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2009 ACVIM Forum & Canadian Veterinary Medical Association Convention

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.*

9:30 am	15	Allyson Berent	The Use of a Percutaneously Controlled Hydraulic Occluder for the Treatment of Urethral Sphincter Mechanism Incompetence in 11 Dogs and 1 Cat
9:45 am	16	Michael Wood	Development of a Ussing Chamber Model for Studying the Canine Urothelial Response to Infection
BREAK			
10:30 am	**17**	**Christine Wu**	**Urodynamic Evaluation of Cats with Feline Interstitial Cystitis (FIC)**
10:45 am	18	Dennis Chew	Randomized, Placebo-controlled Clinical Trial of Pentosan Polysulfate Sodium for Treatment of Feline Interstitial (Idiopathic) Cystitis
11:00 am	19	Herve Lefebvre	Comparison of Plasma Clearance of Exogenous Creatinine and Iohexol in a Canine Population
11:15 am	20	Andrea Zatelli	Effects of Supplementation with Amino Acids in Dogs with Glomerulonephritis
11:30 am	**21**	**Karen Hilling**	**Documentation of Hypercoagulability in Protein-Losing Nephropathy via Thromboelastography in 10 Dogs**
11:45 am	22	Mark Acierno	Measuring Level of Agreement between Directly Measured Blood Pressure and Values Produced by Oscillometric Units in Cats
12:00 pm	**23**	**Carly Bloom**	**Pharmacokinetics and Cardiovascular Effects of Intravenous Fenoldopam in Healthy Awake Dogs**
12:15 pm	24	Bart Maddens	Urinary Immunoglobulin G, C-Reactive Protein and Retinol Binding Protein as Candidate Early Biomarkers for Renal Dysfunction in Dogs with Pyometra ***(ECVIM Award Winner)***

SMALL ANIMAL – HEMATOLOGY

9:00 am	25	Stephanie Smith	Whole Bloodthromboelastometry (TEM) Is Related to Cell Counts and Plasma Coagulation Tests in Healthy Dogs
9:15 am	**26**	**Lara Rose**	**Effect of Canine Hyperadrenocorticism on Thrombelastography Parameters**
9:30 am	**27**	**John Thomason**	**Effects of Cyclosporine on Canine Platelet Procoagulant Activity**
9:45 am	**28**	**Sarah Helmond**	**Treatment of Canine Immune-Mediated Hemolytic Anemia with Individually Adjusted Heparin Dosing: A Pilot Study**
BREAK			
10:30 am	**29**	**Elizabeth Orcutt**	**Comparison of Individually Monitored Unfractionated Heparin versus Low-Dose Aspirin on Survival of Dogs with Immune Mediated Hemolytic Anemia**
10:45 am	**30**	**Kristopher Sharpe**	**Platelet Impedance Aggregometry in Clinically Healthy Dogs with and without Ultralow-Dose Aspirin**
11:00 am	31	Paulo Vilar Saavedra	Hemostatic Effects of Cryoprecipitate in Dogs with Disseminated Intravascular Coagulation
11:15 am	32	Rebecca Kessler	Extended Canine Blood Typing by Gel Column Technique
11:30 am	33	Rebecca Kessler	*Pseudomonas fluorescens* Contamination of Canine and Feline Packed Red Blood Cell Units

SMALL ANIMAL – OTHER

11:45 am	34	Mads Kjelgaard-Hansen	Combination of Inflammatory and Hemostatic Markers in Mortality Model for Critically Ill Canine ICU Patients Significantly Improves Efficacy
12:00 pm	35	Bianka Schulz	Investigation of Doxycycline-Related Side Effects in Dogs

SMALL ANIMAL – PHARMACOLOGY

12:15 pm	36	Fabrice Thoulon	Metabolism and Excretion of Oral Meloxicam in the Cat

SMALL ANIMAL – ENDOCRINOLOGY

9:00 am	**37**	**Arnon Gal**	**Cloning of the Calcium-Sensing Receptor from the Feline Parathyroid Gland**
9:15 am	38	Eric Zini	Hypertriglyceridemia Does Not Impair Whole Body Insulin Sensitivity in Cats, Possibly through Increased Circulating Adiponectin
9:30 am	39	Eric Zini	Role of Hyperglycemia in the Pathogenesis of Pancreatitis in Cats
9:45 am	**40**	**Kirk Sears**	**Use of Lispro Insulin for Treatment of Dogs with Diabetic Ketoacidosis**
BREAK			
10:30 am	41	Kirsten Roomp	Evaluation of Detemir in Diabetic Cats Managed with a Protocol for Intensive Blood Glucose Control
10:45 am	**42**	**Andrew McGraw**	**Determination of the Concentrations of Trilostane and Ketotrilostane that Inhibit *ex vivo* Synthesis of Cortisol in Canine Adrenal Tissues**
11:00 am	43	Ghita Benchekroun	Plasma ACTH Precursors (Pro-OpioMelanoCortin and Pro-Adrenocorticotropin) in Cats with Hyperadrenocorticism
11:15 am	44	Katharine Lunn	Endocrine Function in Cats after Stereotactic Radiosurgery Treatment for Acromegaly
11:30 am	45	John Rossmeisl	Blood-Brain-Barrier Disruption in Chronic Canine Experimental Hypothyroidism
11:45 am	46	Jennifer Wakeling	Induction of Aryl Hydrocarbon Receptors in Cats
12:00 pm	47	Audrey Cook	Prevalence of Hypocobalaminemia in Cats with Hyperthyroidism
12:15 pm	48	Lisa Morrow	Hypertension in Hyperthyroid Cats: Prevalence, Incidence and Predictors of its Development

EQUINE**

9:00 am	49	Natália Rodrigues	Expression of Cyclooxygenase Isoforms in Equine Gastric Ulcers
9:15 am	**50**	**Kati Vainio**	**Primary Gastric Impaction in Horses: A Retrospective Study of 22 Cases (2005-2008)**
9:30 am	51	Frank Andrews	Effect of Volatile Fatty Acid Mixes on Equine Nonglandular Mucosa Bioelectric Properties: Pathogenesis of Gastric Ulcers in Horses
9:45 am	**52**	**Peggy Moreau**	**Study of the Variability of Endoscopic Duodenal Biopsies in Healthy Horses and in Heaves-Affected Horses Fed with Different Diets**
BREAK			
10:30 am	53	Emilie Setlakwe	Sub-Epithelial Fibrosis Is Present in the Peripheral Airways of Heaves-Affected Horses
10:45 am	54	Modest Vengust	Furosemide Does Not Affect Transvascular Fluid Fluxes across the Lung in Exercising Horses
11:00 am	**55**	**James Nicol**	**Partitioning of Pulmonary Resistance in Horses**
11:15 am	56	Julie Dauvillier	Effects of Prolonged Inhaled Corticosteroid Treatment on Cell-Mediated Immunity in Horses
11:30 am	57	Lutz Goehring	EHV-1 Positive PBMC Fractions During Cell-Associated Viremia following Experimental Infection
11:45 am	**58**	**Sonya Wilsterman**	**The Efficacy of Nitazoxanide as an Antiviral Agent for the Treatment of EHV-1 Infection in Horses**
12:00 pm	59	Alexandra Draper	Repeatability and Reproducibility of Transabdominal Ultrasonographic Renal Loci and Dimensions in Thoroughbred Horses
12:15 pm	**60**	**Gemma Tyner**	**Renal Biopsies in Horses: A Retrospective Study of 71 Cases**

** *Also see Equine abstracts 116–129 (Thursday, June 4, 2:15 pm – 6:15 pm) and Equine abstracts 142–143 (Thursday, June 4, 5:45 pm – 6:15 pm)*

SMALL ANIMAL – CARDIOLOGY**

2:15 pm	**61**	**Caryn Reynolds**	**Owner Perceptions and Priorities Regarding Quality of Life and Quantity of Life in Cats with Heart Disease**
2:30 pm	**62**	**Sara Bordelon**	**Validation of Platelet Factor 4, Soluble P-Selectin, β-thromboglobulin, and 11-dehydro-thromboxane B2 ELISAs in the Feline Population**
2:45 pm	63	Karl Jandrey	Plasma PECAM-1 and Endothelin-1 Concentrations Are Elevated in Cats with Hypertrophic Cardiomyopathy
3:00 pm	64	Francesco Porciello	Association of Hypertrophic Cardiomyopathy Phenotype and Genotype in Italian Maine Coon Cats
3:15 pm	65	Denise Schwartz	Pulsed Tissue Doppler Identifies Preclinical Left Ventricular Myocardial Dysfunction in Obese Dogs
3:30 pm	**66**	**Sarah Scruggs**	**Serotonin Transporter Expression Is Locally Down-Regulated in Canine Degenerative Mitral Valves**
3:45 pm	67	Sophia Moesgaard	B-Type Natriuretic Peptide and Cyclic Guanosine Monophosphate Levels Increase whereas Nitric Oxide Bioavailability Decreases with Increasing Degree of Myxomatous Mitral Valve Disease
BREAK			
4:30 pm	**68**	**Adonia Hsu**	**Trans-Thoracic Three-Dimensional Echocardiographic Evaluation of Normal Canine Heart Valves**
4:45 pm	69	Ingrid Ljungvall	Cardiac Troponin-1 Is Associated with Severity of Myxomatous Mitral Valve Disease, Age, Heart Rate and C-Reactive Protein in Dogs
5:00 pm	70	Carl Kirker-Head	Evaluation of a Minimally Invasive Treatment for Mitral Valve Regurgitation
5:15 pm	71	Sonya Gordon	Caval Syndrome: Outcome in 42 Dogs
5:30 pm	72	Brian Maran	Modulation of Inflammatory and Oxidative Stress Markers in Coronary Artery Smooth Muscle Cell Model
5:45 pm	73	Barthel Schmelting	High Definition Oscillometry: A Novel, Non-Invasive Approach for Precise Blood Pressure Determination and Cardiovascular Assessment in Old World and New World Monkeys

** *Also see Cardiology abstracts 1–12 (Thursday, June 4, 9:00 am – 12:30 pm)*

SMALL ANIMAL – NEUROLOGY

2:15 pm	74	Luisa De Risio	Prevalence, Heritability and Genetic Correlations of Congenital Sensorineural Deafness and Pigmentation Phenotypes in 4143 Border Collies
2:30 pm	**75**	**Fiona James**	**Investigation of a New Electroencephalography (EEG) Electrode in Sedated and Awake Dogs**
2:45 pm	76	Simon Platt	Cerebrospinal Fluid Neurotransmitter Concentrations in Dogs with Ischemic Infarction of the Brain
3:00 pm	77	Ronaldo da Costa	Risk Factors for Post-Myelographic Seizures in Dogs – 503 Cases
3:15 pm	78	John Rossmeisl	Effects of Palliative Pharmacotherapy on Survival and Quality of Life in 50 Dogs with Primary Intracranial Neoplasms
3:30 pm	**79**	**Sarah Moore**	**The Pharmacokinetics of Levetiracetam in Dogs Concurrently Receiving Phenobarbital**
3:45 pm	**80**	**Liran Tzipory**	**Metabolism and Action of Nelarabine in Glioma Cell Lines**

BREAK			
4:30 pm	81	Veronika Stein	Expression of Matrix Metalloproteinases and RECK in Microglial Cells in Different Intracranial Diseases
4:45 pm	82	Steven De Decker	Low Field Magnetic Resonance Imaging (MRI) Findings of the Caudal Cervical Region in Clinically Normal Doberman Pinschers and Foxhounds
5:00 pm	83	Ronaldo da Costa	Computed Tomographic Findings In Large and Giant Breed Dogs with Cervical Spondylomyelopathy: 58 Cases
5:15 pm	84	Filippo Adamo	Cervical Arthroplasty in Dogs with Disc Associated Caudal Cervical Spondylomyelopathy and Cervical Disc Herniation: Preliminary Study in Two Cases
5:30 pm	**85**	**Jenny Scarano**	**F Ratio of the Sciatic and Ulnar Nerve in Dogs**
5:45 pm	86	G. Diane Shelton	Peripheral Nerve Pathology in Canine Degenerative Myelopathy with Mutation in Superoxide Dismutase 1 Gene
6:00 pm	87	Joan Coates	An SOD1 Mutation Associated with Degenerative Myelopathy Occurs in Many Dog Breeds

SMALL ANIMAL – ONCOLOGY

2:15 pm	88	Karen Jackson	Lymphocyte Counts and Circulating Lymphoblasts in Canine Lymphoma Patients
2:30 pm	89	Gina Michels	Pharmacokinetic Properties of Toceranib Phosphate (Palladia™, SU11654), a Novel Tyrosine Kinase Inhibitor, in Laboratory Dogs and Dogs with Mast Cell Tumors
2:45 pm	90	Rachel Dean	Feline Injection Site Sarcomas in the United Kingdom: One Hundred and Fifty Seven Cases
3:00 pm	91	Christine Kempf	Nonviral Gene Transfer of the Feline Cytokine Genes IL-2, IFNγ and GM-CSF as Adjuvant Immunotherapy of the Feline Fibrosarcoma
3:15 pm	92	Melani Fork	Immunhistochemical Differentiation of Canine Prostatic Carcinoma
3:30 pm	93	Chick Weisse	Feasibility and Safety Associated with Selective and Superselective Intra-Arterial Carboplatin ± Meloxicam Delivery for Urothelial Tumors in Dogs
3:45 pm	94	Marisa Ames	Effect of Intracavitary Chemotherapy as Treatment of Recurrent Pleural Effusion Following Pericardiectomy: Eight Cases

SMALL ANIMAL – NUTRITION/METABOLISM

4:30 pm	95	Matthew Beal	Technique for Fluoroscopic Nasojejunal Tube Placement in Dogs
4:45 pm	96	Matthew Beal	Technique for Percutaneous Radiologic Gastrojejunostomy in the Dog
5:00 pm	97	John Bauer	Dietary γ-linolenic Acid Supports Arachidonic Acid Enrichment of Feline Red Blood Cell Membranes
5:15 pm	98	Alexander German	Adipokine Expression and Secretion by Canine Adipocytes: Stimulation of Inflammatory Adipokine Production by LPS and TNFα
5:30 pm	99	Anne Chauvet	Exercise and Active Client Motivation Improve Rate of Weight Loss in Obese Dogs
5:45 pm	100	Vincent Biourge	Comparaison of Dietary Strategies on the Perception of Hunger during a Field Feline Weight Loss Program
6:00 pm	101	Joana Nery	Influence of Dietary Protein Source and Content on Fecal Quality and Gene Expression of Water and Electrolyte Transporters of Colon in Miniature Poodles and German Shepherds

SMALL ANIMAL – INFECTIOUS DISEASE

2:15 pm	102	Iain Keir	Influence of Empirical Antimicrobial Choice on Outcome following Septic Peritonitis in Dogs
2:30 pm	103	Dawn Boothe	Detection of Fluoroquinolone Resistance Level in Clinical Canine and Feline *Escherichia coli* Pathogens Using Rapid and Early Real-Time PCR Assay
2:45 pm	**104**	**Ashley Cruse**	**Evaluation of Potential Biomarkers for Canine Sepsis**
3:00 pm	105	Barbara Kohn	Pulmonary Manifestation in Canine Leptospirosis
3:15 pm	106	Susan Little	Seroprevalence of FeLV and FIV in Canada
3:30 pm	107	Desiree Kruse	Prognostic Factors in Cats with Feline Panleucopenia
3:45 pm	**108**	**Elizabeth Lechner**	**Prevalence of Protective Antibody Titers for Distemper Virus and Parvovirus in Dogs Entering a Florida Animal Shelter**
BREAK			
4:30 pm	109	Melissa Beall	Detection of Canine Parvovirus Type 2c by a Commercially Available In-house Rapid Test
4:45 pm	110	Stephanie Janeczko	Prevalence of, Risk Factors for, and Assemblage Types of *Giardia* Infection in Cats Housed in an Animal Shelter
5:00 pm	111	Jody Gookin	Assessment of Reproductive Tract Disease in Cats at Risk for Enteric *Tritrichomonas foetus* Infection
5:15 pm	112	Matt Eberts	Use of Snap®4Dx® Assay to Monitor Tick-Borne Infection in Minnesota: A 4-Year Study
5:30 pm	113	Michael Lappin	Prevalence of Hemoplasma DNA in Field-Caught Mosquitoes in Colorado
5:45 pm	**114**	**Christina Bradbury**	**Topical Imidacloprid and Moxidectin Prevents Flea Transmission of *Bartonella henselae* in Cats**
6:00 pm	115	Katrin Hartmann	Outcome and Prognostic Factors in Dogs with Canine Leishmaniasis in a Non-Endemic Country

EQUINE**

2:15 pm	116	Mary Rose Paradis	The Use of Computer Tomography in the Diagnosis of Septic Arthritis/Osteomyelitis in the Neonatal Foal
2:30 pm	**117**	**Rosa Barsnick**	**Endocrine Energy Response in Septic Foals: Insulin, Leptin and Adiponectin**
2:45 pm	**118**	**Dominic Dawson**	**Opsonization of *Rhodococcus equi* with High Antibody Plasma Decreases Bacterial Viability and Promotes Phagocyte Activation**
3:00 pm	119	Lais Costa	Plasma Endothelin Concentrations in Septic and Non-Septic Neonatal Foals
3:15 pm	120	Jose L. Mendez	Thromboelastography (TEG): An Innovative Technique for Early Detection of Hemostatic Abnormalities in Foals with Septicemia
3:30 pm	121	Gayle Hallowell	Effects of Dobutamine and Atropine and Acetylpromazine on Aortic Valve Function in the Horse
3:45 pm	122	Andrew van Eps	Intravenous 99 m-technetium Liposomes in Horses: A Safety and Biodistribution Study
BREAK			
4:30 pm	123	Jill Muno	Prevalence and Risk Factors for Hyperinsulinemia in Clinically Normal Horses in Central Ohio
4:45 pm	124	Jill Muno	Seasonal Variation in Plasma Insulin and Glucose Concentration in Normal Horses in Central Ohio
5:00 pm	125	Nicholas Frank	Effects of a Supplement Containing Chromium and Magnesium on Insulin Sensitivity in Horses with Equine Metabolic Syndrome
5:15 pm	**126**	**Rebecca Funk**	**Seasonal Changes in the Combined Glucose Insulin Tolerance Test in Normal Aged Horses**
5:30 pm	127	Nicholas Frank	Effects of Sampling Time and Hay Feeding on Blood Glucose, Insulin, and Adrenocorticotropin Hormone (ACTH) Concentrations in Horses
5:45 pm	128	Nicholas Frank	Effects of Endotoxemia and Carbohydrate Overload on Glucose and Insulin Dynamics and the Development of Laminitis in Horses
6:00 pm	**129**	**Teresa Burns**	**Pro-Inflammatory Cytokine and Chemokine Expression Profiles of Various Adipose Tissue Depots of Insulin-Resistant and Insulin-Sensitive Light Breed Horses**

** *Also see Equine abstracts 49–60 (Thursday, June 4, 9:00 am – 12:30 pm) and Equine abstracts 142–143 (Thursday, June 4, 5:45 pm – 6:15 pm)*

FOOD ANIMAL

2:15 pm	130	Jody Gookin	An Animated Model of Reticulorumen Motility
2:30 pm	131	Alexandra Burton	A Retrospective Study of 106 Cases of Bovine Lymphosarcoma
2:45 pm	132	David Francoz	Determination of Dairy Cows Hematological Reference Interval by 3 Methods and Evaluation of Confounding Factors
3:00 pm	**133**	**Anita Varga**	**Correlation of Serum Cardiac Troponin I and Myocardial Damage in Cattle with Monensin Toxicosis**
3:15 pm	**134**	**Anita Varga**	**Validation of the I-STAT® Stallside Immunoassay for the Measurement of Bovine Cardiac Troponin I and its Clinical Relevance**
3:30 pm	135	Baljit Singh	RGD-Conjugated Helical Rosette Nanotubes Inhibit Chemotaxis and Cell Signaling of Cattle Neutrophils
3:45 pm	136	Michel Levy	Experimental Infection of Colostrum-Deprived Calves with Bovine Viral Diarrhea Virus Type-1 Isolated from Free-Ranging White-Tailed Deer (*Odocoileus virginianus*)
BREAK			
4:30 pm	137	Nathalie Kirschvink	Evidence for Transplacental Passage of Bluetongue Virus Seroytpe 8 in Sheep
4:45 pm	138	Nathalie Kirschvink	Long Term Follow up of Rams' Semen Quality after Natural Infection with Bluetongue Virus Serotype 8
5:00 pm	**139**	**Teresa Buchheit**	**Prevalence of BVDV on Alpaca Farms in Eastern and Middle Tennessee**
5:15 pm	140	Mireille Meylan	Seroprevalence of Bovine Viral Diarrhea in New World Camelids in Switzerland and Implications for the Eradication Campaign in the Bovine Population
5:30 pm	141	Mireille Meylan	Tuberculosis Caused by *Mycobacterium microti* in New World Camelids

EQUINE**

5:45 pm	142	Jennifer Davis	Pharmacokinetics of Intravenous Enrofloxacin and its Active Metabolite Ciprofloxacin in Hospitalized Horses
6:00 pm	143	James Belknap	Laminar Leukocyte Accumulation and Epithelial Stress in Horses with Carbohydrate Overload-Induced Laminitis

** *Also see Equine abstracts 49–60 (Thursday, June 4, 9:00 am – 12:30 pm) and Equine abstracts 116–129 (Thursday, June 4, 2:15 pm – 6:15 pm)*

ORAL PRESENTATIONS – Friday, June 5

SMALL ANIMAL – RESPIRATORY

8:00 am	144	Elizabeth Rozanski	The Effect of Tracheal Lavage Administration on Lung Function in Healthy Kittens
8:15 am	145	Laura Nafe	Utility of Biomarkers in Bronchoalveolar Lavage Fluid for Discrimination of Asthma and Chronic Bronchitis in Cats

8:30 am	**146**	**Tekla Lee-Fowler**	**Comparison of Intradermal Skin Testing and Allergen-Specific Serum Immunoglobulin E (IgE) in Experimental Feline Asthma**
8:45 am	**147**	**Jason Eberhardt**	**Chronic Administration of feG-COOH in Experimental Feline Asthma**
9:00 am	148	Ray Dillon	Progression of Circulating Antigen and Antibody Responses in Cats Experimentally Infected with *Dirofilaria immitis*
9:15 am	149	Anne Wooldridge	Isometric Responses of Isolated Intrapulmonary Bronchioles from Cats with and without Adult Heartworm Infection
9:30 am	150	Henna Heikkilä	Matrix Metalloproteinase -2 and -9 in Bronchoalveolar Lavage Fluid of Dogs with Idiopathic Pulmonary Fibrosis
9:45 am	151	Baljit Singh	Recruitment of Pulmonary Intravascular Macrophages in Immune-Mediated Hemolytic Anemia In Dogs

