Combination of inflammatory and hemostatic markers in mortality model for critically ill canine icu patients significantly improves efficacy

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Research Abstract Program of the 2009 ACVIM Forum & Canadian Veterinary Medical Association Convention

Montréal, Québec
June 3 — 6, 2009
Index of Abstracts

ORAL PRESENTATIONS – Thursday, June 4

Time	#	Presenting Author	Abstract Title
SMALL ANIMAL – CARDIOLOGY**			
9:00 am	1	Allison Heaney	Use of Polymerase Chain Reaction to Detect Bacteremia in Dogs with Bacterial Endocarditis
9:15 am	**2**	**Andrea Lantis**	**The Effect of Furosemide and Pimobendan on the Renin-Angiotensin- Aldosterone System (RAAS) in Dogs**
9:30 am	**3**	**Lisa Keller**	**Assessment of Change in Left Ventricular Systolic Function by Tissue Doppler Imaging Methods after Closure of Patent Ductus Arteriosus in Dogs**
9:45 am	4	Giosi Farace	Correlation of N-Terminal Prohormone Brain Natriuretic Peptide with Left Ventricular Outflow Tract in Dogs with Sub Aortic Stenosis
BREAK			
10:30 am	5	Ashley Saunders	NT-proBNP Concentrations in Canine Congenital Heart Disease
10:45 am	6	Gretchen Singletary	Utility of NT-proBNP Assay to Detect Occult Dilated Cardiomyopathy in Doberman Pinschers
11:00 am	7	Gerhard Wess	Evaluation of NT-proBNP in the Diagnosis of Various Stages of Dilated Cardiomyopathy in Doberman Pinschers
11:15 am	**8**	**Sarah Achen**	**Serial Evaluation of NT-proBNP in Dogs with CHF Predicts Clinical Score and the Presence or Absence of Radiographic Pulmonary Edema**
11:30 am	9	Gerhard Wess	The Utility of NT-proBNP to Detect Early Stages of Hypertrophic Cardiomyopathy in Cats and to Differentiate Disease Stages
11:45 am	10	Giosi Farace	Pulmonary Hypertension and N-Terminal Prohormone Brain Natriuretic Peptide in Dogs
12:00 pm	11	Kathryn Meurs	Genome-Wide Association Identifies a Mutation for Arrhythmogenic Right Ventricular Cardiomyopathy in the Boxer Dog
12:15 pm	12	Eva Oxford	Phenotypic Differences in the Ultrastructure of Cardiomyocytes from Boxer Dogs Afflicted with Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

** *Also see Cardiology abstracts 61–73 (Thursday, June 4, 2:15 pm – 6:00 pm)*

SMALL ANIMAL – NEPHROLOGY/UROLOGY			
9:00 am	13	Barrak Pressler	*In vivo* Determination of Canine Cystolith Mineral Composition Using Computed Tomography-Generated Hounsfield Units
9:15 am	14	Allyson Berent	Ureteral Stenting for Feline Ureterolithiasis: Technical and Clinical Outcomes

Boldface type *indicates presentation by award eligible resident. Presentation times are subject to change.*

