

distilled water and sonicated for 20 minutes. Melphalan reference standard was dissolved in distilled water (1 mg/ml) and sonicated for 20 minutes. All solutions were centrifuged for 10 minutes at 1000 g. The supernatant was then diluted to an expected concentration of 5 $\mu\text{g}/\text{mL}$. The reference standard was diluted to 5 $\mu\text{g}/\text{mL}$ in triplicate. Melphalan concentrations in each of the test solutions were compared to the reference standard by high-pressure liquid chromatography. The mobile phase consisted of 12% acetonitrile and 88% distilled water with 0.02% trifluoroacetic acid with a 1 ml/min flow rate. Ultraviolet absorbance was monitored from 190-800 nm with a photodiode array detector and peak heights were quantitated at 262 nm. Separation was achieved with a C-18 column. The injection volume was 50 μL and the retention time was 4.5 minutes. The expected concentrations of the tablets and reference standards were assessed for differences with an analysis of variance (ANOVA).

The mean $\pm$ SD (range) expected concentrations of the reference standard, reference product, and imported drug were $100 \pm 1\%$ (99-101%), $101 \pm 8\%$ (92-107%), and $106 \pm 5\%$ (101-109%), respectively. No significant differences in the concentrations between the reference standard and tablets were present ($p = 0.41$). The absorbance spectrum was similar for all solutions.

The U.S. Pharmacopeia states melphalan tablets should contain 90-110% of the expected drug concentration, which both sources of tablets met. Despite the apparent equivalence of the tablets, *in vivo* bioequivalence cannot be assumed as formulation factors, absorption, and bioavailability may be different. The imported drug arrived sealed in its apparent original packaging and despite being manufactured overseas was manufactured under license of the original manufacturer, thus appearing legitimate. While imported melphalan in this study appeared equivalent, imported drugs should be used cautiously as they may not be equivalent in content or *in vivo* to domestic products.

ABSTRACT #212

SERUM C-REACTIVE PROTEIN CONCENTRATIONS IN DOGS WITH MULTICENTRIC LYMPHOMA DURING CHEMOTHERAPY. A Merlo, SRR Lucas, BCG Rezende, ML Franchini, DMN Simões. Faculty of Veterinary Medicine and Zootecny - University of São Paulo, Brazil.

C-reactive protein (CRP) is an acute-phase protein that usually increases following inflammatory, infectious and neoplastic conditions in dogs. The aim of this study was to evaluate if CRP is a useful marker of relapse in dogs with multicentric lymphoma (ML) and if the use of prednisone in the treatment induces modifications on serum CRP concentrations during chemotherapy. CRP was measured by the use of an immunoassay kit in four groups of dogs during chemotherapy: 4 healthy dogs (control 1) and 10 dogs with ML submitted to COP protocol; and 4 healthy dogs (control 2) and 10 dogs with ML submitted to VCM protocol (without prednisone). Measurement was done once a week during the first month of chemotherapy and each 3-week intervals until the relapse for dogs with lymphoma, and until the 16th week in control dogs. ANOVA test followed by multiple Tukey's tests were used to compare the groups. Mean CRP concentration was significantly higher in dogs with ML at the diagnosis than in healthy dogs. Levels of CRP decreased when lymphoma remission was achieved, but CRP increase was not observed at the relapse. At all other times while dogs were in treatment, CRP concentrations for dogs with lymphoma were not significantly different from controls submitted to chemotherapy. The use of prednisone did not change CRP levels. As conclusion, CRP is not a proper marker of lymphoma relapse in dogs and prednisone does not modify CRP levels when combined with other drugs in a chemotherapeutic protocol.

ABSTRACT #213

SERUM AMYLOID A IN DOGS WITH MULTICENTRIC LYMPHOMA DURING CHEMOTHERAPY. A Merlo, SRR Lucas, BCG Rezende, ML Franchini, PRG Monteiro. Faculty of Veterinary Medicine and Zootecny - University of São Paulo, Brazil.