SMALL ANIMAL – HEPATOLOGY

8:00 am	152	Cynthia Webster	Cyclic AMP Attenuates Fatty Acid Induced Apoptosis in an in vitro Model of Hepatic Lipotoxicity
8:15 am	153	Kenny Simpson	Culture-Independent Detection of Bacteria in Feline Inflammatory Liver Disease

SMALL ANIMAL – IMMUNOLOGY

8:30 am	**154**	**Todd Archer**	**Development of a Flow Cytometric Panel of T-Lymphocyte Biomarkers to Evaluate the Immunosuppressive Effects of Cyclosporine in Dogs**

SMALL ANIMAL – GASTROENTEROLOGY

8:45 am	155	Niels Gruetzner	Evaluation of Serum Concentrations of Immunoglobulin A and Immunoglobulin M in Chinese Shar Peis with Cobalamin Deficiency
9:00 am	156	Aarti Kathrani	Genetical Analysis of Toll-Like Receptor Genes in German Shepherd Dogs Reveals Potential Polymorphism Associated with Inflammatory Bowel Disease
9:15 am	157	Iwan Burgener	Urinary Leukotriene E4 Excretion Is Increased in Dogs with Chronic Enteropathies
9:30 am	158	Melanie Craven	High Throughput Pyrosequencing Reveals Reduced Bacterial Diversity in the Duodenal Mucosa of Dogs with IBD
9:45 am	159	Melanie Craven	*E. Coli* Associated with Granulomatous Colitis of Boxer Dogs Frequently Manifest Resistance to Antibiotics
2:15 pm	**160**	**Susan Simmerson**	**Description of Protein-Losing Enteropathy in Yorkshire Terrier Dogs using the W.S.A.V.A. Gastrointestinal Classification System**
2:30 pm	161	Joshua Hobbs	Doppler Ultrasound Analysis of Gastrointestinal Blood Flow for Differentiating Food Allergic from Non-Food Allergic Pruritic Dogs
2:45 pm	**162**	**Heather Graham**	**Effects of Prednisone or Prednisone with Ultralow-Dose Aspirin on the Gastroduodenal Mucosa of Healthy Dogs**
3:00 pm	163	Panagiotis Xenoulis	Serum Triglyceride Concentrations in Miniature Schnauzers with and without a History of Pancreatitis
3:15 pm	164	Lisa Prior	Serial Evaluation of Canine Pancreatitic Lipase (Spec cPL™) in Dogs with Clinical Signs of Pancreatitis
3:30 pm	165	Marnin Forman	Evaluation of Feline Pancreas-Specific Lipase (Spec fPL™) for the Diagnosis of Feline Pancreatitis
3:45 pm	**166**	**Kelly McCord**	**A Multi-Institutional Study Evaluating Diagnostic Utility of Spec cPL™ in the Diagnosis of Acute Pancreatitis in Dogs**
BREAK			
4:30 pm	167	Suzanne Bailey	Clinical Significance of Increased Serum Feline Pancreatic Lipase Immunoreactivity Concentrations in Cats with Inflammatory Bowel Disease
4:45 pm	168	Dennis O'Brien	Serum D-Lactate Concentrations in Cats with Gastrointestinal Disease
5:00 pm	169	Nora Berghoff	Association of Serum Cobalamin and Methylmalonic Acid Concentrations in Dogs
5:15 pm	**170**	**Kelly McCord**	**Comparison of Gastrointestinal Motility in Dogs Treated with Metoclopramide, Cisapride, Erythromycin or Maropitant Using the SmartPill™**
5:30 pm	171	Frederic Gaschen	Variability Associated with Repeated Measurements of Gastric Emptying using the SmartPill pH.p Wireless Capsule and Scintigraphy in Dogs
5:45 pm	172	Michael Lappin	The Esophageal Transit Time of Tablets or Capsules in Cats following Administration with FlavoRx® Pill Glide or Pill Delivery Treats
6:00 pm	173	Diane Frank	Gastrointestinal Disease in Dogs with Excessive Licking of Surfaces

POSTER PRESENTATIONS

On Display: Thursday, June 4, 7:00 am–10:00 pm Friday, June 5, 7:00 am – 10:00 pm Saturday, June 6, 7:00 am – 12:30 pm

Attended by Author: Thursday, June 4, 7:00 am – 9:00 am; Friday, June 5, 9:50 am – 10:30 am; & Friday, June 5, 6:00 pm – 7:30 pm (Wine & Cheese Reception)

#	Presenting Author	Abstract Title
SMALL ANIMAL – NEUROLOGY		
174	Byeong-Teck Kang	High Resolution Magnetic Resonance Imaging of the Canine Brain at 7 Tesla: A Comparison Study with 0.2 and 1.5 Tesla
175	Alberta de Stefani	Clinical Signs, Magnetic Resonance Imaging Findings and Survival in Dogs with Intracranial Meningiomas and Glial Cell Tumours
176	**Michaela Cautela**	**Oral Hydroxyurea Therapy for Dogs with Suspected Intracranial Meningioma: A Retrospective Cohort Study (2004–2009)**
177	Christopher Mariani	Immune Cell Infiltration into Canine Meningiomas
178	Byeong-Teck Kang	Fluorodeoxyglucose Positron Emission Tomography and Magnetic Resonance Imaging Findings of Non-Suppurative Meningoencephalitis in Seven Dogs
179	Arianna Negrin	Steroid Responsive Meningitis-Arteritis Treatment with Three Potential Different Protocols: Clinical Signs, Laboratory and Long Term Follow Up in 48 Dogs
180	Byeong-Teck Kang	Three-dimensional Time-of-Flight MR Angiography of Intracranial Vessels in a Canine Model of Ischemic Stroke with Permanent Middle Cerebral Artery Occlusion
181	Maria Luisa Suarez	Prevalence of Cerebral Microbleeds in Aged Dogs
182	**Fred Wininger**	**Preliminary Observations between Cox-2 Inhibitor Administration and Acute Cerebrovascular Disease in Dogs**
183	Renee Barber	Multiplex Analysis of Cytokines in the Cerebrospinal Fluid of Dogs after Stroke
184	N. Matthew Ellinwood	Validation of R&D Systems Kits to Detect Cytokine Levels in Canine Cerebral Spinal Fluid
185	Ronaldo da Costa	Magnetic Resonance Imaging Findings in 60 Dogs with Cervical Spondylomyelopathy
186	Hiroaki Kamishina	Phosphorylated Neurofilament NF-H as a Biomarker of Spinal Cord Injury in Dogs
187	Natasha Olby	Epidural and Intradural Scarring Post Laminectomy and Durotomy: A Comparison of Gelfoam and SentrX Film
188	**Allison Haley**	**Breed Specific Polymyositis in the Hungarian Vizsla Dog**
189	Richard Piercy	Mutational Analysis of Dystrophin-Deficient Muscular Dystrophy in Cavalier King Charles Spaniels
190	Edward (Ned) Patterson	Frequency of the Canine Exercise Induced Collapse Gene in Diverse Breeds
191	Charles Vite	BRDU Labeling Pattern of the Rostral Migratory Stream in Normal Canine and Feline Brains
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193	Ji-hey Lim	Neural Stem Cell Sources in Adult Dogs
194	German Santamarina	Dogs with Cortical Beta-Amyloid Pathology Have Less Serotonergic Neurons in the Rostral Raphe Nuclei than Aged Matched Healthy Controls
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198	Emily Marcus	Flow Cytometric Prognostic Factors in Canine B Cell Lymphoma
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200	Timothy Stein	Effects of Mutant Beta-Catenin on Hepatocyte Growth Homeostasis ***(VCS Award Winner)***
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202	Shiguang Yu	Aging Associated Changes in Blood Cells of Cats
203	Rebecca Haviland	Clopidogrel Therapy in Dogs with Immune-mediated Hemolytic Anemia
204	Sharon Center	Platelet P-Selectin (CD62P) Expression and Platelet-Leukocyte Interactions in Clinically Healthy Dogs with and without Ultralow-Dose Aspirin
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209	Beate Egner	Evaluation of HDO® (High Definition Oscillometry) – A New NIBP Technique – In Comparison to Invasive Measurement (HSE – Hydro Sachs Electronics) in Anesthetised Dogs
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212	Alora Carrier	Analytical Validation of a Commercially Available Feline N-Terminal Prohormone Brain Natriuretic Peptide ELISA
213	Katie Cyr	Measurement of Canine B-Type Natriuretic Peptide Using a Human Analyzer
214	**Kristine Yee**	**Intraday Variation in Feline N-Terminal Prohormone Brain Natriuretic Peptide Concentration**
215	Nobuyuki Kanno	Plasma Adrenomedullin as a Biomarker for Severity of Pulmonary Hypertension in Dogs
216	Maria Helena Matiko Akao Larsson	Pulsed Tissue Doppler Evaluation in Dogs with Idiopathic Dilated Cardiomyopathy
217	Maria Helena Matiko Akao Larsson	Evaluation of Left Ventricular Systolic and Diastolic Function Using Pulsed Tissue Doppler in Dogs with Chronic Mitral Valve Insufficiency
218	Miki Nishida	Pathological Findings in Dogs with Mitral Valve Insufficiency: Biopsies during Open Heart Surgery with Cardiopulmonary Bypass
219	Miki Nishida	Long-Term Survival of Two Dogs after Mitral Valve Plasty using Expanded Polytetrafluoroethylene Chords: Pathological Study
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221	Takeshi Mizuno	Intra- and Post-Operative Complications in 47 Dogs that Underwent Mitral Valve Plasty
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224	Yoko Fujii	LV 2D Strain Analysis Pre- and Post- Balloon Valvuloplasty in Dogs with Pulmonary Stenosis
225	Yoshinori Nishijima	Effects of Bisoprolol on Ventricular Myocyte Electrophysiology during Chronic Heart Failure
226	Megan Rector	Heart Rate in Irish Wolfhounds with Lone Atrial Fibrillation & Assessment of Digoxin as Sole Method of Rate Control
227	Jonathan Abbott	Prevalence of the Myosin-Binding Protein C Mutation in Apparently Healthy Cats of Diverse Lineage
228	Ann-Sabin Berli	The Use of Strain, Strain Rate, and Displacement by 2D Speckle Tracking for Assessment of Systolic Left Ventricular Function in Goats: Applicability and Influence of General Anesthesia

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230	Yan-Ann Jhuo	Clinical Application of Barometric Whole-Body Plethysmography and Bronchoprovocation Test in Feline Bronchial Disease
231	**Michelle Patrick**	**The Quality of Canine Bronchoalveolar Lavage Fluid Cytology is Preserved for up to 72 Hours with or without Autologous Serum**
232	Stephanie Pumphrey	Clinical and Laboratory Characteristics of 35 Dogs with Pulmonary Thromboembolism (2002–2008)
233	M Josefa Fernandez Del Palacio	Comparative Study of the Tolerance and Efficiency of Four Different Techniques for Nebulisation Delivery in Cats
234	Carol Reinero	Utility of Tepoxalin, a Dual Lipoxygenase and Cyclooxygenase Inhibitor, in Canine Chronic and/or Eosinophilic Bronchitis
235	Eric de Madron	Treatment of Tracheal Collapse with Vet-Stents® in 36 Dogs: Complications and Long-Term Results

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237	Johanna Rieder	Comparison of Wireless and Conventional Continuous Glucose Monitoring Systems (CGMS) in 4 Dogs
238	Richard Nelson	Extended Use of Protamine Zinc Recombinant Insulin for Treating Diabetes Mellitus in Cats
239	Pascale Smets	Urinary Markers and Plasma Iohexol Clearance in Healthy Dogs and Dogs with Pituitary-Dependent Hyperadrenocorticism

240	Kellie Fecteau	Effect of Phytoestrogens and Melatonin on Adrenal Steroidogenesis Using Human Adrenal Cells as a Model for Canine Hyperadrenocorticism
241	Linda Martin	Effects of Low Doses of Cosyntropin on Cortisol Concentrations in Clinically Healthy Cats
242	Linda Martin	Dose Response of Aldosterone to ACTH Stimulation in Clinically Healthy Cats
243	**Bronwyn Rutland**	**Determination of the Optimal Time for Testing Thyroid Hormones during Treatment with Methimazole in Healthy and Hyperthyroid Cats**
244	Kate Hill	The Efficacy and Safety of a Novel Lipophilic Formulation Methimazole for the Once Daily Transdermal Treatment of Cats with Hyperthyroidism

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246	Debbie Minikhiem	Effect of *Bifidobacterium animalis* AHC7 on Resolution of Acute Diarrhea in the Canine
247	Jose F Garcia	Evaluation of Intra-Stool Variability of Three Lactic Acid Bacterial Genera in Dogs by Quantitative Real-Time PCR
248	**Jodi Nicklas**	**Molecular and Histopathological Characterization of Enteroadherent Bacteria in Failure to Thrive Kittens**
249	Melanie Craven	Absence of a Bacterial Association in Yorkshire Terriers with Protein-Losing Enteropathy and Cystic Intestinal Crypts
250	Panagiotis Xenoulis	Serum fPLI and Spec fPL Concentrations in Cats with Experimentally Induced Chronic Renal Failure
251	**Brier Bostrom**	**Immunohistochemical Characterization of Canine Pancreata with Lymphocytic Infiltration**
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253	Niels Gruetzner	Association Study of Single Nucleotide Polymorphisms with Cobalamin Deficiency in the Chinese Shar Pei
254	Albert Jergens	Apoptosis in the Pathogenesis and Treatment of Canine Inflammatory Bowel Disease
255	Isabelle Ruhnke	Feasibility of a Modified Ussing Air Suction Chamber for the Evaluation of Intestinal Mucosal Function in Endoscopic Biopsies from Dogs and Cats
256	Allison Bradley	Comparison of Gastrointestinal Motility in Dogs Treated with Iberogast™ and Ondansetron using the Smartpill™
257	**Bettina Mayer-Roenne**	**Successful Treatment of Benign Esophageal Strictures with Balloon Dilation and Submucosal Triamcinolone Injection in Five Dogs and One Cat**
258	**Adam Ginman**	**Small Animal Gastrointestinal Endoscopy: A Computer-Aided Instructional Program**

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260	**Andrea Johnston**	**Hepatic Copper Concentrations in Labrador Retrievers with and without Chronic Hepatitis (1980–2008): An Emerging Syndrome or Oversupplementation?**
261	Angela Au	Reduction of Cytokine-Induced Prostaglandin E2 and Chemokine Production in Cultured Canine Hepatocytes by Natural Products
262	Melinda Camus	Serum Protein Electrophoresis Beta-Gamma Bridging Is a Poor Predictor for Hepatic Disease

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264	Ben Young	Influence of Food Consumption on Variation of Urinary Volume, pH, Analytes, and Relative Supersaturation for Calcium Oxalate and Struvite in Healthy Adult Male Hound Dogs
265	Bernhard Gerber	Alpha 1-Acid Glycoprotein, Serum Amyloid A, and Monocte Chemoattractant Protein-1 in Cats with Idiopathic Cystitis
266	Ryan Keegan	Oxidative Stress in Cats with Chronic Renal Failure
267	Marcia Kogika	Erythrocyte Zinc Protoporphyrin Evaluation in Cats with Chronic Renal Disease
268	Christiane Prosser	Sequential Evaluation of Erythrocyte Zinc Protoporphyrin in Cats with Chronic Renal Disease
269	Shigeki Yamano	Endogenous Erythropoietin Levels and Iron Utility in Cats with Chronic Kidney Disease
270	Natalie Finch	Is Cadmium Exposure Associated with Chronic Kidney Disease in Cats?
271	Jerome Seguela	Glomerular Filtration Rate in Healthy English Pointer, English Setter and German Shepherd Dogs
272	David Grant	24-Hour Water Consumption in Healthy Cats using a Fountain versus a Bowl
273	Courtney North	Clinical and Genetic Characterization of a Congenital Entlebucher Mountain Dog Urinary Tract Syndrome
274	Mary Nabity	Proteomic Analysis of Urine from Male Dogs in Early Stages of Progressive Tubulointerstitial Injury Due to X-linked Hereditary Nephropathy (XLHN)

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275	Nolan Frantz	Metabolomic Profiling of Plasma in Arthritic vs Non-Arthritic Dogs
276	Nolan Frantz	Metabolomic Profiling of Plasma in Arthritic vs Non-Arthritic Cats
277	Ryan Yamka	Metabolomic Profiling of Plasma in Lean vs Obese Dogs
278	Yuka Mitsuhashi	Determination of Maintenance Energy Requirement of Cats after Spaying
279	Márcia Jericó	Assessment of Metabolic Syndrome Occurrence in Obese Client-Owned Dogs
280	Amy Chamberlin	A Vegetarian Dietary Oil Source of γ-linolenic Acid Results in Arachidonic Acid Accumulation in Feline Uterine Tissues: Evidence of Delta-5 Desaturation
281	Ji-Houn Kang	Effect of Parenteral L-alanyl-L-glutamine Supplementation on Phagocytic Responses of Canine Peripheral Blood Polymorphonuclear Neutrophilic Leukocytes Exposed to Methylprednisolone Sodium Succinate

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283	Robin Butchin	Bioavailability of a Novel, Bioenhanced Preparation of Curcumin in Dogs
284	Meaghan Whealen	Bioavailability of a Novel Formulation of S-Adenosylmethionine in Beagle Dogs
285	Steven Budsberg	In vivo Effects of Firocoxib, Meloxicam and Tepoxalin Adminstration on Eicosanoid Production in Target Tissues of Normal Cats

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286	Elise Gingrich	Intestinal Parasites of Dogs on the Galapagos Islands
287	Merle Olson	Canadian Prevalence and Diagnosis of Giardia Infection in Dogs and Cats using a Fecal Antigen Test and Fecal Smear
288	Sahatchai Tangtrongsup	Prevalence and Molecular Analysis of *Giardia* spp. Infections in Dogs in Chiang Mai, Thailand: Preliminary Findings
289	Nipattra Debavalyi	Serotypes and Pathogenicity Associated with Antimicrobial-Induced Resistance in Fecal *Escherichia coli* of Healthy Dogs
290	Julia Veir	Comparison of Quantitative PCR and Conventional Endpoint PCR for Amplification of Parvovirus DNA in Blood from Naturally Infected and Recently Vaccinated Dogs
291	**E. Freya Kruger**	**The Epidemiology of *Streptococcus canis* Infections in Dogs and Cats**
292	Jean-Pierre Pages	Bacterial and Fungal Colonization of Peripheral Intravenous Catheters in Dogs and Cats
293	Mary Marcondes	Determination of Four IgG Subclasses in Asymptomatic Dogs with Visceral Leishmaniasis and in Animals Vaccinated against the Disease
294	Regis Krah	Performance Evaluation of Species-Specific Peptide-Based Assays for Detection of Canine Antibodies to *E. canis* and *E. chaffeensis*
295	Ramaswamy Chandrashekar	*Ehrlichia*/*Anaplasma* spp. Infection and Exposure Rates in Dogs from Oklahoma and Arkansas
296	Pedro Paulo Diniz	Efficacy of Doxycycline Treatment in Dogs Naturally Infected with *Anaplasma phagocytophilum*
297	Audra Fenimore	*Bartonella* spp. Associated Endocarditis in Dogs in Colorado and Wyoming
298	**Jeanne Ficociello**	**Detection of *Bartonella henselae* IgM in Cats with Experimentally Induced Bartonellosis**
299	James Eucher	Prevalence of *Bartonella* and Haemoplasma DNA in Samples from Non-Owned Free-Roaming Cats Admitted to Animal Shelters in Colorado and Florida
300	Christian Leutenegger	Association of Pathogenicity and Rate of Coinfection of Three Feline Hemotropic Mycoplasma Strains with Presence and Severity of Anemia
301	Christian Leutenegger	Analytical Sensitivity and Specificity of a Real-Time PCR Assay Detecting Pathogenic Leptospira in Dogs Based on the hap-1 Gene
302	Kristy Dowers	Prevalence of Infectious Agents and Anti-Erythrocyte Antibodies in Cats with Anemia
303	Michael Lappin	*Bartonella henselae* Antigen Recognition Patterns in the Serum and Aqueous Humor of Cats with and without Uveitis
304	Michael Lappin	Effect of Orbifloxacin or Doxycycline Administration on *Bartonella* spp. and Hemoplasma Test Results from Naturally-Exposed Cats
305		Abstract Withdrawn
306	Jacqueline Whittemore	Alpha Enolase and Annexin A2 Antibodies in Cats
307	Kristopher Curtis	Prevalence of Feline Immunodeficiency Virus Antibody and Feline Leukemia Virus Antigen in Samples Submitted to IDEXX Reference Laboratories for Feline Hemotropic Mycoplasma Testing
308	Yasuyuki Endo	An Updated Epidemiological Survey of Feline Immunodeficiency Virus Infection in Japan
309	Mitika Hagiwara	Evaluation of CD4+ and CD8+ T Lymphocytes in Cats Naturally Infected with Feline Leukemia Virus
310	Archivaldo Reche Junior	Cutaneous Mycoflora and CD4+:CD8+Ratio of Cats Infected with Feline Immunodeficiency Virus
311	Archivaldo Reche Junior	Prevalence of Feline Calicivirus and Herpesvirus Type 1 in Maine Coon Cats with Chronic Gingivitis

SMALL ANIMAL – OTHER

312	Sara Zaldivar-Lopez	Disease Prevalence and Causes of Death in American Kennel Club Registered Greyhounds
313	Paulo Vilar Saavedra	Thromboelastographic Changes after Gonadectomy in Retired Racing Greyhounds

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314	Daniela Bedenice	Immunologial Responses in Persistently BVDV Infected Alpacas
315	Claire Whitehead	Humoral Response to Bluetongue Vaccination in Alpacas

FOOD ANIMAL – INFECTIOUS DISEASE

316	**Marcio Costa**	**Incidence of *Clostridium difficile* in Feces of Male Holstein Veal Calves**

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317	**Jennifer Sonis**	**Prairie Rattlesnake Envenomation in 25 New World Camelids**

EQUINE – CARDIOLOGY

318	Marc Kraus	Concentrations of Cardiac Tropinin I Measured with an I-Stat® I Analyzer in Normal Horses Undergoing a Standard Treadmill Performance Examination
319	Rose Nolen-Walston	The Effects of Dehydration on Central Venous Pressure in Adult Horses
320	**Joan Norton**	**Comparison of Water Manometry to Two Electronic Pressure Monitors for Central Venous Pressure Measurement in Horses**
321	**Joan Norton**	**Repeatability and Effect of Head Position on Central Venous Pressure Measurement in Standing Adult Horses**
322	Olga Seco	Cardiac Troponin I Concentrations in Horses Referred for Colic

EQUINE – ENDOCRINOLOGY

323	**Rosa Barsnick**	**Association of Growth Hormone and Ghrelin with the Energy Metabolism and Mortality in Septic Foals**
324	**Rebecca Funk**	**Seasonal Changes in Plasma Alpha-Melanocyte-Stimulating Hormone and Adrenocorticotropic Hormone in Response to Thyrotropin-Releasing Hormone Administration in Normal Aged Horses**
325	John Haffner	Effect of Insulin Infusion into the Digital Artery on Glucose Concentrations in the Digital Vein in Healthy Horses
326	Johan Brojer	Validation of a Species Specific Enzyme-Linked Immunosorbent Assay for Measurement of Serum Insulin in Horses
327	Harold Schott II	Hyperinsulinemia in Horses with Pituitary Pars Intermedia Dysfunction and Effects of Housing on Insulin Response during a Glucose Tolerance Test