prolonged surgery, even in those with no previous hemostatic abnormalities. The severity of bleeding and prognosis are not well correlated with the prothrombin time (PT) or activated partial thromboplastin time (aPTT) (Wiinberg B et al; 2007). Thromboelastography (TEG®) is able to assess *ex vivo* hemostasis using a whole blood sample, since it evaluates most of the components that play a role in the formation of the hemostatic plug *in vivo* (i.e blood cells, platelets, clotting factors). TEG has been proposed as the hemostasis test of choice for monitoring patients receiving plasma components for coagulopathies (Johansson P; 2007). Based on data from previous studies (Fries D et al; 2005, 2006) that showed improvement in clot formation during thrombocytopenia after administration of fibrinogen concentrates, we evaluated the use of canine cryoprecipitate (CRYO) in 3 dogs with postoperative bleeding due to DIC in an attempt to reestablish normocoagulable conditions. Patients were monitored using TEG®. Blood samples were collected via a central venous catheter into a 2.7 ml Vacutainer tube containing 3.2% buffered sodium citrate, and TEG® was done using citrated native technique, as we previously described (Vilar P et al; 2008). Three hypocoagulable ($TEG_{R,K}$ > mean+2SD, and/or $TEG_{angle,MA,G}$ < mean-2SD) dogs with postoperative bleeding associated with DIC were transfused with 50–70 ml/10 kg of CRYO (containing approximately 500 mg/dl fibrinogen). Two dogs had been splenectomized for hemangiosarcoma and 1 had gastric dilation-volvulus. Postransfusion TEGs revealed normocoagulable tracings and increased hematocrits in all cases less than 24 h post-CRYO; no additional blood component transfusions were needed. No clinical thromboembolic complications were detected in any dog. TEG® showed excellent clinical correlation with the bleeding status in all dogs. TEG® is a useful test for monitoring transfusion therapy in patients with coagulopathies; CRYO infusion should be considered in dogs in DIC.

ABSTRACT #32

EXTENDED CANINE BLOOD TYPING BY GEL COLUMN TECHNIQUE. RJ Kessler[1], J Reese[1], D Chang[1], A Hale[2], U Giger[1]. [1]Section of Medical Genetics, University of Pennsylvania (PennVet), Philadelphia, PA. [2]Animal Blood Resources International (ABRI), Stockbridge, MI.

To ensure blood compatibility, safety, and efficacy of transfusions in dogs, typing for the major antigenic blood type DEA 1.1 is recommended by using one of several well established methods. The role of extended typing for other canine blood types is more controversial regarding their clinical importance, and these typing procedures have been hampered by the limited availability of typing reagents and difficulties in both performing the tube typing protocol and interpreting those results due to weak agglutination reactions. Our goal was to develop and standardize a laboratory method of extended typing using polyclonal reagents that minimizes the use of reagent while maximizing sensitivity, specificity, interpretability, and reproducibility.

We utilized available polyclonal typing reagents (ABRI) at optimized concentrations with the saline or canine antiglobulin gel columns (novel GEL; DiaMed, Switzerland) similar to the commercially available gel column DEA 1.1 typing technique (standard GEL). All dogs were also typed using the conventional tube method according to the manufacturer's instructions (TUBE). A total of 54 dogs including 22 patients and 32 blood donors at PennVet were typed for DEA 1.1, 1.2, 3, 4, and 7, as well as the *dal* red cell antigen. Agglutination reactions were graded on a scale of 0 to 4+ and reactions ≥2+ were considered positive.

Of the 43 dogs typed for DEA 1.1, 23 were positive for DEA 1.1 using the standard GEL as well as with the novel GEL method (with antiglobulin) using polyclonal DEA 1.1 and 1.X reagents. Twenty of those 23 were also DEA 1.1 positive with the TUBE. Two of the 3 remaining were positive with the TUBE for 1.X but only 1+ for 1.1 (suggesting a DEA 1.2 type). In addition, 2 dogs tested DEA 1.1 positive using the novel GEL method, but not the standard GEL or TUBE method. All samples tested negative for autoagglutination (in saline±antiglobulin).

All 54 dogs were typed for DEA 3, 4, and 7 using the standard TUBE and novel GEL (saline) method. Only 4 dogs were found to be DEA 3 positive with the TUBE, 3 of which were also positive with the novel GEL method. All dogs tested 3+ or 4+ for DEA 4 via both methods. Twelve dogs tested positive for DEA 7 with the TUBE, half of which were also positive with the novel GEL; an additional 2 dogs were positive by the novel GEL method alone. All 36 dogs typed for the *dal* antigen were positive (no Dalmatians were tested). Only 1 dog was positive for all tested red cell antigens.