Serum amyloid A (SAA) is an acute-phase protein that increases following some inflammatory and neoplastic conditions of human beings. The aim of this study was to evaluate if SAA is a useful marker of relapse in dogs with multicentric lymphoma (ML) and if the use of prednisone in the treatment induces modifications on SAA concentrations during chemotherapy. SAA was measured by the use of an immunoassay kit in four groups of dogs during chemotherapy: 4 healthy dogs (control 1) and 10 dogs with ML submitted to COP protocol; and 4 healthy dogs (control 2) and 10 dogs with ML submitted to VCM protocol (without prednisone). Measurement was done once a week during the first month of chemotherapy and each 3-week

intervals until the relapse for dogs with lymphoma, and until the 16th week in control dogs. ANOVA test followed by multiple Tukey's tests were used to compare the groups. Mean SAA concentration was significantly higher in dogs with ML at the diagnosis than in healthy dogs. Levels of SAA decreased when lymphoma remission was achieved, but SAA increase was not observed at the relapse. At all other times while dogs were in treatment, SAA concentrations for dogs with lymphoma were not significantly different from controls submitted to chemotherapy. The use of prednisone did not change SAA levels. As conclusion, SAA is not a proper marker of lymphoma relapse in dogs and prednisone does not modify SAA levels when combined with other drugs in a chemotherapeutic protocol.

ABSTRACT #214

HMG A EXPRESSION AS A DIAGNOSTIC TOOL IN CANINE PROSTATIC TISSUES. Murua Escobar H.², Winkler S.¹, Meyer B.¹, Eberle N. 2, Simon D.¹, Bullerdiek J.¹ and Noite L.². ¹Centre for Human Genetics, University of Bremen, Bremen, Germany. Small Animal Clinic, School of Veterinary Medicine, Hanover, Germany.

The dog is the only known mammalian species - beside humans - spontaneously developing prostate cancer. Both species show striking similarities in the progress of the disease. Based on the histology of the lesions alone it is often not possible to recognize sufficiently the malignant potential of the tumour in terms of local invasiveness and metastatic spread. Thus, molecular indicators are of considerable interest.

In humans, HMG A overexpression was described to be associated with a highly malignant phenotype of various cancers including prostate cancer and is therefore considered a molecular marker. In previous studies we characterised both canine genes and showed that the human and canine proteins are highly conserved. In both species HMG A proteins are abundantly expressed during embryogenesis and are almost undetectable in most adult tissues. Re-expression was detected in a variety of human malignancies showing correlation of the expression level with the degree of neoplastic cell transformation and metastatic tumour progression.

Herein report on the HMG A2 expression patterns determined by real-time quantitative RT-PCR in prostatic tissues from 16 dogs with different histological findings. The results show that expression of HMG A2 is low in tissues with no abnormality detected, rises in benign neoplasms and increases at least 19-fold in carcinomas. In our study all malignant neoplasias showed expression levels beyond 50,000 transcripts per 250 ng total RNA, whereas none of the non-malignant tissues showed expression levels beyond this value. These results indicate that HMG A2 expression analysis using real-time quantitative RT-PCR may provide a potential tool for better differentiation between varying degrees of malignancy in prostate carcinomas.

ABSTRACT #215

INCIDENCE OF CANINE SERUM ANTIBODY TO KNOWN DOG ERYTHROCYTE ANTIGENS IN POTENTIAL DONOR POPULATION. A.S. Hale and J. Werfelmann; Midwest Animal Blood Services, Inc., Stockbridge, MI.

As blood component therapy for non-emergency purposes becomes a part of routine veterinary practice, factors with the potential of reducing viable red blood cells must be clearly set forth to help clinicians maximize the effectiveness of each transfusion event. There has been ongoing disagreement about the consequence of positive canine donor serum antibody. By using the incidence of positive antibody screen to demonstrate how often unexpected antibody occurs in a potential donor population, the likelihood that such antibody will cause an adverse transfusion reaction may be projected and risk assessed. Antibody screening combines a panel of known-type canine red blood cells with heat-inactivated donor serum. Tubes are incubated (15 minutes at 37°C), spun at 3400 rpm for 30 seconds and read for agglutination, with a 2+ or greater reaction indicating presence of antibody. Based on 2500 antibody screens performed in the Midwest Animal Blood Services, Inc. laboratory over a thirty-month period, the following incidence of positive antibody was found:

Anti-DEA 1.1 0.3%, Anti-DEA 3 1.2 . Anti-DEA 5 0.8%,
Anti-DEA 7 9.8%, nonspecific agglutinating 2.0%

These results reflect a lower incidence of antibody than has been presented in previously published field evidence, which highlights several considerations. 1) In the MABS antibody screening protocol, the effect of antibody against antigen is examined at a single temperature (37°C), identifying antibody that is physiologically active and able to agglutinate at average body temperature, thereby limiting the range of antibody reactivity to that