EQUINE – HEMATOLOGY

328	Susana Macieira	Characterization of the cDNA Sequence Encoding for the Platelet Integrin AlphaIIb and Beta3 in a Horse with Glanzmann Thrombastenia
329	**Maeva May**	**Comparison of Hematologic and Biochemical Values Obtained via Intravenous Catheter Versus Venipuncture in Hospitalized Horses**

EQUINE – IMMUNOLOGY

330	Jennifer Day	Effects of Lipopolysaccharide, Lipoteichoic Acid, and Peptidoglycan on Tumor Necrosis Factor Production in Equine Whole Blood Culture
331	Britta Leise	Laminar Inflammatory Gene Expression in the Carbohydrate Overload Model of Equine Laminitis
332	Brett Sponseller	IL-10 Downregulates Production of Peroxynitrite in Equine Monocyte-Derived Macrophages: Implications for Rhodococcal Infection
333	Jarred Williams	Effect of Intravenous Lidocaine Administration on Leukocyte Emigration in the Black Walnut Extract Model of Laminitis

EQUINE – INFECTIOUS DISEASE

334	Stephanie Bell	The Immune Response of Foals to Natural Infection with Equid Herpesvirus-2 and its Association with Febrile Illness
335	Emir Hodzic	Use of Quantitative Real-Time PCR for the Detection of *Salmonella* sp. in Fecal Samples of Horses Admitted to a Veterinary Medical Teaching Hospital
336	Alma Roy	Comparison of PCR Testing for Management of an Outbreak of EHVI at a Racetrack
337	**Katharyn Mitchell**	**Salmonella Oranienburg in a Large Animal Veterinary Teaching Hospital**

EQUINE – NEPHROLOGY/UROLOGY

338	Harold Schott II	Paradoxical Aciduria in Horses: 37 Cases (2000–2008)

EQUINE – NEUROLOGY

339	Cristina Sogaro-Robinson	Diagnostic Utility of Computed Tomography of the Head in Horses Affected by Neurologic Disorders

EQUINE – NUTRITION/METABOLISM

340	Anna Firshman	The Effect of Metformin on Insulin Sensitivity in Horses
341	David Lewis	The Positive Interaction of Propionate, Insulin, and Cortisol in the Horse
342	Jessica Suagee	Acute Hyperinsulinemia Modulates Expression of Genes Involved in Glucose and Fat Metabolism in Subcutaneous Adipose Tissue of Horses
343	Kibby Treiber	Insulin Sensitivity in Ponies Is Influenced by Season and Pasture

EQUINE – OTHER

344	James Belknap	Mononuclear Leukocytes in the Laminae of Normal Horses and Those with Black Walnut Extract-Induced Laminitis
345		Abstract withdrawn
346	Lee Ann Fugler	Evaluation of Various Matrix Metalloproteinase Inhibitors (MMPIs) in the Horse
347	**Cristobal Navas de Solis**	**Ultrasonographic Measurement of Spleen Volume in Horses**

EQUINE – PHARMACOLOGY

348	Grace Forbes	Effects of Flunixin Meglumine on Leukocyte Activation in a Low-dose Endotoxemia Model in Horses
349	Jennifer Davis	The Pharmacokinetics of Intravenous and Intramuscular Buprenorphine in the Horse
350	Cynthia North	Bioavailability of Detomidine Administered to Horses as an Oromucosal (Sublingual) Gel and Comparison of Absorption of Detomidine by the Sublingual and Intramuscular Routes
351	Elizabeth Davis	Pharmacokinetics of Orally Administered Terbinafine in Horses
352	**Jessica Thompson**	**Assessment of Cardiac Function, Skeletal Muscle Morphology, and Muscle Enzymes in Horses on Long-Term Clenbuterol Therapy**
353	**Dorothy Whelchel**	**Effects of Clopidogrel on Platelet Function in the Horse**

EQUINE – RESPIRATORY

354	Renaud Leguillette	Upper and Lower Airway Endoscopic Findings and Bronchoalveolar Lavage-Induced Lower Airway Collapse in Equine Respiratory Inflammatory Diseases

ABSTRACT #1

USE OF POLYMERASE CHAIN REACTION TO DETECT BACTEREMIA IN DOGS WITH BACTERIAL ENDOCARDITIS. AM Heaney[1], KM Meurs[1], JL Oaks[1], PR Fox[2], TC DeFrancesco[3]. [1]Washington State University, College of Veterinary Medicine, Pullman, WA, [2]The Animal Medical Center, Caspary Institute, New York, NY, [3]North Carolina State University, Raleigh, NC.

Infectious endocarditis (IE) is associated with substantial morbidity and mortality. Outcome is influenced by effectiveness of antimicrobial therapy. The reliability of blood cultures to identify inciting pathogens has been disappointing. Panbacterial polymerase chain reaction (PCR) used on DNA extracted directly from human valve tissue has resulted in a threefold increase in sensitivity compared to blood cultures. Application of panbacterial PCR primers to canine blood samples holds promise to identify the offending bacteria and improve of antimicrobial therapy. Panbacterial PCR amplification (using primers to amplify the conserved 16 s ribosomal bacterial DNA) with direct sequencing to identify the organism would be beneficial if it allows earlier targeted therapy.

Our hypothesis was that PCR can be used to identify circulating bacteria in canine IE patients.

Our objective was to perform PCR with primers targeted to amplify the 16 s ribosomal bacterial DNA from IE suspects' blood samples and compare these findings with those obtained from blood cultures.

Thirteen dogs with IE were studied. Diagnosis was based upon history, physical examination, and echocardiographic examination. All had PCR perfomed and 9 of the 13 also had blood cultures. Two cases were positive using standard blood culture techniques yielding a sensitivity of 22.2%. PCR was positive in 4 of 13 cases yielding a sensitivity of 30.8%

In conclusion, bacteremia was detected by PCR in 4 out of 13 dogs with IE. Panbacterial PCR is a viable and potentially useful test to detect bacteremia in dogs with IE and warrants further investigation. Case enrollment is ongoing.

ABSTRACT #2

THE EFFECT OF FUROSEMIDE AND PIMOBENDAN ON THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM (RAAS) IN DOGS. AC Lantis, CE Atkins, TC DeFrancesco, BW Keene. North Carolina State University College of Veterinary Medicine, Raleigh NC.

We hypothesized that the labeled dosage of pimobendan would not have an additive effect on furosemide-induced RAAS activation. Furthermore, we asked whether furosemide's effect on RAAS would plateau with subacute administration.

Twelve healthy hounds were used. Group 1 (control) received furosemide (2 mg/kg PO, BID) for 10 days. Group 2 received furosemide (2 mg/kg PO, BID) and pimobendan (0.25 mg/kg PO, BID) for 10 days. To measure the response of the RAAS, urinary aldosterone:creatinine ratio (A:Cr) was determined on days 0, 5 and 10.

Urinary aldosterone secretion, as indicated by A:Cr, increased 3-fold with both furosemide monotherapy and the combination of pimobendan and furosemide at both days 5 and 10. There was no evidence of synergism between pimobendan and furosemide in terms of RAAS activation. Furosemide's effect on RAAS appears to plateau at 5 days or sooner but is maintained for at least 10 days.

We have shown that pimobendan, at the labeled dosage, does not accentuate furosemide- induced RAAS activation. We observed a three-fold increase in RAAS activity with furosemide alone and in combination with pimobendan. Therefore, furosemide, with or without pimobendan, is not recommended for chronic use in the absence of concurrent therapy to blunt RAAS activity, such as ACEI, aldosterone receptor blockers, or angiotensin II type I receptor blockers.

ABSTRACT #3

ASSESSMENT OF CHANGE IN LEFT VENTRICULAR SYSTOLIC FUNCTION BY TISSUE DOPPLER IMAGING METHODS AFTER CLOSURE OF PATENT DUCTUS ARTERIOSUS IN DOGS. LJM Keller, M Killich, K Hartmann, G Wess. Clinic of Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

Patent ductus arterious (PDA) initially causes left ventricular (LV) volume overload; systolic dysfunction may develop later. Studies in veterinary medicine showed minimal or no improvement of left ventricular systolic volume indices after PDA closure indicating that myocardial remodeling in PDA is at most only partially reversible. Human long term follow-up studies using LV endsystolic volume measurements and evaluating ejection fraction (EF) showed, however, that systolic function deficits are reversible after closure. The aim of the present study was to determine systolic function in dogs with PDA before and after closure using systolic parameters of Tissue Doppler Imaging (TDI) and comparing them to endsystolic volume indices and EF in dogs.

In 31 dogs, echocardiographic studies including volume measurements and TDI parameters were performed before, one day after, and $\geq$3 months after interventional or surgical PDA closure. To evaluate LV volume indices and EF, the modified Simpson's method was used. Systolic Tissue Velocity, systolic Strain Rate, and Strain using Echo Pac 2D Strain® analysis (General Electric Medical Systems, Horten, Norway) were evaluated in the basal, midventricular, and apical segment of the interventricular septum. Systolic TDI parameters at $\geq$3 months after closure were compared to the TDI parameters of 49 age-matched healthy control dogs.

Systolic Tissue Velocity in all segments as well as systolic Strain Rate and Strain in the apical segment significantly decreased immediately after closure. At the $\geq$3 month follow-up, these parameters had reversed to normal, and there was no significant difference compared to preclosure values. In addition, TDI parameters at the $\geq$3 month follow-up did not show decreased values in comparison to healthy control dogs. Systolic volume indices did not change over the study period, whereas EF decreased and stayed low at long term follow-up.

PDA with a relevant amount of left-to-right shunting, left-to-right shunting causes LV remodeling. This is the first study in dogs that showed, however, that LV remodeling can be reversible after PDA closure using TDI methods. TDI in the present study showed that systolic function deteriorates immediately after closure but recovers within 3 months. Systolic volumes did not show any change over the study period, and EF decrease immediately after closure and stayed low at long-term follow-up. TDI seems to be a more sensitive tool and, therefore, should be preferred over volume measurements for the assessment of LV systolic function in congenital heart diseases.

ABSTRACT #4

CORRELATION OF N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE WITH LEFT VENTRICULAR OUTFLOW TRACT IN DOGS WITH SUB AORTIC STENOSIS. G Farace[1], SJ Ettinger[2], S Forney[3], A Beardow[1], A Carrier[1], K Yeung[1]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA, [3]Las Vegas Veterinary Referral Center, Las Vegas, NV.

In human medicine aortic stenosis has been shown to correlate with N-terminal prohormone brain natriuretic peptide (NTproBNP). This is to be expected because an obstruction in the aorta is likely to cause increased ventricular systolic pressures which in turn will increase the ventricular wall stress and thus cause a release of BNP and NTproBNP. Dogs with sub aortic stenosis (SAS) are likely to undergo similar pathophysioloic changes and so it is probable that there is a correlation between the left ventricular outflow tract (LVOT) peak flow velocity, severity of stenosis, and NTproBNP concentration

To test this hypothesis we retrospectively analyzed samples from 169 normal dogs of various breeds and ages and 69 dogs with varying degrees of SAS. Each dog was given a full cardiac exam including a radiograph and echocardiogram with Doppler flow analysis prior to collecting plasma for NTproBNP testing.

There is a moderate correlation (0.56) between LVOT and NTproBNP and this does not change if only dogs with a diagnosis of SAS are considered.

Grouping the population according to the severity of SAS (none, mild, moderate and severe) results in mean LVOT velocities of 1.46 (95% CI: 1.40–1.52), 2.62 (2.49–2.75), 3.58 (2.99–4.17) and 6.39 (5.83–6.96) m/s respectively and NTproBNP mean values of 361 (307–415), 344 (239–448), 1011 (86–1936) and 2529 (1841–3217) pmol/L. Clearly, there are significant differences in LVOT across the groups while NTproBNP is only significantly different from the normal population once the severity of the SAS reaches a moderate level. This would appear to suggest that while there is a change in the outflow tract velocity in those patients with mild SAS it is not large enough to cause hemodynamic changes resulting in BNP (and NTproBNP) release. However the data does suggest that NTproBNP can differentiate patients with moderate or severe disease from those with no disease or only mild disease. Indeed an ROC analysis gives an AUC of 0.86 and a negative predictive value of 96% at a cut-off of 713 pmol/L.

Mean NTproBNP levels in the moderate and severe groups are extremely high. Interpretive criteria would suggest these dogs were in congestive heart failure. This would make SAS a confounding factor for detecting heart failure associated with acquired cardiac disease were it not for the fact that the 2/3 of the dogs in this study with SAS are under the age of three.

In conclusion, NTproBNP correlates with LVOT and both correlate with severity of SAS.

ABSTRACT #5

NT-PROBNP CONCENTRATIONS IN CANINE CONGENITAL HEART DISEASE. AB Saunders[1], SG Gordon[1], MW Miller[1], SE Achen[1], RM Roland[1], LT Drourr[1], CD Hariu[1] and MA Oyama[2]. [1]The Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, [2]University of Pennsylvania, Philadelphia, PA.

Many canine congenital heart defects (CHD) such as patent ductus arteriosus (PDA) and pulmonic stenosis (PS) can be repaired or palliated with interventional or surgical procedures. Puppies with CHD may have murmurs that are undetected by primary care veterinarians, and CHD such as atrial septal defects (ASD) may go undetected in adult dogs. Echocardiography is indicated for definitive diagnosis, but is not always available to primary care veterinarians who may need to encourage clients to obtain a referral for definitive diagnosis.

The objective of this study was to report NT-proBNP concentrations in dogs with a variety of CHD. Thirty nine dogs with CHD (PDA=13, PS=20, ASD=6) and 6 young, healthy dogs were evaluated. All dogs had echocardiograms. Median NT-proBNP for PDA was 742 pmol/L (range, 50–3000), PS was 746 pmol/L (278–3000), ASD was 833 pmol/L (388–2194) and for normal dogs was 333 pmol/L (161–826). Dogs with PS had Doppler estimated right ventricular systolic pressures consistent with moderate (1/20; 1024 pmol/L) to severe (19/20) PS. Dogs with ASD had hemodynamically significant defects with moderate to severe right atrial and ventricular dilation. Dogs with PDA, PS and ASD had elevated NT-proBNP concentrations when compared to the normal dogs. NT-proBNP >354 and >826 pmol/L possessed sensitivity and specificity for detecting CHD (PDA, PS, ASD) of 89.7% and 66.7% and 46.5% and 100% respectively (area under curve=77.9%).

Further studies are needed to better characterize the clinical utility of NT-proBNP as a screening test for various canine congenital heart diseases.

ABSTRACT #6

UTILITY OF NT-PROBNP ASSAY TO DETECT OCCULT DILATED CARDIOMYOPATHY IN DOBERMAN PINSCHERS. N Morris[1], MA Oyama[2], ML O'Sullivan[3], GE Singletary[2], SG Gordon[4]. [1]Massachusetts Veterinary Cardiology Services, Pembroke MA. [2]University of Pennsylvania, Philadelphia, PA. [3]Ontario Veterinary College, Guelph ONT. [4]Texas A&M University, College Station, TX.

Detection of preclinical (occult) DCM is challenging. Blood testing for occult DCM represents a potential means of screening large numbers of dogs. We sought to determine ability of NT-proBNP to detect occult DCM in Doberman pinschers undergoing screening with Holter and echocardiography.

One-hundred Doberman pinschers were prospectively screened using Holter monitoring, echocardiography, and NT-proBNP assay. Occult disease was defined as LV systolic dimension greater than 38.8, 39.5, 40.2, 40.9, 41.6, 42.3, and 43.0 mm in dogs weighing up to 25, 30, 35, 40, 45, 50, and 55 kg respectively, or >50 VPCs on 24-hour Holter. Median NT-proBNP in dogs with occult DCM (n=33) was significantly higher than normal dogs (n=67) (occult, 552 [IQR, 318–1154] pmol/L; normal, 220 [IQR, 171–315]; $P<0.0001$). NT-proBNP >194, >315, and >478 pmol/L possessed sensitivity and specificity for detecting occult DCM of 90.9% and 40.3%, 78.8% and 76.1%, and 57.6% and 91.4%, respectively (area under curve=82.3%). With regards to how diagnosis of occult DCM was achieved, 5 dogs fulfilled echo criteria alone, 16 dogs fulfilled Holter criteria alone, and 12 dogs fulfilled both. NT-proBNP detected dogs with abnormal echo (AUC=91.6%) or abnormal echo and Holter (AUC=95.3%) better than dogs with only abnormal Holter (AUC=69.7%). NT-proBNP >478 pmol/L had 88.2% sensitivity and 91.4% specificity for detecting the 17 dogs with abnormal echo. The use of abnormal Holter or NT-proBNP >478 pmol/L possessed 86.4% sensitivity, 96.8% specificity, and 93% accuracy for detecting occult cases, suggesting that use of these two diagnostics could potentially be used to detect occult DCM.

ABSTRACT #7

EVALUATION OF NT-PROBNP IN THE DIAGNOSIS OF VARIOUS STAGES OF DILATED CARDIOMYOPATHY IN DOBERMAN PINSCHERS. G. Wess, V. Butz, M. Killich, A. Schulze, K. Hartmann. Clinic of Small Animal Internal Medicine, Ludwig Maximilian University of Munich, Germany.

Dilated cardiomyopathy (DCM) in Doberman pinschers is an autosomal inherited diseases with a very high prevalence in this breed. The disease is usually divided into three disease stages. The first stage consists of a genetic defect that cannot be diagnosed with current diagnostic tools. This stage is followed by an occult phase, during which dogs appear to be clinically healthy. The occult phase is characterized by ventricular arrhythmias, leading to sudden death in about 1/3 of the dogs. In the occult phase, dogs may or may not show abnormalities on echocardiography. This stage is followed by the clinical phase with typical echocardiographic findings of DCM. Previous studies have shown elevated NT-proBNP in dogs with congestive heart failure and in dogs with heart diseases without heart failure. The purpose of this study was to evaluate NT-proBNP in the diagnosis of DCM in Doberman pinschers in various disease stages.

The study included 484 NT-proBNP measurements of 324 dogs (187 male and 137 female Doberman pinschers, mean age 4,7 years). Samples were collected from dogs at various rechecks in different disease stages. Based upon echocardiography and 24-hour-ECG, the following groups were compared: healthy controls with normal echocardiographic exam and <50 VPCs in 24-hours-ECG (n=329), only ventricular premature contractions (VPCs) (n=64), only echocardiographic changes (n=26), VPCs and echocardiographic changes (n=31), decompensated stage (n=13) and finally the stage "still normal" consisting of dogs that were considered to be free of disease at the first exam, but that developed the disease later (n=17). Nt-proBNP was measured in plasma samples using an ELISA (VETSIGN Canine CardioSCREEN Nt-proBNP, Guildhay Ltd, UK).

NT-proBNP levels were significant different the between the control group and the group with only VPCs, the group with only echocardiographic changes, the group with both VPCs and echocardiographic changes and the decompensated group. Between the control group and the "still healthy" group only a trend, but no significant difference was detected. NT-proBNP had a sensitivity of 76,1% and a specificity of 76,9% using a cut-off value of 400 pmol/l to detect the occult phase of cardiomyopathy. Using the same cut-off value, the sensitivity reached 90%, the specificity 79% to detect dogs with echocardiographic changes. NT-proBNP increased with progressing disease stages. Even VPCs without echocardiographic changes led to increased NT-proBNP values. NT-pro-BNP is a useful additional test to screen for Doberman cardiomyopathy, but should not replace conventional tests.

ABSTRACT #8

SERIAL EVALUATION OF NT-proBNP IN DOGS WITH CHF PREDICTS CLINICAL SCORE AND THE PRESENCE OR ABSENCE OF RADIOGRAPHIC PULMONARY EDEMA. SE Achen[1], SG Gordon[1], RM Roland[1], AB Saunders[1], MM Boggess[2] and MW Miller[1]. The Michael E. DeBakey Institute, [1]College of Veterinary Medicine and Biomedical Sciences & [2]College of Science, Texas A&M University, College Station, TX.

Human studies have found NT-proBNP useful in clinical decision making in CHF. The objective of this prospective study was to evaluate serial NT-proBNP in dogs with CHF due to chronic valve disease (CVD). Evaluations included thoracic radiographs, blood work, BP, echocardiogram, NT-proBNP, and therapy changes.

Statistical analysis evaluated the predictive value of the change in NT-proBNP (ΔNT-proBNP) against clinical score (CS; −1= ↓ furosemide± ↑ pimobendan±fluid therapy, 0=no therapy change, 1= ↑ furosemide± ↑ pimobendan±parenteral furosemide) and radiographic cardiogenic infiltrate score (RIS; 0=none, 1=cardiogenic infiltrate). Eighteen dogs had 58 total serial evaluations. NT-proBNP between successive visits was calculated, giving 38 observations on 14 dogs. Median NT-proBNP was −170 pmol/L (range −2,000–3,700 pmol/L).

Using logistic and multinomial logistic models, with Huber-White standard errors to account for correlation of observations on the same dog, NT-proBNP is significantly predictive of both CS and RIS ($p<0.05$). For a ↓ in NT-proBNP ≥2,000 pmol/L, a CS of −1 was likely, with an 80% probability. For NT-proBNP between −1,000 and 2,000 pmol/L, a CS of 0 was likely, with a probability > 70%. For NT-proBNP >2,000 pmol/L, a CS of 1 was likely, with a 60% probability. A NT-proBNP >2,000 pmol/L, predicted an RIS=1 while a ↓ in NTproBNP, or an ↑ <2,000 pmol/L, predicted an RIS=0.

These results suggest serial evaluation of NTproBNP may facilitate management of CHF due to CVD.

ABSTRACT #9

THE UTILITY OF NT-PROBNP TO DETECT EARLY STAGES OF HYPERTROPHIC CARDIOMYOPATHY IN CATS AND TO DIFFERENTIATE DISEASE STAGES. G. Wess, P. Daisenberger, J. Hirschberger, K. Hartmann. Clinic of Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats. In practice, it is currently necessary to use echocardiography to confirm the diagnosis and to assess disease severity. Early stages of the disease often show no or only subtle clinical symptoms and might therefore not be detected if echocardiography is not available. N-terminal proBNP (Nt-proBNP) has no physiological activity, but is more stable than BNP and therefore easier to measure. Measurement of NT-proBNP concentration is helpful to distinguish cardiac from non-cardiac causes of dyspnea in cats, dogs, and humans. Previous studies have shown elevated NT-proBNP concentrations even in the occult phase of cardiomyopathy.

The purpose of this prospective study was to evaluate the utility of Nt-proBNP to detect early disease stages of HCM in cats and to assess whether a differentiation between mild, moderate, and severe disease stages is possible using NT-proBNP measurements.