In conclusion, this gel column technique was able to detect the DEA 1.1, 3, 4, and 7 as well as the *dal* antigen using available polyclonal antibodies. The agglutination reactions for extended typing were stronger and more readily interpreted with the novel GEL than the TUBE method. Few discrepancies between the TUBE and GEL methodology remain and require further investigation.

ABSTRACT #33

***PSEUDOMONAS FLUORESCENS* CONTAMINATION OF CANINE AND FELINE PACKED RED BLOOD CELL UNITS.** RJ Kessler, S Young, DA Oakley, S Rankin, U Giger. Departments of Clinical Studies and Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

While rigorous screening programs have drastically reduced the risk of infectious disease transmission in human and veterinary blood banking, bacterial contamination of blood products has emerged as a major cause of morbidity and mortality in human transfusion medicine. In particular, packed red blood cells (pRBCs) and platelet concentrates stored at 4 °C and 20 °C, respectively, have been found to be contaminated with *Streptococcus spp* (platelets), *Yersinia spp*, and *Pseudomonas spp* (pRBCs). In veterinary medicine, there has been only sparse mention of bacterial contamination of canine and feline blood products. We describe here the discovery of a *P. fluorescens* (*Pf*) contamination of a unit of feline pRBCs which led to further investigations of experimentally *Pf*-contaminated units and utilization of a semi-quantitative real-time 16S bacterial ribosomal DNA PCR test (16S PCR) for blood product screening.

A unit of feline pRBCs that turned black on the 22nd day of storage at 4 °C was removed from the blood bank and further examined. There was red cell lysis and cytology showed many free bacteria, however, aerobic culture of the blood at 37 °C was negative. Real-Time 16S PCR testing was strongly positive, and isolated DNA was sequenced and identified as *Pf*. An aliquot of the unit grew when cultured at 20 °C (room temperature). Extensive evaluations failed to identify a source of this contamination, nor has a similar contamination or color change been observed with any other stored pRBCs in our blood bank in the year prior to or in the 4 months after this incident.

Units of canine pRBCs (25 ml each) were inoculated with 0, 5, and 25 µl of *Pf*-rich pRBCs from the sentinel feline unit and stored at either 4 °C or 20 °C for 48–72 hr. Some units were then switched from 4 °C to 20 °C after 48 hr. Prior to and immediately post-inoculation all 16S PCR results were negative; separately spiked 25 ml pRBCs only became positive after adding ≥100 µl *Pf*-rich pRBCs. Only *Pf*-spiked pRBC units became 16S PCR positive (≥8 hr [25 µl at 20 °C]; 48 hr [25 µl at 4 °C and 5 µl at 20 °C]) and showed a color change as early as 24 hr; cultures confirmed the presence of a pure culture of *Pf*. One spiked unit [5 µl at 4 °C] that was 16S PCR negative at 48 hr became positive within 4 hr at 20 °C.

In conclusion, *Pf* appears to have the unique capacity to grow in feline and canine pRBCs slowly when stored cold and rapidly at room temperature. Screening of blood products with 16S PCR is a simple, rapid, and sensitive test method to detect bacterial contamination before a gross color change is noted or bacterial culture results are positive, and thus may be useful for routine screening of pRBCs. While a source of contamination and potential accidental exposure to 20 °C for our sentinel unit was not determined here, aseptic collection and processing methods and temperature controlled storage along with regular visual evaluation of units is recommended.

ABSTRACT #34

COMBINATION OF INFLAMMATORY AND HEMOSTATIC MARKERS IN MORTALITY MODEL FOR CRITICALLY ILL CANINE ICU PATIENTS SIGNIFICANTLY IMPROVES EFFICACY. M Kjelgaard-Hansen[1], B Wiinberg[1], AL Jensen[1], E Rozanski[2], AT Kristensen[1]. [1]Department of Small Animal Clinical Sciences, University of Copenhagen, Copenhagen, Denmark. [2]Cummings School of Veterinary Medicine at TUFTS University, North Grafton, MA.

