mg/dL. The Free Glycerol Determination Kit from Sigma was found to be accurate, linear, precise, rugged, specific, and stable. Serum from multiple species does not interfere with accurate quantitation of glycerol. Corrected triglyceride (triglyceride concentration – "triglyceride equivalent" of free glycerol concentration) was also determined to be accurate. The free glycerol assay together with the con-