Nt-proBNP was measured in plasma samples from 159 cats using an ELISA (VETSIGN Feline CardioSCREEN Nt-proBNP, Guildhay Ltd, UK). The cats were classified according to echocardiography into one of the following groups: (1) clinical healthy (control) group (n=33), (2) mild HCM with focal hypertrophy of the left ventricular (LV) free wall or septal hypertrophy between 5.5 and 5.9 mm (n=13), (3) moderate HCM with focal or generalized LV free wall or septal wall between 6.0 and 7.0 mm and normal left atrial size (LA) (n=12), and (4) severe HCM with LV free wall or septal wall > 7.0 mm and enlarged LA (n=43) and clinical symptoms.

Nt-proBNP was significantly higher in all HCM groups compared to healthy control cats. Mean NT-proBNP was 58±65 pmol/l in the control group (1), 333±244 pmol/l in the mild group (2), 433±299 pmol/l in the moderate group (3) and 835±314 pmol/l in the severe group (4). Values of the mild (2) and moderate (3) group cats were significantly lower then those of the severe (4) group cats, but there was no difference between the values of the mild (2) and moderate (3) group. It was shown that the recommended cut-off value of 49 pmol/l had a high specificity of 97.1% but only a specificity of 56% to differentiate healthy cats from cats with HCM. This low cut-off value revealed too many false positive results. Using a cut-off value of 100 pmol/l, Nt-proBNP had a sensitivity of 94.2% and a specificity of 81.3% for the differentiation of control and HCM cats and might therefore be preferable for use in practice. In conclusion, this feline ELISA Nt-proBNP assay is helpful in the diagnosis of even early cases of HCM in cats. Cats with elevated levels, therefore, should be further worked up with echocardiography.

ABSTRACT #10

PLUMONARY HYPERTENSION AND N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE IN DOGS. G Farace[1], SJ Ettinger[2], S Forney[3], A Beardow[1], A Carrier[1], K Yeung[1]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA, [3]Las Vegas Veterinary Referral Center, Las Vegas, NV.

N-terminal prohormone brain natriuretic peptide (NTproBNP) is usually viewed as a marker of left ventricular dysfunction but there is a significant body of literature in human medicine that shows a correlation between pulmonary hypertension (PH), right ventricular dysfunction and NTproBNP. In dogs it has been reported that B-type natriuretic peptide (BNP) is elevated in cases of PH but the degree of elevation was not correlated to the ISACHC class. PH may, therefore be a confounder if NTproBNP alone is used to diagnose disease. If the NTproBNP level correlates with the severity of PH it might be useful in staging disease.

The aim of this retrospective study was two-fold: firstly to determine if NTproBNP levels are elevated in dogs with PH independent of disease severity and to determine if NTproBNP correlates to PH severity.

374 dogs had their maximum tricuspid regurgitation pressure gradient (max TRPG) measured together with a blood draw upon entry into the California Animal Hospital Veterinary Specialty Group (CAHVSG)/IDEXX Cardiac Study. Those with congenital disease were excluded. (10) The remainder was divided according the magnitude of the max TRPG. Dogs with a max TRPG <30 mmHg were classified as normal; 31–50 mmHg were considered to have mild PH; 51–75 mmHg moderate and >75 mmHg had severe PH.

Plotting max TRPG against NTproBNP concentration gives a weak but significant correlation (0.17, $p=0.001$) thus indicating a relationship between these two measures. Comparing those dogs with PH to those with no PH shows a significant difference (1289 vs. 1076 pmol/L, $p=0.02$). However controlling for disease severity (according to the CAHVSG) there is no significant difference between the two groups at any individual grade.

The mean NTproBNP levels for the four groups of PH were: none – 1076 pmol/L (n=193), mild – 1185 pmol/L (n=132), moderate – 1686 pmol/L (n=24) and severe – 1573 (n=15). While this suggests that there is an increase in NTproBNP as PH increases significant overlap between groups occurs and only those dogs with moderate PH are significant elevated ($p=0.04$) over the dogs with no PH. Controlling for the disease severity fails to identify any significant difference between the four groups.

In conclusion, this study concurs with the study on BNP. NTproBNP is elevated in the presence of PH but that there is no correlation to the severity of cardiac disease or severity of PH when the evaluation is controlled for disease severity.

ABSTRACT #11

GENOME-WIDE ASSOCIATION IDENTIFIES A MUTATION FOR ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN THE BOXER DOG. KM Meurs[1], E Mauceli[2], G Acland[3], K Lindblad-Toh[2,4]. [1]Washington State University College of Veterinary Medicine, Pullman, WA, [2]Broad Institute of MIT and Harvard, Cambridge, MA, [3]Cornell University, Ithaca, NY, [4]Uppsala University, Uppsala, Sweden.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) in the Boxer dog may result in syncope, sudden cardiac death and the development of congestive heart failure. ARVC is more common in the boxer than in other breeds, suggesting a genetic predisposition in

this breed. There is a familial inheritance, possibly suggesting a major dominant gene. Based on the new tools available with the canine genome project, it is now possible to perform genome wide association with a canine 50k SNP array. The objective of this study was to identify the genetic alteration(s) associated with the disease.

A 10 Mb region of genome-wide significant association was identified on chromosome 17 (p genome < 0.02) using DNA samples from 48 adult boxer dogs with ARVC (>500 ventricular premature complexes (VPC)/24 hours) and 48 unaffected boxers (<100 VPC/24 hours). Fine-mapping of 24 additional SNPs within the region localized a signal to between two candidate genes of potential cardiac importance. All exons of the candidate genes and non-coding conserved elements within this region were re-sequenced in affected and unaffected dogs.

A 7 base pair deletion within a non-coding conserved element in a regulatory region of a calcium modulating gene was identified and observed to be highly associated with the disease status ($p<0.0001$). The deletion was not observed in unaffected Boxer dogs or in any of 31 dogs from other breeds tested. Affected dogs were either homozygous or heterozygous for the deletion. Homozygous dogs (N=15) had a statistically greater number of VPC/24 hours than heterozygous (mean of 7860 and 2845, respectively) ($p<0.05$).

We conclude that this 7 base pair deletion is associated with the development of Boxer ARVC and that dogs that are homozygous for the mutation appear to have a more severe degree of arrhythmias.

ABSTRACT #12

PHENOTYPIC DIFFERENCES IN THE ULTRASTRUCTURE OF CARDIOMYOCYTES FROM BOXER DOGS AFFLICTED WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY (ARVC). Eva M. Oxford[1], Karen Maass[2], PhilipR. Fox[3], Mario Delmar[4], and N. Sydney Moise[1]. [1]Cornell University, College of Veterinary Medicine, Ithaca, NY. [2]NYU Langone Medical Center, NY, NY. [3]Animal Medical Center, NY, NY [4]Center for Arrhythmia Research, U. of Michigan, Ann Arbor, MI.

ARVC is an inherited myocardial disease associated with sustained monomorphic ventricular tachycardia, sudden cardiac death (SCD), and replacement of myocardium with fatty or fibro-fatty tissue. Our previous work demonstrated altered localization of intercalated disc (ID) proteins in afflicted boxers. In this study we used transmission electron microscopy (TEM) to assess the ultrastructure of the ID and sarcomere in ARVC-afflicted boxers.

Afflicted boxers were identified by 24-hour Holter monitoring and histopathological examination of the heart. Samples from the right and left ventricles (RV and LV) of 2 unafflicted dogs (1 beagle, 1 German shepherd), and 7 ARVC afflicted boxers were collected and examined by TEM (data from 3 boxers reported here). Samples from control tissue showed normal morphology, normal alignment of the sarcomeres and myofibrils, and attachment of myofibrils to the ID. Samples from ARVC-afflicted boxers displayed myofibral disorganization, with fewer attachments to ID. Furthermore, electron dense areas up to one sarcomere in length were observed radiating from Z bands (Z-band streaming) in ARVC afflicted samples. Within RV and LV longitudinal sections, the following parameters were measured from an average of 120 μm of ID per ventricle: (1) average length of desmosomes (DSM), adherens junctions (AJ), and gap junctions (GJ), and (2) number of DSM, AJ, and GJ per 10 μm of ID. No significant differences were found in the length (all measurements in μm) of AJ or GJ from RV or LV samples of ARVC versus control. AJ length was 0.27 ± 0.01 (RV) and 0.25 ± 0.02 (LV) in control samples versus $0.26\pm.04$ (RV) and 0.23 ± 0.02 (LV) in afflicted animals (pNS). GJ length in control samples was 0.48 ± 0.03 (RV) and 0.48 ± 0.02 (LV). In afflicted animals, GJ length was 0.40 ± 0.02 and 0.47 ± 0.13, for RV and LV respectively (pNS). In contrast, the average length of DSM was significantly reduced in afflicted animals. DSM length was 0.22 ± 0.05 (RV) and 0.20 ± 0.02 (LV) in control. DSM length in afflicted samples was 0.16 ± 0.003 (RV) and 0.15 ± 0.03 (LV) ($p< 0.05$). In addition, our data indicated a significant ($p< 0.05$) decrease in the number of GJ per 10 μm ID in the ARVC samples (1.8 ± 0.12 RV, 1.5 ± 0.27 LV in control; 0.4 ± 0.02 RV, 0.6 ± 0.24 LV in ARVC). No decrease in the number of DSM or AJ was observed. Our results indicate that ARVC in the boxer is associated with reduced length of DSM and reduction of GJ at the intercalated disc; the latter which may act as a substrate for ventricular arrhythmias. Furthermore, Z band streaming suggests a novel concept: genes associated with the cardiomyocyte contractile apparatus may play an etiological role in the development of ARVC in the boxer dog.

ABSTRACT #13

***IN VIVO* DETERMINATION OF CANINE CYSTOLITH MINERAL COMPOSITION USING COMPUTED TOMOGRAPHY-GENERATED HOUNSFIELD UNITS**. BM Pressler, LG Adams, HG Heng, JJ Rohleder, GE Moore. Purdue University, West Lafayette, IN.

Determination of mineral composition is essential for effective management of urolithiasis in dogs. Composition of uroliths retrieved from people has been determined *in vitro* by computed tomographic peak attenuation measurements (Hounsfield units, HU), and HU allow *in vivo* differentiation of the most common types of uroliths. In a previous study the three most common cystolith mineral types recovered from dogs could be differentiated *in vitro* using CT-generated HU with $>90\%$ accuracy. This study was designed to determine the accuracy of CT-generated HU for differentiation of canine cystolith mineral composition *in vivo*.

Forty-eight cystoliths of known composition were placed in a phantom array (15 struvite, S; 15 calcium oxalate, O; 11 urate, U; 7 mixed, M) and scanned at 80 kVp/400 mAs using two different CT scanners. Mean HU were calculated for each cystolith on each CT scanner by two radiologists; region-of-interest (ROI) for HU calculation was determined using the largest cystolith cross-section, with a manually-centered oval ROI encompassing two-thirds to three-fourths of the cystolith. Reference ranges were constructed using receiver operating curves to optimize sensitivity and specificity of mineral identification. To determine *in vivo* applicability of these HU reference ranges, 20 dogs with confirmed cystolithiasis were anesthetized and CT scans (1 mm slices) were obtained of their bladders immediately prior to removal of cystoliths. Each radiologist calculated mean HU using the largest urolith cross-section, and mineral composition was predicted. True cystolith mineral composition was determined using standard techniques by a veterinary urolith center.

The 20 enrolled dogs were determined to have 12 calcium-containing cystoliths (9 pure O, 1 pure calcium phosphate, 2 cystoliths with 80–85% O and 15–20% silica), 6 S-containing cystoliths (5 pure S, 1 85% S/15% calcium phosphate), and 2 U cystoliths. 9 of 12 (75%) of calcium-containing cystoliths were correctly identified by both radiologists; 2 were misidentified as S by one radiologist, and one was misidentified as S by both radiologists. 2 of 6 (33%) S cystoliths were correctly identified by both radiologists; 3 were incorrectly identified as U by both radiologists, 1 was identified as O by both radiologists. 2 of 2 (100%) U cystoliths were correctly identified by both radiologists. In total, the two radiologists were in agreement in their analysis of likely mineral composition in 18 of the 20 (90%) cystoliths. Overall, 75% and 88% of non-dissolvable and dissolvable uroliths, respectively, were correctly identified.

These preliminary results suggest that CT-generated HU may be used to differentiate canine cystolith mineral composition *in vivo*. Accuracy is highest for calcium-containing and urate cystoliths. Ongoing studies will increase the number of enrolled dogs and determine if alternative methods of reference range calculation may improve correct identification of cystolith mineral composition.

ABSTRACT #14

URETERAL STENTING FOR FELINE URETEROLITHIASIS: TECHNICAL AND CLINICAL OUTCOMES. A Berent[1], C Weisse[1], D Bagley[2], C Adan[1], K Todd[1], J Solomon[1]. [1]Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA. [2]Thomas Jefferson University Hospital, Philadelphia, PA.

Ureteral stenting has been performed for a variety of benign and malignant disorders, bypassing an obstruction while directing urine from the renal pelvis to the urinary bladder. The most common ureteral dilemma in feline patients is ureterolith-induced obstruction, of which traditional therapy is associated with excessive morbidity/mortality. The authors' objective is to describe the tech-

nical and clinical outcome of ureteral stenting in feline patients with ureterolith induced obstructions.

Twenty-two cats (25 ureters) had stent placement (3 french double pigtail) attempted for nephro-ureterolithiasis. Access was attempted endoscopically (12) and/or surgically (17). 14 female and 8 male cats, weighing 2.1–6.2 kg, were included. Surgical placement was possible in 14/17 (82.4%) and endoscopic in 4/12 (30%), with an overall success in 18/25 ureters (72%). 18/18 stents (100%) resulted in ureteral/pelvis decompression and azotemia stabilization. Procedure associated complications included: temporary stranguria (5), imperfect stent location (2) and a ureteral tear/trauma (1). One of two displaced stents required adjustment 3 days later. All other procedure associated complications resolved spontaneously without further intervention.

Seventeen of 18 stents remained in place and patent long-term (range: 2->780 days). There was no reported need for pre-mature removal. 4/16 cats with stents developed urinary tract infections (2/4 while a nephrostomy tube was present). All infections were successfully cleared. One cat had evidence of intermittent hematuria without associated dysuria, infection, azotemia, or pyelectasia. Further investigation was declined.

Overall, ureteral stenting is possible, safe, and effective for the treatment of feline ureterolithiasis, maintaining ureteral patency regardless of stone number or size.

ABSTRACT #15

THE USE OF A PERCUTANEOUSLY CONTROLLED HYDRAULIC OCCLUDER FOR THE TREATMENT OF URETHRAL SPHINCTER MECHANISM INCOMPETENCE IN 11 DOGS 1 CAT. A Berent[1], C Weisse[1], C Adan[1], K Todd[1]. [1]Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA.

Refractory urinary incontinence, where traditional medical/surgical techniques fail to resolve urinary leakage, is a major problem in animals with urethral sphincter mechanism incompetence (USMI), particularly after fixation of ureteral ectopia. The authors' objective is to describe the technical and clinical outcome using a percutaneously controlled hydraulic occluder (HO) for refractory incontinence secondary to USMI in small animal patients.

Eleven dogs and 1 cat were included. 8/11 dogs had a history of ectopic ureters. All patients were females ranging from 2.3 kg–37.3 kg, and 6–55 months. All patients failed medical management with phenylpropanolamine (12), diethylstilbestrol (6) and/or collagen injections (6). An uninflated silicone ring (HO) was surgically placed around the proximal urethra and connected to a subcutaneous injection port. Cystopexy was performed when necessary (6/12). Saline injections, providing extraluminal urethral compression, were performed if necessary. Median follow-up time was 7 months (range 2–10 months).

Seven of 11 dogs and 1/1 cat (66.7%) were continent after HO placement. 2/4 incontinent dogs were not yet injected. The two remaining dogs had severe anatomical abnormalities (intrapelvic/hypoplastic bladder [2], urethral dysplasia [1], short/wide urethra [2]). All incontinent dogs were reported to have dramatic improvements (50–80%). Two urethral stenoses occurred (1 extraluminal stricture; 1 intraluminal webbing) 3 weeks after placement requiring removal (n=2) and replacement (n=1) with a larger HO and intraluminal urethral stent. One dog returned to incontinence after removal.

Overall, the use of a HO for refractory USMI may result dramatic improvement toward urinary continence. The risk of urethral narrowing should be considered.

ABSTRACT #16

DEVELOPMENT OF A USSING CHAMBER MODEL FOR STUDYING THE CANINE UROTHELIAL RESPONSE TO INFECTION. M. Wood, E. Breitschwerdt, M. Suyemoto, M. Stone, J. Gookin. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

The urothelium prevents pathogen access to the body. Tight junctions linking umbrella cells of the urothelium serve as a physical blockade passively preventing infectious agents from accessing the underlying submucosa. These junctions assist in maintaining a measurable transepithelial resistance (TER) across viable tissue. The urothelium also serves active roles. During the acute phase of urinary tract infections the epithelium can produce cytokines including IL-6. In-vitro studies have demonstrated that cultured urothelial cells will produce IL-6 when challenged with bacteria. To date however, a precise understanding of the role of IL-6 during the acute phase response to urinary pathogens has eluded researchers. While cell culture systems identify the response of individual cells, the mucosal response of the tissue may be more complex. With the ultimate goal of characterizing the global urothelial response to infection, the described work sought to develop an in-vitro model that preserves the microenvironment constructed by the urothelium and lamina propria. To achieve this aim bladder mucosa from recently euthanized dogs was stripped of its muscularis layer and mounted within Ussing chambers. This construct created independent luminal and submucosal compartments. Each side of the tissue was bathed with an oxygenated, 37 °C, physiological saline solution. A 10 mM glucose substrate added to the submucosal side provided nutrient support. Model success was defined as a demonstration of intact barrier function and cytokine production over a minimum course of 5 h. Barrier function was measured using voltage clamps to record the potential difference and short circuit currents traversing the tissue over time. These values were used to calculate the TER over a 10 h period for tissue treated on the mucosal side with *E. coli* J96 or saline control. Additional chambers received PMA/ionomycin treatment for 3 h as a positive control for cell death. Tissue from each of these time course treatments were formalin fixed and stained with H&E to visually identify evidence of cell death. The second criterion of model success was evidence of active urothelial function as evidenced by cytokine production. Fluid from both the serosal and mucosal reservoirs was sampled at 3, 5, 7 and 10 h after mucosal *E. coli* and saline treatments. IL-6 concentrations were determined via a validated canine specific IL-6 ELISA. Results indicated that both saline and *E coli* treated tissue remained viable beyond 5 h as the TER increased over this time period and there were no indicators of cell death via histology. This contrasted with the PMA/ionomycin treated tissue in which the TER decreased and there was evidence of apoptosis within 3 h. *E. coli* treatment significantly increased submucosal IL-6 concentrations at 3 and 5 h when compared to the saline control. These data provide evidence that canine bladder mucosa sustains normal barrier function, remains histologically intact, and responds biologically to *E. coli* infection by synthesis of IL-6 in an ex-vivo model system. The model generated represents a powerful tool for the study of molecular mechanisms of the urothelial response to urinary tract infection.

ABSTRACT #17

URODYNAMIC EVALUATION OF CATS WITH FELINE INTERSTITIAL CYSTITIS (FIC). Wu CH[1], Westropp JL[1], Buffington CAT[2]. [1]School of Veterinary Medicine, University of California, Davis, CA and [2]The Ohio State University College of Veterinary Medicine.

Although urodynamic studies of humans with interstitial cystitis have been reported, similar evaluations of cats with FIC have not been reported to our knowledge. The purpose of this study was to compare urodynamic parameters from 12 female cats with FIC with previously published data from six healthy female cats using the same anesthetic regimen.

All cats were anesthetized with propofol for cystometrogram (CMG) and urethral pressure profile (UPP) measurements. Threshold pressure (TP) and volume were recorded for CMGs. If a detrusor reflex was not evident, TP was recorded when urine passively leaked, or intravesical pressure exceeded 85 cmH_20. The maximal urethral closure pressure (MUCP) was evaluated during the UPP.

Four cats had obvious detrusor reflexes, but none had evidence of detrusor overactivity (DO). Bladder compliance during the first CMG was significantly lower in FIC than in healthy cats (0.3±0.2 vs. 0.6±0.2; p=0.04). Compliance of cats with FIC increased significantly on the second CMG compared to the first (0.4±0.3 vs. 0.3±0.2 mls/cm H20; p=0.007). The maximal urethral closure pressure (MUCP) was significantly higher in FIC than in healthy cats on the first and second UPP evaluated (first MUCP obtained: 158±48 vs. 91±23 cm H20; p=0.003, second: 152±45 vs. 86±29).

Although a decreased compliance was documented, it was not a result of DO; therefore drugs aimed at this problem do not seem

warranted in cats with FIC. Because higher MUCP values were noted, selective alpha adrenoceptor antagonism may be warranted, particularly if these problems are found in male cats.

ABSTRACT #18
RANDOMIZED, PLACEBO-CONTROLLED CLINICAL TRIAL OF PENTOSAN POLYSULFATE SODIUM FOR TREATMENT OF FELINE INTERSTITIAL (IDIOPATHIC) CYSTITIS. DJ Chew[1], JW Bartges[2], LG Adams[3], JM Kruger[4], CAT Buffington[1]. [1]The Ohio State University, Columbus, OH. [2]University of Tennessee, Knoxville, TN. [3]Purdue University, West Lafayette, IN. [4]Michigan State University, East Lansing, MI.

The objective of this study was to evaluate the treatment effect of pentosan polysulfate (PPS) compared to placebo on lower urinary tract signs (LUTS) in cats with feline interstitial (idiopathic) cystitis (FIC). This study was a multicenter, double-blinded, placebo-controlled, randomized clinical trial that involved 107 cats with at least two episodes of LUTS within the past six months, cystoscopic findings of glomerulations, and absence of an alternative diagnosis.

Cats were randomly assigned to 0.0 (vehicle placebo), 2.0, 8.0 or 16.0 mg/kg PPS orally twice daily for 26 weeks. Owners rated hematuria, stranguria, pollakiuria, periuria and vocalization during voiding attempts on a scale of 0–3 (none, mild, moderate, severe) weekly. Glomerulations were counted at the end of the study, and 3 of the 4 sites also recorded urothelial friability, edema and vascularization. Data were analyzed within and between groups using repeated measures; ANOVA; $P \leq .05$ was considered significant.

All treatments were well tolerated. Owner-recorded average symptom scores decreased by 75% in both groups; recurrent episodes occurred in approximately 40% of cats in each group. No statistically significant differences were observed between any of the groups based on the owner's evaluation of clinical signs or overall improvement in cystoscopic score. A statistically significant decrease in friability was observed at the 16.0 mg/kg dose.

Clinical improvement occurred in all cats, regardless of the dose of PPS administration or changes in cystoscopic appearance of the bladder. These results indicate that PPS is equivalent to placebo for treatment of FIC, and demonstrate the importance of placebo controlled clinical trials for evaluation of therapy for FIC.