Key inflammatory and hemostatic markers have been identified as possible prognostic markers in critically ill canine patients; serial

measurements of Protein C and Antithrombin (AT) was reported to correlate to survival of septic dogs (deLaforcade et al., 2008) and the level of IL-6 correlated to survival time in dogs with SIRS and sepsis (Rau et al., 2007). However, studies combining inflammatory and hemostatic markers are rare. Recent human studies have reported significant improvement in prognostic efficacy by addition of simple hemostatic and/or inflammatory markers to traditional risk assessment scores (e.g. SOFA and SAPS-II). The objective of the present study was to investigate the effect of combining key inflammatory and hemostatic markers in prognostic models for critically ill canine ICU patients.

Fifty critically ill dogs admitted to the ICU at Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA (April to July 2006) were included prospectively. Citrated whole-blood, serum and citrated plasma were obtained by standardized procedures. Tissue-factor Thromboelastography (TEG [MA, G, R, K and α]) and platelet count was performed within 30 minutes, while serum and plasma was stored and analyzed in batches for AT, D-dimer, Protein C, Protein S, PT, aPTT, Plasminogen, Plasminogen inhibitor, C-reactive protein (CRP) and IL-10. All methods were validated for use in dogs. Twenty-eight day survival was recorded.

Multiple logistic regression analysis was performed with survival as outcome variable based on A) Inflammatory markers, B) Hemostatic markers and C) all markers, using backwards exclusion and forward inclusion ($P > 0.1$). Area under Receiver operation characteristic curves (ROC-AUC) was used to evaluate and compare model discriminative efficacies, and optimized sensitivity and specificity were assessed.

Two dogs were excluded (missing data). Mortality was 42% (20/48). Final models and results were (significant parameters included, (ROC-AUC), [sensitivity and specificity], respectively: A) CRP, (0.71), [0.75, 0.68], B) MA, G, AT (0.80), [0.70, 0.89] and C) MA, G, AT, PT, aPTT, CRP, (0.91), [0.75, 0.93]. Model C) had significantly better discriminative efficacy than A) [$P=0.019$] and B) [$P=0.046$], while no difference was found between A) and B) [$P=0.32$].

In conclusion, prognostic models for 28-day mortality was developed by means of key inflammatory and hemostatic markers, available and validated for use in dogs. Significantly improved discriminative efficacy was observed when both inflammatory and hemostatic markers were included in the model. Future studies refining prognostic modeling in critically ill dogs are recommended to include key markers of both inflammation and hemostasis.

ABSTRACT #35
INVESTIGATION OF DOXYCYCLINE-RELATED SIDE EFFECTS IN DOGS

B. Schulz, S. Hupfauer, K. Hartmann. Clinic for Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

Doxycycline is a commonly used antibiotic in small animal practice with a broad spectrum of activity against bacteria, *Rickettsia*, *Mycoplasma*, and *Chlamydophila* species. Although there are many data published on pharmacokinetics and toxicology in humans and laboratory animals, little information is available on side effects in dogs. Side effects described in single case reports include gastrointestinal problems, hepatotoxicity, and injection site reactions. In young animals bone and dental problems can occur.

Aim of the retrospective study was to assess the incidence of doxycycline-related side effects (anorexia, vomiting, diarrhea, pyrexia, and increased liver enzyme activities) in dogs and to investigate correlations between side effects and signalement, dose, duration of treatment, frequency of application, doxycycline preparation, and use of additional drugs. Statistical comparison was performed using likelihood ratio tests and logistic regression analyses.