ABSTRACT #19
COMPARISON OF PLASMA CLEARANCE OF EXOGENOUS CREATININE AND IOHEXOL IN A CANINE POPULATION. C Collignon[1], R Heiene[2], N. Harran[1], U Risoen[2], D. Balouka[1], C Germain[1], M Faucher[1], K Eliassen[2], Y Queau[1], B Reynolds[1], HP Lefebvre[1]. [1]Clinical Sciences, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France. [2]Companion Animal Clinical Sciences, Norwegian School of Veterinary Science, Oslo, Norway.

Plasma clearance of exogenous creatinine (PCEC) and iohexol (PCI) have been validated for measurement of glomerular filtration rate (GFR) in dogs. The aim of this prospective study was to compare PCEC and PCI in a canine population.

Simultaneous intravenous administration of iohexol (300 mg I/kg) and creatinine (40 mg/kg) was performed in 50 healthy and renal-impaired Anglo Francais dogs. Blood samples were then obtained at 5, 10, 30, 60, 120, 240, 360, and 480 min.Plasma creatinine and iohexol were assayed by enzymatic method and HPLC, respectively. Exo-iohexol concentrations were used. The method effect on the GFR value was tested using ANOVA.

Plasma clearances (mean±SD) of PCEC and PCI were 3.0±0.7 and 3.4±0.8 mL/min/kg. Their ranges were 1.1–5.7 and 1.2–5.1 mL/min/kg, respectively. A significant effect of the method on GFR value was evident ($P=0.001$; $R^2=0.767$). Nevertheless, both methods identified the same dogs with abnormally low GFR value, discrepancies being observed for higher GFR values. 34/50 dogs showed a difference between both clearances <0.6 mL/min/kg and only 5/50 dogs a difference >1 mL/min/kg.

In conclusion, both methods provide similar results for screening patients with low GFR values but may differ for higher GFR values.

ABSTRACT #20
EFFECTS OF SUPPLEMENTATION WITH AMINO ACIDS IN DOGS WITH GLOMERULONEPHRITIS. A Zatelli[1], F Nizi[1], E Zini[2]. [1]Clinica Veterinaria Pirani, Reggio Emilia, Italy; [2]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Severe malnutrition is frequent in dogs with renal proteinuria and is likely associated with increased morbidity and mortality. Diets with reduced protein content are routinely used in the management of dogs with kidney disorders, including glomerulonephritis. They were shown to reduce disease progression, delay uremia onset and increase survival. However, in dogs with glomerulonephritis renal diets may not adequately meet protein requirements, thus leading to malnutrition. Aim of this study was to investigate the effects of oral amino acid supplementation on body weight (BW), serum albumin, creatinine and urea levels, and urine protein-to-creatinine ratio (UPC) in dogs with glomerulonephritis treated with enalapril and a renal diet.

Forty-seven dogs with glomerulonephritis were allocated to receive the amino acid supplement (29 dogs, Group A), or not (18 dogs, Group B). The daily amount (mg) of amino acids (IT IS, ACME, Reggio Emilia, Italy) was calculated with the following formula: BW (kg)×UPC×20. All dogs were treated with enalapril (0.5 mg/kg, q24 h) and received a commercial renal diet. BW, serum albumin, creatinine and urea, and UPC were determined at baseline and after 4–8 weeks in all dogs. In Group A the same parameters were also recorded at 16–20 weeks. Statistical differences in each group were determined with paired t-tests.

At 4–8 weeks, BW of dogs in Group A increased on average 3.1% ($P<0.01$) whereas in Group B was stable. Albumin levels increased of 0.25 g/dl ($P<0.05$) in Group A and did not differ in the other. Urea levels and UPC significantly decreased only in Group B (average decrease of urea: 16.0 mg/dl; and of UPC: 1.91). In both groups creatinine levels did not vary. At 16–20 weeks in Group A, compared to 4–8 weeks, creatinine levels increased of 0.45 mg/dl ($P<0.05$) and UPC decreased of 0.84 ($P<0.05$). BW and levels of albumin and urea remained stable.

Treating dogs affected by glomerulonephritis with enelapril and a renal diet, in addition to lowering proteinuria, is beneficial because maintains stable BW and albumin levels. Even though supplementation with amino acids increases BW and albumin levels, it delays the decrease of proteinuria and prevents lowering of urea, one of the major toxins in chronic renal failure. In light of these findings, supplementation with amino acids should be regarded with caution in dogs with glomerulonephritis. Whether amino acids may have a place in the treatment of dogs with more severe hypoalbuminemia is currently explored.

ABSTRACT #21
DOCUMENTATION OF HYPERCOAGULABILITY IN PROTEIN-LOSING NEPHROPATHY VIA THROMBOELASTOGRAPHY IN 10 DOGS. KM Hilling, MA Labato, AM de Laforcade, S Shaw. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

Protein-losing nephropathy (PLN) has been previously associated with hypercoagulable states, including the presence of fatal pulmonary thromboemboli. A sensitive method for detecting Hypercoagulablility (HC) in affected dogs is warranted, as the appropriate use of either anti-platelet or anti-thrombotic therapies may improve patient survival and limit complications of PLN. Conventional tests of coagulation are insensitive at detecting HC. Serum albumin has previously been shown to correlate with antithrombin (AT) activity in PLN; reduced AT is associated with thrombotic disease. The thromboelastography (TEG) assay has recently been re-introduced as a point-of-care test of the global clotting function. The purpose of this pilot study was to evaluate the TEG assay as a reliable and early predictor of HC in canine PLN patients, including those with evidence of thromboembolism.

Kaolin-activated TEG was performed in accordance with manufacturer's recommendations, using the TEG 5000 Haemostasis Analyzer (Haemoscope Co). The results of TEG variables *R, K, α, MA* and *G* for study dogs were compared to established local reference ranges. Based on the TEG *G* value, dogs were categorized as hypo- (<5.7 K), normo- ($G=5.7$–6.9 K), and hypercoagulable

(G > 6.9 K); absolute numbers of the TEG variables *R, K, α, MA* and *G* were also evaluated as was serum albumin. All dogs with PLN were eligible for study inclusion; dogs were divided into uncomplicated PLN (PLN-uc) and PLN with thromboembolic disease (PLN-TED). Results between groups were compared using a t-test with a $p < 0.05$ considered significant.

Ten dogs were enrolled; all dogs were hypercoagulable. There was no correlation between G and albumin values. Three dogs had TED, albumin was significantly higher in dogs with PLN-TED then PLN-uc ($p=0.02$), but there was no other difference in any TEG variables between groups.

Variable	R	K	α	MA	G	Albumin
Ref range	3–4	1–2	61–66	54–58	5.7–6.9	2.8–4.0
PLN	5.4±2.2	1.5±0.6	69.9±5.6	68.7±7.3	11.8±3.9	2.5±0.7
PLN-TED	6.8±3.7	2.0±1.0	66.3±8.4	71.4±10.0	13.8±5.6	3.1±0.1
PLN-uc	4.9±1.2	1.3±0.4	71.4±3.8	67.6±6.4	10.9±3.1	2.2±0.6

These data confirm that dogs with PLN are hypercoagulable as assessed by TEG. However, in contrast to antithrombin activity, the degree of HC does not correlate to serum albumin. Further studies are warranted to delineate coagulation changes in PLN, as well as define optimal anticoagulation strategies.

ABSTRACT #22

MEASURING LEVEL OF AGREEMENT BETWEEN DIRECTLY MEASURED BLOOD PRESSURE AND VALUES PRODUCED BY OSCILLOMETRIC UNITS IN CATS. MJ Acierno[1], DC Seaton[1], MA Mitchell[2], A da Cunha[1]. [1]The Louisiana State University, Baton Rouge, LA. [2]University of Illinois, Urbana, IL.

While the Doppler detector has been validated for use in dogs and cats, studies involving the use of oscillometric units have shown that these devices consistently underestimate feline blood pressure. Recently, several oscillometric devices that claim to have been specifically optimized for use in cats have been introduced The goal of this study is to compare three of these newly designed oscillometric devices.[a,b,c] to directly measured blood pressure in cats undergoing anesthesia.

A 22 gauge catheter was placed in the dorsal pedal artery of 15 client owned cats receiving anesthesia in preparation for surgery. The arterial catheter was connected to a continuous multifunction monitor[c] via a disposable pressure transducer system.[d] The directly obtained blood pressure readings were checked for stability and consistency while each of the oscillometric devices were setup and calibrated in accordance with the manufacturers' directions. A series of four paired blood pressure measurements (directly vs. oscillometric) was then made for each device.

Agreement between the directly and indirectly obtained systolic, diastolic and mean arterial pressure measurements were determined by use of the Bland-Altman method for multiple observations. Good agreement was defined as a bias and limit of agreement (LOA) within 10% of the expected mean. Results for 15 cats were available for units 1[a] and 2[b], and 13 for unit 3[c]. Unit 1[a] demonstrated a systolic bias of −7.0 mmHg (LOA −52.2 to 38.2), a diastolic bias of 9.2 mmHg (LOA −36.47 to 54.87), and a MAP bias 7.0 mmHg (LOA −35.5 to 49.5). Unit 2[b] demonstrated a systolic bias of −13.18 mmHg (LOA −53.75 to 27.39), a diastolic bias of 0.03 mmHg (LOA −26.8 to 26.9), and a MAP bias −4.1 mmHg (LOA −28.2 to 19.98). Unit 3[b] demonstrated a systolic bias of −9.7 mmHg (LOA −41.5 to 22.1), a diastolic bias of 5.6 mmHg (LOA −35.5 to 41.7), and a MAP bias −4.6 mmHg (LOA −36.6 to 27.4).

Results suggest that there is significant disagreement between all of the oscillometric devices and the directly measured blood pressure. At this time, use of these oscillometric devices in cats cannot be recommended.

a- VET HDO, Vetline LLC, Saint Kitts and Nevis, WI.
b-PetMap, Ramsey medical, Tampa FL.
c- Cardell Max-1, Sharn Veterinary, Tampa, Fla.
d - DTX Plus, Becton Dickinson, Sandy, Utah.

ABSTRACT #23

PHARMACOKINETICS AND CARDIOVASCULAR EFFECTS OF INTRAVENOUS FENOLDOPAM IN HEALTHY AWAKE DOGS. CA Bloom[1], MA Labato[1], MH Court[2], S Hazarika[2]. [1]Tufts University Cummings School of Veterinary Medicine, North Grafton, MA. [2]Tufts University School of Medicine, Boston, MA.

The purpose of this study was to collect pharmacokinetic data on fenoldopam in healthy awake dogs. Fenoldopam is a selective dopamine-1 receptor agonist that causes peripheral arterial vasodilation, increased renal blood flow, and diuresis. Main uses in human medicine are acute hypertensive crisis and non-polyuric renal failure. Enthusiasm exists for the use of fenoldopam in non-polyuric renal failure in dogs, and while pharmacodynamic studies have been performed, there is no pharmacokinetic data.

Six healthy, awake beagles were given a 180-minute fenoldopam CRI at 0.8 mcg/kg/min, followed by a 120-minute washout period. Citrated blood was collected at minutes 0, 5, 10, 20, 30, 60, 90, 120, 150, and 180 during infusion, and minutes 182, 185, 190, 210, 225, 240, 270, and 300 following infusion. HR, RR, and BP via Doppler were concurrently measured.

All dogs completed the study uneventfully. There was no significant change in HR, RR or BP.

Steady state fenoldopam concentrations averaged 19.9 ng/ml and were achieved within 10 minutes of starting infusion. Area under the curve averaged 3590 ng/ml×minute. Clearance averaged 40 ml/min/kg. Elimination was rapidly achieved in all dogs. Three dogs had no fenoldopam in the plasma at time 180, within seconds of terminating the infusion; therefore, elimination data were not determined for these dogs. Elimination half-life ranged from 1.1–6.7 minutes in 3 dogs, and volume of distribution ranged from 30–727 ml/kg in 3 dogs. Clinical studies of fenoldopam in dogs with non-polyuric renal failure using a fenoldopam CRI at 0.8 mcg/kg/min are warranted.

ABSTRACT #24

URINARY IMMUNOGLOBULIN G, C-REACTIVE PROTEIN AND RETINOL BINDING PROTEIN AS CANDIDATE EARLY BIOMARKERS FOR RENAL DYSFUNCTION IN DOGS WITH PYOMETRA. B.E.J. Maddens[1], S. Daminet[2], P. Smets[2], K. Demeyere[1], H. de Rooster[2], E. Meyer[1]. [1]Department of Pharmacology, Toxicology, Biochemistry and Organ Physiology; [2]Department of Medicine and Clinical Biology of Small Animals, both Faculty of Veterinary Medicine, Ghent University, Belgium.

Renal dysfunction in dogs has been associated with pyometra. Nevertheless, the mechanism and type of renal injury remain controversial. Routine measurement of serum urea nitrogen and creatinine detects renal injury only in a late irreversible stage and does not indicate the localisation of the damage. We hypothesize that urinary immunoglobulin G (uIgG), C-reactive protein (uCRP) and retinol binding protein (uRBP) may serve as superior renal biomarkers. Both IgG and CRP are high molecular weight proteins indicating glomerular lesions, whereas RBP may serve as a tubular damage marker.

In this study, 14 dogs with *Escherichia coli* (*E. coli*) pyometra (P) without concurrent other diseases were included. Age-matched clinically healthy bitches (H) served as controls (n=14). At ovariohysterectomy (P, H) or ovariectomy (H) blood, urine and uterine swabs were taken. Serum biochemical analysis, CBC, urinanalysis and uterine bacteriological culture were performed. Commercial canine ELISAs were validated and used to quantify uIgG and uCRP; uRBP concentrations were determined with a human RBP ELISA kit validated for use in the dog. All concentrations were related to urinary creatinine concentration (uC) and expressed as ratios.

In P dogs, uIgG/C (196±45.2 mg/g) and uCRP/C (706±298 μg/g) (mean±SEM) ratios were significantly increased compared to those in H bitches (2.1±0.4 mg/g respectively 0.1±0.1 μg/g) ($P<0.01$). Furthermore, uRBP/C ratios were significantly higher in P (43.7±10.1 μg/g) than in H dogs (16.7±5.5 μg/g) ($P<0.05$). A positive correlation at the 0.05 level was found between the urinary concentrations of all three proteins. Urinary total protein/C ratios (uTP/C) were significantly higher in P (0.77±0.19) than in H dogs (0.16±0.06) ($P<0.05$). A highly significant positive correlation was found between uTP/C and uIgG/C (R=0.79) or uCRP/C (R=0.72), but not between uTP/C and uRBP/C.

Concentrations of uIgG and uCRP, the latter for the first time examined in the dog, are strongly elevated in P bitches indicating glomerular dysfunction immediately after *E. coli* pyometra. The milder increase in uRBP is suggestive for tubular dysfunction, possible secondary to the proteinuria related to immune-mediated glomerulonephritis. We suggest that the observed increases in the candidate renal markers uIgG, uCRP and uRBP might be indicative of renal dysfunction at the glomerular and tubular level, associated with *E. coli* pyometra.

Previously presented at ECVIM in Belgium, September 2008.

ABSTRACT #25

WHOLE BLOODTHROMBOELASTOMETRY (TEM) IS RELATED TO CELL COUNTS AND PLASMA COAGULATION TESTS IN HEALTHY DOGS

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TEM use is increasing. An understanding of the relationships between TEM and other hematologic values is needed. Blood samples were collected from 78 apparently healthy dogs. Hematocrit (Hct), Platelet count (Plat), prothrombin time (PT), activated partial thromboplastin time (aPTT), and Fibrinogen (Fib) were performed using standard methods. Citrated whole blood was evaluated using Rotem (Pentapharm) and either the manufacturer supplied Extem (tissue factor) or Intem (contact activator). TEM parameters included clot time (CT), clot formation time (CFT), alpha angle (α), and maximum clot firmness (MCF). Strength of correlations was determined using Pearson Product Moment Correlation (r) calculated with Sigma Stat.

Comparisons between Extem (Ex) and Intem (In) parameters indicated that there was no significant relationship between ExCT and InCT. r values for CFT (0.77^{***}), α (0.69^{***}), and MCF (0.52^{*}) indicated that Ex values and In values were highly correlated. Additional r values:

	ExCT	ExCFT	Exα	ExMCF
PT	**0.34***	0.19	−0.18	−0.23
APTT				
Plat	−0.16	**−0.36****	**0.44****	**0.35***
Fib	−0.21	**−0.51****	**0.50*****	**0.50*****
Hct	**0.34***	**0.49*****	**0.45*****	**−0.39****

*p < 0.05, **p < 0.005, ***p < 0.0005

	InCT	InCFT	Inα	InMCF
PT				
aPTT	**0.78***	0.08	−0.07	−0.03
Plat	−0.20	**−0.36***	**0.29***	**0.58****
Fib	−0.29	**−0.53***	**0.53***	**0.62****
Hct	0.28	**0.66****	**−0.66****	**−0.53****

*p < 0.05, **p < 0.005, ***p < 0.0005

CT is a function of time to initial fibrin polymerization, and is primarily dependent on thrombin formed in the initiation phase of coagulation. CT is related to the type and strength of trigger used and consequently correlates with plasma coagulation testing utilizing a comparable trigger. In contrast, CFT, α, and MCF are related to thrombin generated (and fibrin polymerized) during propagation. These parameters are correlated with platelet count and fibrinogen concentration, and independent of the type of clot trigger used. Interestingly, Hct was correlated with all parameters, with the exception of In-CT, despite the presence of normal red cell mass in all dogs (Hct range 41–60%). Dogs with higher Hct were relatively hypocoagulable, while those with lower Hct were relatively hypercoagulable. The significant impact of Hct on TEM results may be an *in vitro* artifact associated with relative under- or over-citration of blood samples, or may be an indication of interference of red cell mass with clot development. TEM parameters obtained from anemic or polycythemic dogs may need to be assessed in reference to HCT adjusted reference ranges.

ABSTRACT #26

EFFECT OF CANINE HYPERADRENOCORTICISM ON THROMBELASTOGRAPHY PARAMETERS

L Rose, M Dunn, C Bédard. University of Montreal Faculty of Veterinary Medicine, Saint-Hyacinthe, Quebec.

Hyperadrenocorticism (HAC) is associated with an increased risk of thromboembolism in dogs. The purpose of this prospective clinical study was to use thrombelastography (TEG®) to detect and qualify the hypercoagulable state in canine patients with HAC. We hypothesized that the presence of HAC in dogs would result in a hypercoagulable profile on TEG® tracings that is reversible with management of the disease.

Seventeen dogs suffering from HAC as diagnosed by ACTH stimulation test, low dose dexamethasone suppression test or complete adrenal profile were included. TEG® tracings were obtained before treatment in all dogs and after normalisation of ACTH stimulation and improvement of clinical signs in 4 dogs. 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.

When individual dogs were compared to the normal reference range, no dog was considered hypercoagulable. Furthermore, a paired t test showed no significant difference in TEG® values before and after treatment. However, a t test for values with unequal variance was used to compare the population of HAC dogs with a population of healthy beagles and showed that the mean values of healthy dogs were significantly higher for R ($p < 0.0001$) and K ($p < 0.0001$) and significantly lower for alpha ($p < 0.0001$) and MA ($p < 0.0001$) suggesting that HAC dogs tend toward hypercoagumshy;lable TEG® tracings.

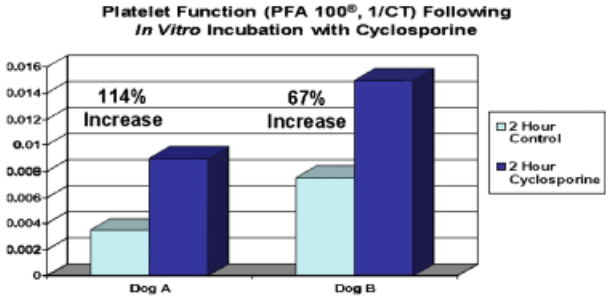
ABSTRACT #27

EFFECTS OF CYCLOSPORINE ON CANINE PLATELET PROCOAGULANT ACTIVITY

JM Thomason, K Lunsford, A Mackin, L Pinchuk, S Pruett, C Langston. Mississippi State University College of Veterinary Medicine, Starkville, MS.

Cyclosporine has become increasingly popular for treating canine immune-mediated diseases such as immune-mediated hemolytic anemia (IMHA). Cyclosporine has recently been shown to increase platelet procoagulant activity in humans, potentially predisposing to thrombosis. Our study was designed to evaluate the effects of cyclosporine on canine platelet procoagulant activity.

Platelet reactivity was quantified by flow-cytometric evaluation of activated platelet surface markers P-Selectin and phosphatidylserine, platelet function was assessed via a point-of-care analyzer (PFA-100®), and clot formation was evaluated using viscoelastometry (SonoClot®). Evaluation of platelet reactivity in two healthy dogs after cyclosporine PO BID for 1 week, and in a third dog after a cyclosporine IV CRI for 1 day (dose adjusted to attain target blood levels) revealed increased P-Selectin expression (mean increase 95%, range 37–138%), decreased phosphatidylserine expression (mean decrease 35%, range 5–64%), and variable Sonoclot® signatures. *In vitro* platelet exposure to cyclosporine resulted in decreased PFA-100® closure times (mean change 96 seconds, range 41–170 seconds), and no consistent change in P-Selectin or phosphatidylserine expression or Sonoclot® signatures.



Our results suggest that cyclosporine may increase platelet procoagulant activity in some dogs. Individual variations in canine platelet thromboxane receptor function, thromboxane-mediated degranulation, and P-selectin expression may explain variable responsiveness to cyclosporine.

Cyclosporine may potentially predispose some dogs to thrombotic complications such as pulmonary thromboembolism, possibly negating the benefits of the drug in patients with prothrombotic conditions such as IMHA. Further investigation of the potential effects of cyclosporine on platelet procoagulant activity in both normal dogs and clinic patients is warranted.

ABSTRACT #28

TREATMENT OF CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA WITH INDIVIDUALLY ADJUSTED HEPARIN DOSING: A PILOT STUDY. SE Helmond[1], DJ Polzin[1], PJ Armstrong[1], M Finke[1], SA Smith[2]. [1]University of Minnesota College of Veterinary Medicine, St Paul, MN. [2]University of Illinois College of Medicine, Urbana, IL.

A major cause of mortality and morbidity in canine Immune-Mediated Hemolytic Anemia (IMHA) is thrombotic disease. Previous studies have suggested that unfractionated heparin (UH) may not be effective at increasing survival in IMHA; however, subtherapeutic dosing may explain the seeming lack of efficacy. We hypothesized that providing appropriate therapeutic plasma levels of UH by individually adjusting doses based on anti-factor Xa activity would improve survival in dogs with IMHA.

To investigate this hypothesis, a randomized, prospective, masked, placebo-controlled clinical trial was conducted using 15 dogs with newly diagnosed primary IMHA. Dogs were recruited following diagnosis and received standardized therapy for IMHA. In addition, dogs were treated with either a Constant Dose (CD) (150 U/kg SQ q 6 h) or an Individually Adjusted Dose (IAD) of UH. UH dosing was monitored via an Anti-Xa chromogenic assay modified for use in dogs and adjusted according to a nomogram developed for dogs. UH dose was adjusted daily until day 7, and then once weekly until day 28, after which heparin dosing was tapered over 1 week. Dogs were followed for 6 months (180 days).