Three hundred eighty six dogs, that had received doxycycline, were included in the study. Of these, 2.5% (8/314) developed anorexia during therapy. The longer dogs were treated, the less frequently anorexia was observed (reduced by factor 0.83; $p=0.050$). Vomiting developed in 18.3% (63/344) of dogs, and the risk increased with age by factor 1.06 per year ($p=0.030$). Diarrhea was documented in 7.0% (24/342) of dogs. None of the dogs developed fever. An increase in ALT was observed in 39.4% (26/66), an increase in ALP in 36.4% (16/44) of patients receiving doxycycline therapy. The factors sex, dose, frequency of application, doxycycline preparation, and application of additional drugs did not have an influence on occurrence of side effects.

In conclusion, vomiting and increase in liver enzyme activities represent the most frequently observed doxycycline-related side effects.

ABSTRACT #36
METABOLISM AND EXCRETION OF ORAL MELOXICAM IN THE CAT

Thoulon F[1], Narbe R[2], Johnston L[2], Ingwersen W[3], Watson P[2]. Boehringer Ingelheim Animal Health. [1]Reims, France [2]Ingelheim, Germany [3]Burlington, Ontario, Canada.

The objective of this study was to investigate the metabolic pathways and routes of excretion of oral meloxicam in the cat. Many nonsteroidal antiinflammatory drugs (NSAIDs) are metabolised by the hepatic glucuronyl transferase enzyme pathway (i.e. glucuronidated), before excretion. Cats are relatively and variably deficient in this enzyme activity, making prolongation of NSAID half life and toxic accumulation a possibility. Meloxicam is metabolised via oxidation in all other species previously studied.

A mean oral dose of 0.75 mg/kg or 397.55 kBq/kg of oral [^{14}C]-meloxicam was administered to 3 fasted male cats. The cats were fed 4 hours after administration. No concurrent medication was administered. Urine, faeces, vomit and cage washes were collected over the following 144 hour period. Blood was collected pre-dosing and at 3 and 12 hours post-dosing. Metabolites were identified by HPLC/MSMS. Where possible a metabolic structure was proposed for each metabolite detected.

Only the unchanged parent compound was identified in plasma. In urine 5 major metabolite peaks were detected and in faeces 4 major metabolite peaks were detected, which were identified by HPLC/MSMS as products of oxidative metabolism. No conjugated metabolite was detected.

Elimination occurred early (34% during the first 24 hours, 61% during the first 48 hours).

% Recovered dose in first 48 hours	Meloxicam	Metabolites	Total
Urine	2	19	21
Faeces	49	30	79

The results indicate that the major route of excretion of meloxicam in the cat is faecal. The findings indicate that, as with other species investigated, the main pathway of biotransformation of meloxicam in the cat is oxidation. This results in predictable pharmacokinetics, making meloxicam suitable for long-term administration in the cat.

ABSTRACT #37
CLONING OF THE CALCIUM-SENSING RECEPTOR FROM THE FELINE PARATHYROID GLAND

A Gal, T Ridge, TK Graves. University of Illinois College of Veterinary Medicine, Urbana, IL.

Gain- or loss-of-function mutations in the plasma membrane-bound calcium-sensing receptor (CaSR), which is responsible for detecting extracellular concentraions of calcium ion, can result in aberrant calcium regulation. Such mutations cause familial benign hypocalciuric hypercalcaemia, neonatal severe primary hyperparathyroidisim, and autosomal dominant hypoparathyroidisim in people. To enable future study of these disorders in cats, we cloned and sequenced CaSR mRNA from the feline parathyroid gland.

Total RNA was extracted from cat parathyroid gland, and reverse-transcribed cDNA was used for PCR amplification of the CaSR. Plasmids containing CaSR amplicons were transferred to *E. coli*, which were grown on selective media. Sequenced inserts were used to design specific exonic CaSR PCR primers. Peripheral blood leukocytes of 5 healthy normocalcemic cats were used for amplification and sequencing of the six CaSR exons.

The feline CaSR has one positive reading frame and consists of 3243 base pairs. Its overall homology to homology to canine, bovine