The primary endpoint was 6 month survival. Secondary endpoints were: 1) frequency of thromboembolism (TE), 2) frequency of hemorrhage, 3) the dose of UH required to maintain a therapeutic plasma level in the IAD group.

At Day 180, 7 out of 8 dogs in the IAD group were alive; 1 dog out of 7 in the CD group was alive ($p=0.01$). Median survival time for the IAD group was 180 days, and 68 days for the CD group. Five dogs in the CD group had confirmed TE events, three of which were fatal. Two dogs in the IAD group had suspected TE events; one of these dogs died of TE. There were no hemorrhagic complications in either group. The dose of UH required to maintain therapeutic plasma levels (0.3–0.7 IU/mL) in dogs in the IAD group ranged from 180 IU/kg to 712 IU/kg q6–8 h. There were marked inter- and intra-individual variations in UH doses, with no correlations between dose and anti-Xa activity. Anti-Xa activity in the CD group ranged from <0.1 IU/mL to 0.33 IU/mL (median 0.1 IU/mL).

Results of this pilot study suggest that IAD UH therapy, using anti-Xa monitoring, reduces mortality in canine IMHA. This is the first study to demonstrate successful adjustment of therapeutic heparin levels in dogs with IMHA. In addition, we found that fixed, low dose UH therapy resulted in poor survival rates, as has been suggested in other studies.

ABSTRACT #29

COMPARISON OF INDIVIDUALLY MONITORED UNFRACTIONATED HEPARIN VERSUS LOW-DOSE ASPIRIN ON SURVIVAL OF DOGS WITH IMMUNE MEDIATED HEMOLYTIC ANEMIA. ES Orcutt[1], DJ Polzin[1], PJ Armstrong[1], SE Helmond[1], S Smith[2]. [1]University of Minnesota College of Veterinary Medicine, St Paul, MN. [2]University of Illinois, Urbana, IL.

A previous retrospective study on low-dose aspirin therapy (LDA) in dogs with immune mediated hemolytic anemia (IMHA) reported a survival rate of 75% in LDA treated dogs. As a result, LDA has become the standard anticoagulant therapy for IMHA because of its perceived effectiveness, lack of need for special monitoring, minimal cost and availability. However, multiple studies in human patients comparing the TE prophylactic effect of aspirin versus heparin have shown marginal to no benefit with aspirin therapy compared to a significant reduction in TE or vascular events in patients treated with heparin. We hypothesized that subcutaneous administration of unfractionated heparin with dosage individually adjusted using anti-Xa monitoring (IAH) is superior to LDA in limiting mortality in dogs with IMHA managed with glucocorticoids. Study objectives were: (1) to evaluate 6 month survival in dogs treated with LDA versus IAH and (2) to evaluate the incidence of TE in both groups.

Medical records from the U of MN Veterinary Medical Center (VMC) were searched for dogs with IMHA treated with low-dose aspirin therapy (0.5 mg/kg/day). Inclusion criteria were: 1) regenerative anemia, 2) one or more of the following: a) autoagglutination, b) spherocytosis, or c) positive Coomb's test; 3) platelet count $>40{,}000/\mu l$; and 4) glucocorticoid therapy for IMHA. Dogs from a study at the VMC evaluating IAD and selected using the same inclusion criteria were used as the comparison group. Data collected included signalment, biochemical and hematological result, treatment regimes, TE complications and outcome.

Twenty five dogs met inclusion criteria for the LDA group and 8 dogs from the IAD study served as the comparison group. Ten of 25 dogs treated with LDA survived and 7 of 8 dogs treated with IAD survived ($p=0.084$). Seven of 25 LDA treated dogs and 2 of 8 IAD treated dogs developed TE. Six of 7 LDA dogs developing TE died; 1 of 2 IAD dogs developing TE died.

Results of this retrospective study suggest that IAD may be superior to LDA in reducing mortality in canine IMHA. However, dogs enrolled in the IAD study may have fared better due to closer monitoring occurring in the prospective clinical trial.

ABSTRACT #30

PLATELET IMPEDANCE AGGREGOMETRY IN CLINICALLY HEALTHY DOGS WITH AND WITHOUT ULTRALOW-DOSE ASPIRIN. KS Sharpe, SA Center, JF Randolph, MB Brooks, KL Warner. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Improved long-term survival in dogs with IMHA has been achieved when ultralow-dose aspirin (ULDAsp, 0.5 mg/kg/day PO) is combined with glucocorticoids and azathioprine. Whether this survival benefit solely reflects antithrombotic influences on platelets, or modulatory effects on vascular endothelium, systemic inflammation, or drug protein-binding, remains unclear.

This study was conducted to evaluate the efficacy of ULDAsp as an antithrombotic agent in the dog using a relevant *in vitro* platelet aggregation methodology. Our hypothesis was that ULDAsp would inhibit *in vitro* platelet aggregation in clinically healthy dogs.

Impedance platelet aggregation was studied in 19 dogs without and with ULDAsp administered orally once daily for 2 days. Blood was collected into 3.2% sodium citrate (1:9 dilution) 24-hrs after the last treatment. Each dog had a platelet count $\geq 250{,}000/\mu L$. Blood was maintained at 37 °C during aggregation assessments. Agonists evaluated included 20, 10, and 2 μmol/L ADP and 10, 5, and 2 μg/mL bovine collagen. Onset (seconds) and rate (ohms/second) of the aggregation response and maximum aggregation (ohms) were recorded. Aggregation parameters were normally distributed. Differences with and without ULDAsp were determined using a paired T-test with $\alpha=0.05$.

ULDAsp significantly delayed aggregation-onset elicited with ADP by 54–104%, but no change occurred with collagen. ULDAsp significantly slowed and attenuated the maximum aggregation response by 23–25% and 25–29%, respectively, with ADP and higher concentrations of collagen.

Findings substantiate that ULDAsp impairs platelet aggregation in healthy dogs. This data will serve as a platform for future functional assessment of platelet reactivity in dogs with IMHA.

ABSTRACT #31

HEMOSTATIC EFFECTS OF CRYOPRECIPITATE IN DOGS WITH DISSEMINATED INTRAVASCULAR COAGULATION. P Vilar, R Ball, N Westendorf, MC Iazbik, L Marín, and CG Couto. The Ohio State University, Veterinary Clinical Sciences, Columbus, OH.

Hemorrhagic thrombocytopenic complications (e.g disseminated intravascular coagulation-DIC, severe acute hemorrhage) are frequently observed in trauma patients and in those undergoing

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

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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*, M*ycoplasma*, and C*hlamydophila* 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

and human CaSR is 92%, 90% and 91%, respectively. The amino acid sequence is as follows:

MAFYSCCLILLAITWCTSAYGPDQRAQKKGDIILGGLF
PIHFGVAAKDQDLKSRPESVECIRYNFRGFRWLQAMIFAI
EEINSSPVLLPNMTLGYRIFDTCNTVSKALEATLSFVAQN
KIDSLNLDEFCNCSEHIPSTIAVVGATGSGISTAVANLLGL
FYIPQVSYASSSRLLSNKNQFKSFLRTIPNDEHQ9MAFYSC
CLILLAITWCTSAYGPDQRAQKKGDIILGGLFPIHFGVAA
KDQDLKSRPESVECIRYNFRGFRWLQAMIFAIEEINSSPVL
LPNMTL MAFYSCCLILLAITWCTSAYGPDQRAQKKGDIIL
GGLFPIHFGVAAKDQDLKSRPESVECIRYNFRGFRWLQA
MIFAIEEINSSPVLLPNMTLGYRIFDTCNTVSKALEATLSF
VAQNKIDSLNLDEFCNCSEHIPSTIAVVGATGSGISTAVAN
LLGLFYIPQVSYASSSRLLSNKNQFKSFLRTIPNDEHQ9MA
FYSCCLILLAITWCTSAYGPDQRAQKKGDIILGGLFPIHFG
VAAKDQDLKSRPESVECIRYNFRGFRWLQAMIFAIEEINS
SPVLLPNMTL MAFYSCCLILLAITWCTSAYGPDQRAQKKG
GDIILGGLFPIHFGVAAKDQDLKSRPESVECIRYNFRGFRW
LQAMIFAIEEINSSPVLLPNMTLGYRIFDTCNTVSKALEAT
LSFVAQNKIDSLNLDEFCNCSEHIPSTIAVVGATGSGISTAV
ANLLGLFYIPQVSYASSSRLLSNKNQFKSFLRTIPNDEHQ9
MAFYSCCLILLAITWCTSAYGPDQRAQKKGDIILGGLFPIH
FGVAAKDQDLKSRPESVECIRYNFRGFRWLQAMIFAIEEI
NSSPVLLPNMTL MAFYSCCLILLAITWCTSAYGPDQRAQK
KGDIILGGLFPIHFGVAAKDQDLKSRPESVECIRYNFRGF
RWLQAMIFAIEEINSSPVLLPNMTLGYRIFDTCNTVSKALE
ATLSFVAQNKIDSLNLDEFCNCSEHIPSTIAVVGATGSGIS
TAVANLLGLFYIPQVSYASSSRLLSNKNQFKSFLRTIPNDE
HQ9MAFYSCCLILLAITWC

This is the first report of the feline CaSR sequence. This sequence may serve to elucidate mechanisms of disorders of calcium regulation in cats.

ABSTRACT #38

HYPERTRIGLYCERIDEMIA DOES NOT IMPAIR WHOLE BODY INSULIN SENSITIVITY IN CATS, POSSIBLY THROUGH INCREASED CIRCULATING ADIPONECTIN. E Zini[1], D Konrad[2], M Osto[3], M Franchini[4], M Ackermann[4], TA Lutz[3], CE Reusch[1]. [1]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland. [2]University Children's Hospital, Zurich, Switzerland. [3]Institute of Veterinary Physiology, [4]Institute of Virology, Vetsuisse Faculty, University of Zurich, Switzerland.

Obesity and hyperlipidemia impair insulin sensitivity in human type 2 diabetes mellitus, partly due to activation of a mild inflammatory response. Because obesity-induced insulin resistance predisposes cats to diabetes and hyperlipidemia is a frequent concurrent finding, we propose that excess lipids impair insulin sensitivity in this species. We hypothesize that hyperlipidemia may be accompanied by decreased circulating adiponectin and increased pro-inflammatory markers, such as α_1-acid glycoprotein (AGP) and monocyte chemoattractant protein-1 (MCP-1) and, in insulin sensitive tissues, by decreased mRNA of adiponectin, glucose transporter-4 (GLUT4), peroxisome proliferative activated receptor-γ1 (PPARγ1) and PPARγ2, and increased resistin and GLUT1.

Healthy cats (n=6) were infused with lipids (Lipovenoes 10%, Fresenius Kabi, Switzerland) for 10 days to clamp their blood triglycerides at the approximate level of untreated feline diabetes (265–620 mg/dl). Controls received saline (n=5). On day 10, adiponectin and pro-inflammatory markers were measured in plasma. An intravenous glucose tolerance test was performed and whole body insulin sensitivity was calculated. Specimens were collected from subcutaneous and omental fat, liver and skeletal muscles. Tissue mRNAs of glucose metabolism-related genes were quantified. Statistical differences were determined with non parametric tests.

Whole body insulin sensitivity did not differ between lipid- and saline-infused cats. Compared to cats on saline, cats infused with lipids had 50% higher plasma adiponectin ($P<0.05$) and 2–3 times higher AGP and MCP-1 ($P<0.01$ and $P<0.05$, respectively). Lipid-infused cats had decreased resistin and GLUT1 mRNA and increased GLUT4 in the omental fat. GLUT1 mRNA was decreased and PPARγ2 increased in subcutaneous fat. Adiponectin and PPARγ1 expression did not differ in any tissue.

Although hyperlipidemia induced systemic inflammation in cats, whole body insulin sensitivity was not impaired. Increased circulating adiponectin levels may have contributed to preventing insulin resistance, possibly by increasing GLUT4 and PPARγ2 transcripts and decreasing resistin expression in subcutaneous or omental fat. The source of increased plasma adiponectin is unknown.

ABSTRACT #39

ROLE OF HYPERGLYCEMIA IN THE PATHOGENESIS OF PANCREATITIS IN CATS. E Zini[1], M Osto[2], M Franchini[3], F Guscetti[4], M Ackermann[3], TA Lutz[2], CE Reusch[1]. [1]Clinic for Small Animal Internal Medicine, [2]Institute of Veterinary Physiology, [3]Institute of Virology, [4]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland.

Pancreatitis is commonly diagnosed in cats and humans with diabetes mellitus. It is characterized by infiltration with neutrophils and macrophages and by varying degrees of fibrosis. Based on studies in diabetic humans, various hypotheses have been put forward to explain concurrence of exocrine and endocrine disease, such as the spread of an inflammatory disorder from the former to the latter, a common immune-mediated inflammation or a local vascular damage. We propose that diabetic hyperglycemia or hyperlipidemia promotes inflammation of the exocrine pancreas in cats.

Healthy cats were infused with glucose (n=5) or lipids (n=6) for 10 days to clamp their blood concentrations at the approximate level of untreated feline diabetes (glucose: 450–540 mg/dl; triglycerides: 265–620 mg/dl). Controls received saline (n=10). On day 10, blood samples and tissue specimens were collected. Levels of amylase, lipase, and feline pancreatic lipase and trypsin-like immunoreactivity (fPLI and fTLI, respectively) were measured in serum samples. To quantify neutrophils in the exocrine pancreas, pancreatic sections were immunostained with insulin and myeloperoxidase. Myeloperoxidase was also used to quantify neutrophils in liver and omental fat sections. Statistical differences between groups were determined with non parametric tests.

Plasma amylase was lower than the reference range in 4 hyperglycemic cats. Control and hyperlipidemic cats had normal amylase. Lipase, fPLI and fTLI concentrations were similar between groups. Compared to controls, hyperglycemic cats had three times more neutrophils in the exocrine pancreas per high-power field ($P<0.001$). The number of neutrophils did not differ in the liver and omental fat. Control and hyperlipidemic cats had similar neutrophil counts in all examined tissues.

Hyperglycemia selectively increases the number of neutrophils in the exocrine pancreas of cats, possibly predicting the development of pancreatitis. As shown in experimental pancreatits in cats, low amylase may represent an early marker of pancreatic inflammation. Based on the present feline model, hyperlipidemia does not seem to affect the exocrine pancreas.

ABSTRACT #40

USE OF LISPRO INSULIN FOR TREATMENT OF DOGS WITH DIABETIC KETOACIDOSIS. KW Sears, KJ Drobatz, RS Hess. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

The goals of this study were to characterize the use of lispro insulin (LI) in dogs with diabetic ketoacidosis (DKA) and to compare the rate of resolution of hyperglycemia and ketoacidosis in dogs treated with LI to these rates in dogs treated with regular insulin (RI).

A prospective randomized study of dogs with DKA (blood glucose (BG) concentration >250 mg/dl, venous pH <7.35, and beta hydroxybutyrate concentration >2.0 mmol/L) was performed. Dogs received either LI or RI as an intravenous (IV) continuous rate infusion according to a previously published protocol. IV fluid therapy, adjustment of the insulin infusion, antibiotic therapy, and electrolyte supplementation were also standardized. Insulin therapy was begun after 6 hours of IV fluid therapy. BG was measured q2 hours and electrolytes and venous pH were measured q6 hours.

Ten dogs have been enrolled in the study: four received LI and six received RI. Median BG concentrations at the time of admission and at the time that insulin treatment was begun were not significantly different between LI (392 mg/dl and 311 mg/dl) and RI

(500 mg/dl and 369 mg/dl) groups; however, median BG in all 10 dogs at the time insulin treatment began (328 mg/dl) was significantly lower than at admission (400 mg/dl, p=0.025). There was no significant difference between LI and RI groups in the median time from initiation of insulin therapy to the time BG dropped below 250 mg/dl (3 and 7 hours, respectively). Venous pH was not significantly different between LI (7.26) and RI (7.23) groups at the time of admission and there was no significant difference in the time it took for venous pH to reach 7.35 (28 and 44 hours, respectively). Similarly, beta hydroxybutyrate concentration was not significantly different between LI (4.4 mmol/L) and RI (4.7 mmol/L) groups at the time of admission and there was no significant difference in the time it took for beta hydroxybutyrate concentration to reach 2.0 mmol/L (29 and 40 hours, respectively). Length of hospitalization was not different between LI (85 hours) and RI (108 hours) groups. Adverse effects to insulin therapy were not identified in any of the dogs.

It is concluded that LI is a safe and effective alternative to RI treatment in dogs with DKA.

ABSTRACT #41

EVALUATION OF DETEMIR IN DIABETIC CATS MANAGED WITH A PROTOCOL FOR INTENSIVE BLOOD GLUCOSE CONTROL. K Roomp[1], JS Rand[2]. [1]Max Planck Institute for Informatics, Germany. [2]Centre for Companion Animal Health, Uni of Queensland, Australia.

There are no reported studies of long-term use of detemir in diabetic cats. The aim of this study was to report outcomes using detemir and a protocol aimed at intensive blood glucose control with home monitoring in diabetic cats, and to compare the results to a previous study using the same protocol with glargine.

Eighteen cats diagnosed with diabetes mellitus were included in the study. Cats diagnosed with acromegaly were excluded. Data were provided by owners who joined the online German Diabetes-Katzen Forum, and followed an intensive blood glucose regulation protocol for a minimum of 5 months or until remission was achieved. Detemir was administered twice daily and a low carbohydrate wet food diet was fed. The insulin dose was adjusted aiming to achieve euglycemia (50–100 mg/dL as measured using a portable blood glucose monitor calibrated for human blood). Owners performed an average of 5±2 blood glucose measurements per day in the stabilization period, and supplied spreadsheets recording daily insulin dosages, blood glucose concentration and clinical information.

Seventeen cats in the cohort were initially treated with another insulin type (16 with porcine lente insulin) for a median of 9 weeks, but failed to achieve remission prior to switching to detemir. Most (15/17) of these cats were fed a low carbohydrate diet while on the other insulin.

The overall remission rate was 67% (12/18). For cats that began the protocol within 6 months of diagnosis, the remission rate was 81% (9/11) and for those that began 6 months after diagnosis, the remission rate was 42% (3/7). The median time to remission was 1.7 months after beginning the intensive protocol (range=10 days to 5.3 months). Nine cats of 12 cats (75%) achieving remission remained off insulin, and the median duration of remission was 12.3 months (range=6.4 months to 2 years). Three cats (25% of remission cats) relapsed and required insulin again. Only one of these relapsed cats achieved a second remission.

Six of 18 cats (33%) in the cohort required insulin throughout the study to control blood glucose concentrations and did not achieve remission. The median length of time on the protocol was 10.3 months (range=5.4 months to 1.2 years). The majority (83%; 5/6) of long-term diabetics were considered well regulated with a median blood glucose concentration of ≤150 mg/dL and 17% (1/6) were moderately well regulated (median blood glucose ≤200 mg/dL). Clinical hypoglycemia was rare, with only a single event in one cat which had mild signs. The median maximum insulin dose administered to cats in the study was 1.75 IU twice daily.

These results are comparable to those of the glargine study. No significant differences were identified between outcomes for glargine and detemir, with the exception of a lower maximal dose for detemir (p-value=0.045). The median maximum glargine dose was 2.5 IU (range=1.0 to 9.0 IU) compared with a median detemir dose of 1.75 IU (range=0.5 to 4.0 IU).

ABSTRACT #42

DETERMINATION OF THE CONCENTRATIONS OF TRILOSTANE AND KETOTRILOSTANE THAT INHIBIT *EX VIVO* SYNTHESIS OF CORTISOL IN CANINE ADRENAL TISSUES. AL McGraw[1], EM Whitley[2], DM Boothe[3], EN Behrend[1], HP Lee[1]. [1]Departments of Clinical Sciences, [2]Pathobiology and [3]Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL.

Interest has been stimulated recently regarding use of trilostane in the treatment of canine hyperadrenocorticism. Trilostane competitively inhibits 3-β-hydroxysteroid dehydrogenase, an essential enzyme in the cortisol production pathway. Pharmacodynamic data for trilostane in dogs is lacking, and the potency of ketotrilostane, a metabolite of trilostane, relative to trilostane in dogs is unknown. The purpose of this study was to evaluate the *ex vivo* ability of trilostane and ketotrilostane to inhibit cortisol secretion as a model for future study of the drug and to determine whether trilostane or ketotrilostane is more potent in dogs.

For each trial (n=4), adrenal gland tissues were sliced, placed in culture and stimulated with ACTH for the first 3 hrs. From 3–7 hrs of incubation, slices were incubated with ACTH alone (none) or with one of 5 different concentrations of trilostane or ketotrilostane (T1-T5=140, 700, 1,400, 4,200 and 7,000 ng/mL and K1-K5=40, 200, 400, 1,200 and 2,000 ng/mL). In each trial, a single concentration of each compound was used in 6 tubes (total 24 tubes each for trilostane and ketotrilostane). At the end of 0, 1, 2, 3, 5 and 7 hr(s), tubes were harvested, and media and tissue slices were divided for different assays (cortisol and potassium concentrations, respectively). An adrenal slice exposed to each drug concentration was submitted for histopathology to judge tissue health. For each drug concentration, % inhibition between 3 (maximal secretion) and 7 hrs (maximal inhibition) in secreted cortisol concentration per mg tissue was calculated and compared using an ANOVA. Significance was set at $p<0.05$.

All tissue slices were judged to be healthy. The mean % inhibition when no drug was present was 2.8%. The mean % inhibition ranged from 19.5%–77.3% for T1-T5 and from 36.1%–65.2% for K1 through K5. When comparing % inhibition between the 5 trilostane concentrations and no drug, a significant difference was detected ($p<0.0001$). The % inhibition was significantly less when no drug or the lowest concentration of trilostane was present compared to T2 through T5. When comparing % inhibition between the 5 ketotrilostane concentrations and no drug, a significant difference was detected ($p<0.0056$). The % inhibition was significantly less when no drug was present compared to all ketotrilostane concentrations.

Both trilostane and ketotrilostane inhibit cortisol secretion in the *ex vivo* model. Ketotrilostane appears to be more potent than trilostane.

ABSTRACT #43

PLASMA ACTH PRECURSORS (PRO-OPIOMELANOCORTIN AND PRO-ADRENOCORTICOTROPIN) IN CATS WITH HYPERADRENOCORTICISM. G. Benchekroun[1], P. de Fornel Thibaud[1,2], M. Dubord[3], M. Le Chevoir[1], C. Petit[4], O. Dossin[5], F. Fracassi[6], F. Garnier[7], C. Maurey-Guenec[1] and D. Rosenberg[1]. [1]Internal Medicine Unit, [2]Veterinary Anticancer Center, Maisons Alfort, France [3]Veterinary Biochemistry Unit, [4]Parasitology-Dermatology Unit, [5]Internal Medicine Unit, [6]Veterinary Clinical Dpt, Faculty of Veterinary Medicine, Bologna, Italia and [7]Biochemistry Unit, National Veterinary School of Lyon, Marcy l'Étoile, France. [1,3]National Veterinary School of Alfort, Maisons Alfort, France. [4,5]National Veterinary School of Toulouse, Toulouse, France.

Feline hyperadrenocorticism (FH) is a rare condition. Pituitary-dependant hyperadrenocorticism (PDH) is observed in approximately 80% of cases. Most of the cases are associated with diabetes mellitus. Diagnosis of FH is quite difficult. First, clinical signs are non specific, except for the non systematic feline skin fragility syndrome. Secondly, the specificity of all tests validated for cats is questionable as well. Recently, an ACTH precursor (POMC/pro-ACTH) assay has been validated in cats (Benchekroun *et al.*, J Vet Intern Med 2008; 22, 794 (abstract). The aim of this preliminary study is to evaluate prospectively the plasma concentration of

ACTH precursors in a small cohort of cats with PDH and estimate its usefulness in its diagnosis.

Three groups of cats were defined. Group 1 included cats with PDH. The diagnosis of FH was based on clinical data and low-dose dexamethasone suppression test (LDDST). PDH was demonstrated by adrenal and pituitary gland CT scan. Group 2 and 3 included diabetic and apparently healthy cats respectively. For the two groups, FH was excluded by LDDST or Urine Cortisol:Creatinine Ratio (UCCR).

Whole blood was collected from the cats on EDTA-tubes; after immediate centrifugation at 4 °C, plasma was promptly frozen (−80 °C) until assay.

Six cats were included in group 1. 5 cats had a large pituitary tumor with a height ranging from 6 mm to 27 mm. No pituitary tumor was visualised in the remaining cat. Plasma ACTH precursor concentrations ranging from 229 to 1412 pmol/L were measured in PDH cats with large tumors; the remaining cat of group 1 had an ACTH precursor concentration <53 pmol/L. Group 2 and 3 included 8 and 13 cats respectively. Plasma ACTH precursors ranged respectively from <53 to 96 pmol/L and from <53 to 99 pmol/L.

Although being found in a small number of cats, these results suggest that large corticotropic tumors are associated in that species with large plasma concentration of ACTH precursors like in dogs. No plasma POMC/pro-ACTH concentration above 100 pmol/L was found in cats free of PDH. The specificity of high plasma ACTH precursor concentration in cats with PDH has to be confirmed on a larger cohort. If confirmed, given the high prevalence of large pituitary tumors, the introduction of this tool could offer a gain of specificity in the general approach of feline PDH.

Previously presented at ECVIM in Belgium, September 2008.

ABSTRACT #44

ENDOCRINE FUNCTION IN CATS AFTER STEREOTACTIC RADIOSURGERY TREATMENT FOR ACROMEGALY. KF Lunn, SM LaRue. College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Four cats received stereotactic radiosurgery (SRS) for treatment of acromegaly. Endocrine testing was performed for up to 26 weeks after SRS, to document changes in pituitary function. All cats had typical clinical signs of acromegaly, including diabetes mellitus with insulin resistance. A macroscopic pituitary tumor was detected on CT in 3 of 4 cats. SRS was administered in 2–4 fractions over 4 days using a Varian Trilogy™. Dose was based on normal tissue constraints. Serum IGF-1, thyroid hormones, endogenous ACTH, and cortisols before and after ACTH stimulation were measured before and 2, 6, 12, and 26 weeks after SRS.

All cats had elevated IGF-1 levels prior to SRS. Insulin resistance decreased markedly by 26 weeks in 2 cats, and in 1 of these, IGF-1 levels fell to 50% of baseline. At all time points evaluated, thyroid panels were unremarkable, and ACTH stimulation tests showed elevated pre- and post-ACTH cortisol values in all cats, however there were no clinical signs of hyperadrenocorticism. Endogenous ACTH levels did not indicate SRS-induced pituitary hypofunction. There was no evidence of diabetes insipidus and electrolyte values remained normal in all cases. No adverse effects of SRS were noted in any patient.

These results indicate that SRS is well-tolerated in cats with acromegaly, and may lead to improved clinical signs within 6 months. Pituitary function remains normal at least for 26 weeks post-SRS, but IGF-1 levels may improve within this time. These patients should be monitored beyond 26 weeks, as continued endocrine changes are likely.

ABSTRACT #45

BLOOD-BRAIN-BARRIER DISRUPTION IN CANINE CHRONIC EXPERIMENTAL HYPOTHYROIDISM. Rossmeisl JH, Panciera DL. VA-MD Regional College of Veterinary Medicine, Blacksburg, VA.

Hypothyroidism has been documented to cause central nervous system (CNS) abnormalities, most commonly central vestibular disease. The purpose of this study was to evaluate the effects of chronic hypothyroidism on CNS vasculature. It was hypothesized that canine hypothyroidism would cause blood-brain-barrier (BBB) abnormalities that would be detectable using indirect peripheral biomarkers.

Eighteen clinically normal, euthyroid, mixed breed females were studied. Hypothyroidism was induced by ^{131}Iodine administration in nine dogs; nine served as untreated controls. Clinical and routine cerebrospinal fluid (CSF) analyses, blood and CSF protein/lipoprotein electrophoresis, vascular endothelial growth factor (VEGF) and S-100beta concentrations, and CSF albumin quota (AQ) were performed at baseline and 6, 12, and 18 months after induction of hypothyroidism. Data were analyzed using repeated measures ANOVA.

At baseline, no differences between groups were detected for any variable. Throughout the study, controls dogs remained free of neurologic disease, and had test variables that remained within reference ranges. Two hypothyroid dogs developed CNS signs during the study, associated with necropsy evidence of brain infarction. At 12 and 18 months, the CSF total protein, VEGF, and S-100beta, and fractional albumin concentrations as well as AQ were significantly higher ($p<0.04$) in hypothyroid dogs than controls. Among test variables assayed in blood the only significant difference was a higher S-100beta concentration in hypothyroid dogs ($p=0.003$) compared to controls at 18 months.

Chronic hypothyroidism results in multiple alterations compatible with BBB disruption that may be asymptomatic, which may have clinical implications for pharmacologic treatment of coincident conditions. Further studies are needed to identify risk factors for the development of clinically apparent CNS dysfunction in hypothyroid dogs with cerebrovascular disease.

ABSTRACT #46

INDUCTION OF ARYL HYDROCARBON RECEPTORS IN CATS. J. Wakeling, J. Elliott, H. Syme. Royal Veterinary College, London, UK.

Hyperthyroidism is a common disease of older cats and is insidious in onset. Recent studies have suggested that persistent organic pollutants (POPs) may be implicated in the development of this disease. POPs are ubiquitous halogenated aromatic industrial pollutants that have many effects on reproductive and thyroid function. The most toxic POP is 2,3,7,8-tetracholodibenzo-p-dioxin (2,3,7,8-TCDD). When given to pregnant rats 2,3,7,8-TCDD decreases tT4 concentrations leading to a sustained excessive secretion of TSH followed by hyperplasia of the thyroid follicular cells in the offspring. POPs are thought to induce many of their endocrine disrupting effects by binding to the cytoplasmic aryl hydrocarbon receptor (AhR). It is possible to quantify POP binding to the AhR in a patented cell-culture system (CALUX®; Chemical Activated Luciferase Gene Expression), allowing an estimation of the total burden of POPs in a sample. This retrospective pilot study tested the hypothesis that cats accumulate POPs throughout their lifetime and that this accumulation is related to development of hyperthyroidism.

AhR induction was measured in fat samples collected from cats either at post mortem (senior cats, >8 years; n=17) or at the time of ovariohysterectomy (young cats; n=6). The luminescence detected in the CALUX® assay was used to generate results expressed as a toxic equivalence (TEQ) benchmarked to a 2,3,7,8-TCDD standard curve. Total thyroxine and TSH concentration were measured in all cats; blood samples were collected within 6 months of death or at the time of neutering, depending on the group. Senior cats were further divided into three groups; euthyroid (tT4<55 nmol/l) with TSH concentration below the limit of quantification of the assay (<0.03 ng/ml; Undetectable TSH group; n=6), euthyroid cats with detectable TSH concentrations (≥0.03 ng/ml; Detectable TSH group; n=5) and cats with hyperthyroidism confirmed ante mortem (Hyperthyroid group; n=6). Thyroid histopathology was performed in all senior cats and nodular hyperplastic changes were graded as previously described. Where data were available, the percentage daily weight loss was calculated for each senior cat in the months prior to death.

Percentage weight loss and TEQ pg/g fat were compared between groups using the Kruskal Wallis test. Normal distribution was assessed using the Kolmogorov Smirnov test. The TEQ pg/g fat was normally distributed when logarithmically transformed. Correlations between normally distributed continuous variables were tested using the Pearson's Correlation coefficient.

Median [IQ range] TEQ for all cats was 17 [8–30] pg/g fat, similar to median values reported in humans using the same assay. There was no significant difference in the induction of the AhR (TEQ pg/g fat) between the four groups of cats ($p=0.72$) or in the amount of weight loss between the three senior cat groups ($p=0.21$). There was no significant correlation of Log [TEQ] pg/g fat with histopathological thyroid grade ($p=0.25$); TSH concentration ($p=0.64$) or age ($p=0.71$).

These limited data do not provide evidence that POPs accumulate with age in cats, or that accumulation of POPs is a risk factor for the development of hyperthyroidism in this species.

ABSTRACT #47

PREVALANCE OF HYPOCOBALAMINEMIA IN CATS WITH HYPERTHYROIDISM. AK Cook[1], JM Steiner[1], JS Suchodolski[1], JE Robertson[2]. [1]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX. [2]IDEXX Laboratories, West Sacramento, CA.

Serum cobalamin concentrations are routinely measured in cats with suspected gastrointestinal disorders, and hypocobalaminemia is commonly associated with exocrine pancreatic insufficiency and diffuse distal small intestinal disease. In human medicine, abnormalities in serum cobalamin concentrations are commonly noted in geriatric individuals and those with thyroidal disease, but similar associations have not been reported in feline patients. The goal of this study was to determine the prevalence of hypocobalaminemia in cats with spontaneous hyperthyroidism.

Serum cobalamin concentrations were measured using an automated chemiluminescence assay in 76 cats with total thyroxine (T4) concentration $\geq$8.0 μg/dl (range: 8.0–30.2 μg/dl; median 11.9 μg/dl).

Cobalamin concentrations ranged from $<$150 ng/L to $>$1000 ng/L, with a median value of 409 ng/L. Thirty one cats (40%) had a serum cobalamin concentration below the reference range of 290–1,500 ng/L. There was no correlation between serum cobalamin concentrations and T4 concentrations. In addition, the mean T4 concentration for hypocobalaminemic cats and those with serum cobalamin concentrations within the reference range were not significantly different.

This study indicates that hypocobalaminemia is a common finding in cats with hyperthyroidism. The mechanism is unknown, but possible explanations include compromised cobalamin uptake due to changes in gastrointestinal transit time or changes in cobalamin requirements or metabolism. It is unknown at this time if serum cobalamin concentrations normalize when a euthyroid state is achieved, or if some cats remain cobalamin deficient following successful therapy for their hyperthyroid state.

ABSTRACT #48

HYPERTENSION IN HYPERTHYROID CATS: PREVALENCE, INCIDENCE, AND PREDICTORS OF ITS DEVELOPMENT. LD Morrow[1], VJ Adams[2], J Elliott[1], HM Syme[1]. [1]Royal Veterinary College, University of London, UK. [2]Animal Health Trust, Newmarket, UK.

Hypertension and azotemic chronic kidney disease (aCKD) can be diagnosed concurrently with hyperthyroidism and have also been detected after initiation of treatment. The aims of this study were to estimate the prevalence of hypertension in cats at first diagnosis of hyperthyroidism, to estimate its incidence after treatment for hyperthyroidism and to identify predictors of the development of hypertension in hyperthyroid cats.

Hyperthyroidism was diagnosed when total plasma thyroxine concentration was $>$55 nmol/l. Systolic blood pressure (SBP) was measured using the indirect Doppler method and the mean of five consecutive measurements was calculated. Hypertension was diagnosed when a cat had SBP greater than 170 mm Hg on two separate occasions or when it had SBP greater than 170 mm Hg on one occasion and compatible ocular signs. Hyperthyroid cats were treated medically, surgically or with a combination of these methods. A cat was diagnosed as having aCKD if it had a plasma creatinine concentration of $>$2.0 mg/dl and a urine specific gravity of $<$1.035 on the same visit. Three hundred and twenty four cats newly diagnosed as hyperthyroid at two first opinion geriatric cat clinics in London, UK between April 1, 1999 and June 18, 2008 were included in this retrospective analysis of longitudinal data using Kaplan-Meier survival analysis and Cox regression with time-varying covariates. The time-varying data that were analysed represented a total of 2205 visits to the clinic for 215 cats.

Of 324 cats diagnosed with hyperthyroidism, 21 were already receiving amlodipine for treatment of hypertension and were excluded from further study. Thirty nine of the remaining 303 cats were hypertensive at time of diagnosis of hyperthyroidism; a prevalence of 12.9% (95% CI: 9.4–17.3). The incidence with which hypertension developed in hyperthyroid cats following treatment was 22.8% (49/215, 95% CI: 17.5–29.1). Hypertension developed a median of 5.3 months (95% CI: 3.2–9.9) after treatment began. Of the newly hypertensive cats for which renal status data were available, 15 out of 42 (35.5%, 95% CI: 22.0–52.0) had a creatinine concentration of $>$2.0 mg/dl. Both urine and blood samples had been taken at diagnosis of hypertension in 31 of the cats; of these, 11 (35.5%, 95% CI: 19.8–54.6) had aCKD. Overall survival did not differ between hypertensive and normotensive cats ($p=0.4$). Age and treatment for hyperthyroidism were predictive of the development of hypertension.

We conclude that although hypertension can present concurrently with hyperthyroidism, in many cats hypertension develops after anti-thyroid treatment. In addition, hypertension does not always develop in association with the unmasking of underlying renal disease. These findings suggest that it would be prudent to assess hypertensive status in both newly diagnosed and treated hyperthyroid cats.

ABSTRACT #49

EXPRESSION OF CYCLOOXYGENASE ISOFORMS IN EQUINE GASTRIC ULCERS. Natália L. F. Rodrigues, Monique Doré and Michèle Y. Doucet. Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.

In order to characterize cyclooxygenase-1 and cyclooxygenase-2 isoforms expression in naturally occurring equine gastric ulcers, 38 ulcerated and 10 healthy equine stomachs were collected from a local abattoir. Two gastric squamous mucosa samples were taken from each stomach. When ulcers were present, samples were collected in the immediate periphery of the lesion. The first sample from each site was fixed in 10% formalin for immunohistochemical preparation and the other was frozen at -70 °C for immunoblotting analysis. The inflammatory reaction present in ulcerated tissues was evaluated on standard histology sections using a scoring system from 0 to 2, where 2 characterized the most severe reaction. Immunnoreactivity to two antibodies, MF-241 (selective for COX-1) and MF-243 (selective for COX-2), was evaluated using a scoring system from 0 to 3, where 0 denoted the absence of COX expression and 3 corresponded to the maximum expression of either COX isoform. COX-1 and COX-2 characterizations were confirmed by immunoblotting analyses. Fifty-five percent (55%) of the ulcerated gastric mucosa samples presented an inflammation score of 2 while 37% were scored 1. All normal gastric mucosa samples strongly expressed COX-1. The expression of COX-1 was significantly lower and the expression of COX-2 was significantly higher in ulcerated compared to normal mucosae ($p<0.0001$). An increased expression of COX-2 in equine squamous gastric ulcers suggests a role for this enzyme in gastric ulcer healing as observed in other species. The use of specific COX-2 inhibitors should therefore be used with caution in horses with EGUS.

ABSTRACT #50

PRIMARY GASTRIC IMPACTION IN HORSES: A RETROSPECTIVE STUDY OF 22 CASES (2005–2008). KME Vainio, BW Sykes. Hyvinkaa Horse Hospital, Hyvinkaa, Finland.

Gastric impaction is a disease characterized by excessive accumulation of ingesta in the stomach after appropriate fasting. The objective of this retrospective study was to describe the clinical findings and outcome of horses with a primary gastric impaction in a referral population. Diagnosis of a primary gastric impaction was made if the horse had been fasted for a minimum of 16 hours, a ball of ingesta precluded visualization of the margo plicatus, and there was no evidence of concurrent intestinal pathology.

Hospital records were examined, and 22 horses with a primary gastric impaction treated between 2005 and 2008 were identified. The median age of horses was 8 years (range 8 months to 25 years). Twelve of 22 horses were geldings, 9 of 21 were mares, and 1 of 21 was a stallion. Half (50%) of the patients were Warmblood riding horses. This is comparable to the hospital population (54%). Finnhorses (27%) appeared to be overrepresented compared to the hospital population (17%). Fourteen of 22 horses presented on an emergency basis. The most common complaint was inappetence (59%) followed by acute colic (32%) and recurrent colic (32%) On admission, 36% of horses were tachycardic and 36% had subjectively decreased gastrointestinal borborygmi. The most common laboratory findings included leucopenia (29%), granulocytosis (29%) and hyperfibrinogenemia (29%).

All horses were treated with enteral fluid therapy with or without analgesia and laxatives. The median dose of fluids administered per day was 5 doses of 2–10 liters of isotonic electrolyte solution (range 1 to 8 doses). The mean length of treatment until resolution was 2.1 days (range 1 to 5 days). The median duration of hospitalization was 5 days (range 2 to 9 days). The majority of horses (91%) survived to discharge. One horse was euthanized due to gastrointestinal tract rupture and one due to a non-resolving impaction. The condition reoccurred in two cases; one of these horses was euthanized and the other one was treated successfully a second time. Primary gastric impaction appears to be a condition with clinical signs of inappetence and mild abdominal discomfort. It has a good prognosis. Enteral fluid therapy may be of value in treating gastric impactions.

ABSTRACT #51
EFFECT OF VOLATILE FATTY ACID MIXES ON EQUINE NONGLANDULAR MUCOSA BIOELECTRIC PROPERTIES: PATHOGENESIS OF GASTRIC ULCERS IN HORSES

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Gastric ulcers are common in horses and volatile fatty acids (VFAs), fermentation products from the action of gastric bacteria on high grain diets, have been implicated in their cause. The purpose of this study was to measure nonglandular (NG) tissue bioelectric properties in an Ussing Chamber system following exposure to normal Ringers solution (NRS), a VFA mixture containing subthreshold VFA concentrations found 2 hours after feeding a sweet feed diet (0.5 Kg/Kg BW) and 3 VFA mixtures containing high acetic (40 mM), high butyric (40 mM) and high propionic (40 mM) acids in NRS, at pH 1.5, 4, or 7. After NRS or VFA exposure, tissues were examined under light microscopy for histopathologic evidence of cell swelling and necrosis.

Horses (n=10) were euthanatized and NG gastric tissues obtained within 30 min.Tissues were then mounted in an Ussing Chamber system and NRS or VFA mixtures, at pH 1.5, 4 or 7 added to the mucosal side of the chamber and NRS, at pH 7 was added to the serial side. Tissues were allowed to acclimate for 30 mins, then pH on the mucosal side was adjusted to pH 1.5, 4 or 7 and exposed for 330 mins. Tissue short circuit current (Isc) and potential difference (PD) were measured ever 15 mins and resistance (R) and conductance (G) calculated. After exposure, tissues were removed from the Ussing Chamber and placed in 10% formalin and stained with hematoxylin and eosin for histopathologic examination.

NG mucosa exposed to HCl alone (pH 4 and 1.5) in NRS caused an immediate significant decrease in PD, followed by a decrease in Isc and R. NG mucosa, when exposed to the sub-threshold VFA mixture ($\leq$20 mM), showed no significant change in PD or Isc, when compared to tissues exposed to NRS at the same pH. However, NG mucosa exposed to VFA mixtures containing high acetic, propionic and butyric acids, caused an immediate significant decrease in PD and Isc. Results suggest that a sub-threshold mixture of VFAs, found after feeding a grain diet (0.5 Kg/Kg BW) do not cause bioelectric changes in the equine NG mucosa other than that caused by HCl alone. However, higher concentrations of VFAs in gastric juice from excessive grain feeding, at pH$\leq$4.0 results in changes in barrier function and may lead to gastric ulcers. Histopathologic changes of cell swelling were noted only in tissues exposed to the VFA mixtures containing high VFA concentrations. This study confirms that when stomach VFA concentrations are at or below threshold ($\leq$20 mM), tissue barrier function is maintained; whereas if gastric juice VFA concentrations exceed threshold concentrations ($>$20 mM), as found after feeding grain in excess of 0.5 Kg/Kg BW, damage may occur in NG mucosa resulting in ulcers.

ABSTRACT #52
STUDY OF THE VARIABILITY OF ENDOSCOPIC DUODENAL BIOPSIES IN HEALTHY HORSES AND IN HEAVES-AFFECTED HORSES FED WITH DIFFERENT DIETS

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Endoscopic duodenal biopsies are currently used in equine medicine to evaluate small intestinal diseases. However, there is limited information concerning the normal duodenal histology of horses. A recent study reported minimal to moderate infiltration of inflammatory cells in the duodenal lamina propria of healthy horses. Diet, anti-inflammatory drugs or systemic inflammatory diseases are factors possibly contributing to mucosal inflammatory cell infiltration. The objectives of the study were: 1) to evaluate duodenal histology with healthy horses fed with different diets and 2) to compare duodenal histology in healthy horses with that of horses with heaves, a condition associated with systemic inflammation.

Glucose absorption tests, endoscopic duodenal biopsies and rectal biopsies were performed on six healthy horses fed with three different diets (sweet feed/hay, fat and fibre/hay, sweet feed/alfalfa pellets). Ten horses with heaves were evaluated during symptomatic and asymptomatic periods, when fed with different diets (sweet feed/hay or sweet feed/alfalfa pellets), and with or without inhaled corticosteroids administration. Morphology (villous stunting, epithelial injury, crypt distention, lacteal dilatation, mucosal fibrosis, lymphoid follicles) and inflammation (lymphocytes, eosinophils, plasma cells, macrophages and neutrophils in epithelium and/or in lamina propria) were assessed in a blind fashion by a board certified pathologist. Inflammatory cell infiltration was graded as minimal, mild, moderate or severe.

Glucose absorption tests and rectal biopsies were considered normal in all horses and were not different with types of food, diseases (heaves) or inhaled corticosteroids. Duodenal biopsies showed minimal to moderate infiltration with lymphocytes/plasma cells (43% of biopsies), eosinophils (11%), mixed eosinophils and lymphocytes/plasma cells (27%) with variability between horses and for a same horse, but not associated with different types of diets, heaves or corticosteroids.

In conclusion, variability of duodenal histology in horses was confirmed, but neither types of diets, heaves or inhaled corticosteroids were identified as significant factors of variability.

ABSTRACT #53
SUB-EPITHELIAL FIBROSIS IS PRESENT IN THE PERIPHERAL AIRWAYS OF HEAVES-AFFECTED HORSES

EL Setlakwe, M Leclère, JP Lavoie. Université de Montréal, St-Hyacinthe, QC.

It has been proposed that extra-cellular matrix remodelling, when present, could either serve as a protective mechanism or a contributing factor to airway obstruction or airway hyperresponsiveness. The aim of this study was to evaluate, using morphometric techniques, whether sub-epithelial fibrosis is present in the peripheral airways of heaves-affected horses.

Peripheral lung biopsies acquired under thoracoscopic guidance were obtained from 6 heaves-affected horses and 5 controls, both before and after a 30 day antigenic exposure. All horses had been kept in an antigen poor environment for at least 3 months prior to the study. Lung function parameters were derived from an esophageal ballon catheter and a heated pneumotachograph. Lung biopsies were microscopically examined using picrosirius red, a collagen specific histological staining technique. Collagen area in the sub-epithelial layer, e.g. the region between the airway smooth muscle and the epithelial layer, was measured and corrected for airway size using standard morphometric techniques.

At baseline, all horses had a normal lung function, while only heaves-affected horses developed airway obstruction after antigenic challenge. In comparison with controls, horses with heaves had a nearly doubled collagen deposition in the airway sub-epithelial layer before challenge. No further increase in sub-epithelial fibrosis was observed in diseased horses after the 30 day antigenic challenge.

Sub-epithelial fibrosis is present in the peripheral airways of heaves-affected horses and is unchanged by a 30 day continuous antigenic challenge. Further studies will be required to determine the role of fibrosis in equine obstructive lung disease.

ABSTRACT #54

FUROSEMIDE DOES NOT AFFECT TRANSVASCULAR FLUID FLUXES ACROSS THE LUNG IN EXERCISING HORSES. M. Vengust[1], C. Kerr[2], H. Staempfli[2], J. Pringle[3], G. Heigenhauser[4], L. Viel[2]. [1]University of Ljubljana, Veterinary Faculty, Slovenia; [2]University of Guelph, Ontario Veterinary College, Canada; [3]Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine and Animal Science, Uppsala, Sweden; [4]McMaster University Medical Centre, Hamilton, Ontario, Canada.

Use of intravenous furosemide is used in some racing jurisdictions for purported effect in racehorses experiencing Exercise-Induced Pulmonary Hemorrhage. The effects of furosemide appear to be a reduction in pulmonary transcapillary pressures. In addition furosemide inhibits Cl^- and HCO_3^- exchange, which will effect erythrocyte volume regulation. Erythrocyte volume regulation and increased pulmonary transcapillary pressures during exercise are major contributors to pulmonary transvascular fluid fluxes. For this study it is hypothesised that furosemide treatment would attenuate transpulmonary fluid fluxes in horses during high intensity exercise.

Six Standardbred horses were exercised twice on a high-speed treadmill (Säto Sweden) at 80% VO_2 peak until fatigue. Horses were randomly assigned treatment with 250 mg of furosemide (FurTr) or placebo (Con) intravenously 4 hours prior to exercise, with cross over treatment used at the repeated exercise test (8 days later). Resting arterial and mixed venous blood, as well as CO_2 elimination and O_2 uptake, were sampled simultaneously 5 minutes apart. During exercise, the sampling was performed at 60 sec intervals until fatigue. Erythrocyte and plasma volume changes across the lung were calculated from changes in hemoglobin and hematocrit values in venous and arterial blood. Cardiac output (Q) was calculated using Fick equation. Erythrocyte and plasma fluid fluxes, and transvascular fluid fluxes across the lung were calculated using Q and erythrocyte, plasma, and whole blood volume changes across the lung. Variables were analyzed using two-way repeated-measures ANOVA ($P<0.05$).

At rest furosemide had no effect on Q, erythrocyte and plasma fluid flux, and transvascular fluid fluxes across the lung. During exercise Q increased in Con and in FurTr, with Q being lower in FurTr at fatigue (301.8 (mean)±8.5 (SE) L/min) compared to Con (336.5±15.6 L/min) ($P<0.006$). Fluid flux from erythrocytes increased during exercise in Con and FurTr and at fatigue reached (14.6±2.3 L/min) and (11.8±2.2 L/min), respectively ($P=0.2$). There were no plasma fluid fluxes observed in either group. Transvascular fluid flux from the pulmonary circulation increased and at fatigue reached 14.1±3.5 L/min in Con and 11.8±3.4 L/min FurTr ($P=0.7$).

At the standard dose furosemide is given as premedication to race horses, it appears that changes in pulmonary transcapillary pressures and Cl^- and HCO_3^- exchange blockage do not have significant effects on erythrocyte and plasma volume changes across the lung, and transpulmonary fluid fluxes.

ABSTRACT #55

PARTITIONING OF PULMONARY RESISTANCE IN HORSES. JA Nicol, R. Léguillette. Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta.

The relative contribution of larger and smaller airways to total lung resistance is unknown in horses. The purpose of this study is to determine the relative contribution of central airway (bronchi binomial numbering: #1.1), large bronchi (first generation), small bronchi (third generation), and smaller airways embedded in the lung tissue to the total lung or lobar resistance.

To address this question we measured lung mechanics ex-vivo using 5 horses' lungs. The lungs were obtained from an abattoir, then only their cranial lobes were used and their central airway (#1.1) was cannulated. Two retrograde catheters were then blindly inserted respectively in a larger first generation bronchus, as well as a smaller third generation bronchus. One alveolar capsule was also glued to the surface of each lobe. The lobes were then suspended in a sealed box and subjected to quasi-sinusoidal pressure changes. A pneumotachograph was inserted at the outlet of the main bronchus and used to measure airflow and calculate volumes. Five pressure transducers were used to measure transpulmonary, central airway, peripheral airway, and alveolar pressures. The lungs were ventilated in the box at 3 different transpulmonary pressures (Ptp) (15, 20, 30 cmH_2O) and frequencies (10, 20, 30 breaths/min). Total lobar resistance (RL) and elastance (EL) were calculated. Individual resistances to airflow from the central airway (Rc), the two different size catheterized airways (RpLarge and RpSmall) and lower tissue elastance (Et) and resistance (Rt) were calculated and expressed as relative values of RL. The diameter of the catheterized airways was measured using a standard method after the lung mechanics.

The measured diameter of the catheterized airways was 5.7 mm (±0.9 SD) for the larger airways and 2.5 mm (±1.1 SD) for the smaller airways.

Effect of an increase in Ptp: The following results were obtained when Ptp was increased from 15 cm H_2O to 30 cm H_2O at the different respiratory rates mentioned above:

RL and EL are increasing significantly for all respiratory rates. The relative contribution of Rc, RpLarge and RpSmall to RL significantly decreased for all respiratory rates. Conversely, the relative contribution of Rt to RL significantly increased for all respiratory rates. Furthermore, the relative contribution of first and third generation peripheral airway to RL was not significantly different for all respiratory rates and Ptp values.

Effect of an increase in respiratory rate: The change in respiratory rate from 10 breath/min to 25 breaths/min had no significant affect on RL, Rc, Rp and Rt. The increase in EL with increasing respiratory rate was not significant.

The effect of Ptp on lung mechanics was predominant on the change in respiratory rate. The most important contributor to RL is Rt. The relative contributions of Rc, RpSmall and RpLarge to RL decrease when Ptp increases, whereas the relative contribution Rt to RL does the opposite.

ABSTRACT #56

EFFECTS OF PROLONGED INHALED CORTICOSTEROID TREATMENT ON CELL-MEDIATED IMMUNITY IN HORSES. Julie Dauvillier[1], M. Julia B.F. Flaminio[2], Jean-Pierre Lavoie[1]. [1]Faculté de Médecine Vétérinaire, Université de Montréal, Saint Hyacinthe, QC, Canada. [2]College of Veterinary Medicine, Cornell University, Ithaca, NY.

A single therapeutic dose of systemically administered corticosteroids significantly modifies cell-mediated immunity in horses. Thus, inhaled medications have been used in an attempt to attenuate systemic side effects when the prolonged administration of corticosteroids is necessary for the treatment of chronic inflammatory pulmonary diseases. The objective of this study was to determine whether a prolonged administration of inhaled corticosteroids altered the cell-mediated immunity in horses.

Ten adult horses with heaves were divided into 2 groups: 5 horses received therapeutic dosages of inhaled fluticasone while 5 others received no medication. Nine months after the beginning of treatment, the following parameters were measured in peripheral blood using flow cytometry: lymphocyte subpopulation distribution (CD4+ T lymphocytes, CD8+ T lymphocytes, B lymphocytes), lymphocyte function-associated antigen-1 (LFA-1) and major histocompatibility complex (MHC) class II expression on lymphocytes and monocytes, and *in vitro* lymphocyte proliferation when stimulated by pokeweed mitogen or concanavalin A. Values of treated and untreated horses were compared using a Student t-test. A complete blood count was performed to evaluate total leukocyte subpopulations before (T0), and after 2, 6 and 11 months of treatment. A repeated-measures linear model, with group as between-subject factor and months of treatment as within-subject factor, was

used and comparisons of means were conducted with the sequential Bonferroni procedure.

Results of the different immunologic tests were comparable between groups ($p > 0.05$). Moreover, no significant differences were observed in the leukocytes subpopulations, neither between groups nor within groups between different time points. In addition, none of the horses developed signs of infection that could have resulted from immunosuppression. These results suggest that prolonged administration of inhaled fluticasone is not associated with major alteration in cell-mediated immunity in horses.

ABSTRACT #57

EHV-1 POSITIVE PBMC FRACTIONS DURING CELL-ASSOCIATED VIREMIA FOLLOWING EXPERIMENTAL INFECTION

S Wilsterman, LV Ashton, RJ Callan, DP Lunn, S Rao, LS Goehring. College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Viremia during Equid Herpesvirus type 1 (EHV-1) infections is a prerequisite for horses to develop neurological disease. Cell-associated viremia occurs in peripheral blood mononuclear cells (PBMC), which consist of 4 cell subsets: CD4, CD8, B lymphocytes and monocytes. The goal of this study was to identify the virus-infected PBMC subsets during EHV-1 viremia.

PBMC were collected from 2 independent infection studies between days 4–9 *post infection*. The first study included 8 cross-bred yearling horses, infected with EHV-1, strain Ohio 03. The second study used 7 cross-bred yearling ponies, and EHV-1 strain Ab4. PBMC were isolated, labeled using subset-specific monoclonal antibodies, and separated into 4 fractions by using a magnetic bead technique. Fractions from each animal on each collection day were submitted for quantitative PCR analysis using 2 PCR assays. PCR EHV1 gB provided viral genome copy numbers detected in each sample, and PCR equine β-actin estimated the number of nucleated cells in a sample. Results were expressed as qualitative data, absence or presence of viremia in a fraction, and as quantitative data as viral genome copy numbers/2.5×10^5 cells. Multivariable linear regression analysis was used to determine the association of 'cell subset' and 'day post infection' with 'EHV-1 gB copy number'. The viral load of the monocyte cell population, and 'day 4' were used as references for 'cell subset' and 'day post infection', respectively. Poisson regression was used to analyze the average number of viremic days in each fraction.

Neurological disease was mild and transient in 3 horses, and absent in all ponies. Duration of viremia in horses (days 5–9) was longer than in the ponies (days 5–7), and was undetectable in 2 horses, and in 1 pony. Total horse viremic days for each subset were 17 (CD8), 7 (B lymphocyte), 3 (monocyte) and 2 (CD4). Virus quantity was significantly higher in CD8 when compared to the reference. Viral load in CD4 and B lymphocytes was not significantly different from the monocyte reference. Total pony viremic days were 12 (CD8), 10 (B lymphocytes), 7 (monocytes) and 5 (CD4). In the pony study virus quantities were not different between CD8, B lymphocytes and monocytes. CD4 lymphocytes consistently contained fewer viral genome copies than the reference.

Differences in number of viremic days, virus preference and quantity within a PBMC subset may depend either on the strain that was used for infection (Ohio 03 *versus* AB4), or on the host (horse *versus* pony). Both experiments show that the CD8 subset contained the highest cumulative viral load followed by B lymphocytes, monocytes and CD4. The results on subset preferences do not suggest a mechanism on how endothelial cells become infected. Future studies will be necessary to clarify this mechanism.

ABSTRACT #58

THE EFFICACY OF NITAZOXANIDE AS AN ANTIVIRAL AGENT FOR THE TREATMENT OF EHV-1 INFECTION IN HORSES

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There is currently no specific antiviral medication for the treatment of EHV-1 myeloencephalopathy. The purpose of this study was to determine whether or not treating horses with nitazoxanide (Navigator®) paste prior to and during infection with EHV-1 was efficacious in protecting the treated horses against clinical disease, and whether or not this treatment diminished the nasal shed of virus or decreased the extent of viremia.

16 yearling horses were randomly assigned to a treatment (n=8) or control group (n=8). Horses were verified EHV-1 seronegative prior to the experiment. The treatment group was treated with 25 mg/kg of nitazoxanide by mouth twice per day starting two days prior to EHV-1 infection for a total of 25 days. The control group received a placebo. All horses were challenged with a 5×10^7 PFU of EHV-1 (Ohio 03). Horses were evaluated daily by a veterinarian who did not have knowledge of the treatment groups, a clinical score was determined (0=no abnormalities). Viremia and the nasal shed of virus were determined using quantitative PCR for the viral glycoprotein B gene. Clinical score data was analyzed using a Wilcoxon non-parametric test. Physical exam parameters, viremia, and nasal shedding were analyzed by linear regression.

There were no significant differences in the clinical scores or physical exam parameters between the treatment and control groups following EHV-1 infection. The log peak titer of viremia was lower in the treatment group but not significantly different. There were no significant differences noted between the treatment and control groups in regard to the peak nasal titer, the duration of nasal shed of virus or the duration of viremia.

The treatment of horses with nitazoxanide does not significantly decrease viremia, nasal viral shed or ameliorate the clinical signs associated with EHV-1 infection.

Previously presented at Conference of Research Workers in Animal Diseases.

ABSTRACT #59

REPEATABILITY AND REPRODUCIBILITY OF TRANSABDOMINAL ULTRASONOGRAHIC RENAL LOCI AND DIMENSIONS IN THOROUGHBRED HORSES

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Transabdominal renal ultrasonography is the most widely utilised imaging modality for the investigation and diagnosis of renal disease but to the authors' knowledge no reference ranges or reports of repeatability and reproducibility have previously been published in horses. The aims of this study were to report intercostal space (ICS) visibility of Thoroughbred kidneys, normal references ranges for various kidney dimensions in longitudinal (Long) and transverse (Trans) section which include total kidney, cortex, medulla and pelvic length and width dimensions and to calculate repeatability and reproducibility of these dimensions using transabdominal ultrasonography. Seven Thoroughbred horses (500±30 kg) were scanned in triplicate by two scanning technicians over three consecutive days. Images were analysed on three separate occasions by one observer (to assess repeatability) and once by a second observer (to assess reproducibility). Reproducibility was also assessed using a questionnaire sent to 10 clinicians; this consisted of 10 ultrasound images (5 normal and 5 abnormal kidneys) from clinical cases (diagnosis was confirmed by post-mortem examination). Statistical analysis included ANOVA, repeated measures ANOVA and *post-hoc* Bonferoni test and paired T-test. Both kidneys were visible in the paralumbar fossa (PLF) and 17th, 16th and 15th ICS, with the right kidney rarely visible in 14th ICS. Mean values (±SD) were obtained for all renal dimensions and renal length and width are displayed in table 1. There were significant differences between renal dimensions in different ICS on both left and right sides ($p=0.03$) except between the 17th and 16th ICS on both the left and right sides. Good repeatability and reproducibility was demonstrated from the horses examined with an intra-class correlation coefficient (ICC) of >0.9 for all renal dimensions. Reproducibility of normal and abnormal renal dimensions from the questionnaire was also deemed to be excellent ($ICC > 0.93$) as was identification of normal versus abnormal. In conclusion a normal reference range for various renal dimensions in Thoroughbred horses has been established. Excellent repeatability and reproducibility of renal measurements and identification of abnormal versus normal mean that this is a valuable clinical modality for examination of this organ.

Table 1. Dimensions for the left (L) and right (R) kidneys displayed as mean (±SD).

	PLF		17th ICS		16th ICS		15th ICS	
	Long	Trans	Long	Trans	Long	Trans	Long	Trans
L Length (cm)	12.3	13.1	10.9	11.4	13.9	12.5	12.7	12.1
	(1.2)	(2.1)	(2.9)	(3.1)	(1.3)	(1.4)	(2.2)	(1.6)
Width (cm)	7.7	8.8	7.6	8.0	6.6	7.5	7.2	7.7
	1.1)	(1.4)	(2.2)	(1.6)	(0.7)	(1.1)	(1.2)	(2.1)
R Length (cm)	14.7		13.7	11.6	14.8	10.3	16.0	11.0
	(0.8)		(2.1)	(1.0)	(1.8)	(0.6)	(0.4)	(1.2)
Width (cm)	7.5		7.5	9.6	7.4	4.3	7.9	8.5
	(0.6)		(0.8)	(0.9)	(0.7)	(0.3)	(0.5)	(0.9)

ABSTRACT #60

RENAL BIOPSIES IN HORSES: A RETROSPECTIVE STUDY OF 71 CASES. GA Tyner[1], S Lindborg[1], A Gruntman[2], L Javsicas[3], C Sommardahl[4], LC Fennell[5], N Menzies-Gow[6], L Coutëil[7], T Norman[8], L Hardefeldt[9], RD Nolen-Walston[1]. [1]New Bolton Center, University of Pennsylvania, Kennett Square, PA. [2]Tufts University, Grafton, MA. [3]University of Florida, Gainesville, FL. [4]University of Tennessee, Knoxville, TN. [5]University of Melbourne, Melbourne, Aust. [6]Royal Veterinary College, London, UK. [7]Purdue University, West Lafayette, IN. [8]Texas A&M University, College Station, TX. [9]University of Wisconsin, Madison, WI.

Renal biopsies are infrequently performed in horses due to concerns regarding complication rate and poor diagnostic yield. This multi-center retrospective analysis was performed to determine the utility and safety of renal biopsies in equids. Nine institutions identified a total of 71 horses that had undergone renal biopsy. The median age was 8 years (range 11 days-26 years), consisting of 25 mares, 33 geldings, and 13 stallions. Presenting complaints included chronic anorexia (28% of cases), azotemia (27%), weight loss (27%), colic (15%), hematuria (15%), and polyuria/polydipsia (15%). Azotemia was present in 69% of cases (median creatinine 3.0 mg/dl, range 0.4–29.0), 44% were anemic, and 30% were hypoproteinemic. Electrolyte abnormalities included hypochloremia (59%), hypocalcemia (42%), hyponatremia (32%) and hypercalcemia (19%). Urinalysis results revealed isosthenuria in 35%, and an increased fractional excretion of sodium in 26%. Diagnostic ultrasonography was performed in 94% of cases prior to biopsy. The principal sonographic findings were increased echogenicity (23%), renomegaly (15%), loss of corticomedullary distinction (15%), renal atrophy (8%), and renal mass (9%). The biopsy was taken from the right kidney in 77% of cases, the left in 15%, and both kidneys in 7%. A median of 2 samples (range 1–4) were taken per kidney, and one specimen was obtained laparoscopically.

A histologic diagnosis was obtained for 79% of samples, 12% revealed no lesion, and 9% of samples were inadequate for diagnosis. Complications occurred in 11% (8/71) cases; most were mild and self-limiting, and included colic (2/8), hematuria or hematoma not requiring transfusion (4/8), pain (1/8), and abortion (1/8). No cases received a blood transfusion. One case died as a result of the biopsy procedure (1/71), resulting in a mortality rate of 1.4%. This outcome occurred in an adult gelding that underwent biopsy of a left kidney mass and died of exsanguination associated with arterial disruption. The majority of cases were discharged alive (77%), although 10% were euthanized at home within one month. A total of 22% of cases were euthanized in hospital; 13% due to poor prognosis, 3% from worsening of clinical signs, 3% for various comorbidities, and 2% due to financial constraints. In conclusion, renal biopsies in the horses were found to be associated with a low rate of serious complications, and provided a definitive histopathologic diagnosis in most cases. Further investigation is warranted to establish if data generated by renal biopsy significantly alters therapeutic plan or ability to provide accurate prognostic information.

ABSTRACT #61

OWNER PERCEPTIONS AND PRIORITIES REGARDING QUALITY AND QUANTITY OF LIFE IN CATS WITH HEART DISEASE. CA Reynolds[1], MA Oyama[1], D Brown[1], J Rush[2], E Rozanski[2], P Fox[3], D Adin[4], R Williams[5], K MacDonald[6], R Malakoff[7], KE Schober[8], ML O'Sullivan[9], GE Singletary[1]. [1]University of Pennsylvania, Philadelphia, PA. [2]Tufts University, North Grafton, MA. [3]The Animal Medical Center, New York, NY. [4]Veterinary Specialists of Rochester, Rochester, NY. [5]Veterinary Emergency Clinic, Toronto, ON. [6]The Animal Care Center, Rohnert Park, CA. [7]Angell Animal Medical Center, Boston, MA. [8]The Ohio State University, Columbus, OH. [9]Ontario Veterinary College, University of Guelph, Guelph, ON.

Owners' perceptions and priorities regarding quality of life (QoL) are important considerations given the unknown efficacy of many commonly administered medications, stress of hospital visits, difficulties providing home care, and personal choices involving euthanasia. We sought to better understand these issues through questionnaire-based study of owners of 182 cats with a variety of heart diseases from 8 sites. Appetite, owner interaction, sleep patterns, and litterbox habits were deemed significantly more important to QoL than pet-pet interaction, play habits, and desire to go outdoors. Concern over pet suffering was significantly greater than life expectancy. Using an 11-point scale ranging from 0 (low QoL, long lifespan) to 10 (high QoL, short lifespan), owners desired a median balance between these priorities of 8. 94% of owners were willing to trade survival time for comfortable QoL; 54% of these were willing to trade up to 6 mo. On univariate analysis, factors associated with willingness to trade 6 mo included owner age, study site, clinician, ISACHC class, stress of hospital visit, number of cats in the household, and current medications. On multivariate analysis, factors associated with increased willingness to trade were study site, cats not receiving medications, and older age of owners. Owner concern whether stress of administering medications at home would decrease QoL increased with number and frequency of medications. These results indicated that QoL is more important to owners of cats with heart disease than longevity. The various priorities and concerns of cat owners should be taken into account in order to provide optimal care.

ABSTRACT #62

VALIDATION OF PLATELET FACTOR 4, SOLUBLE P-SELECTIN, β-THROMBOGLOBULIN, AND 11-DEHYDRO-THROMBOXANE B2 ELISAS IN THE FELINE POPULATION. SB Bordelon, AM Heaney, OL Nelson, TB Wills, KM Meurs. Washington State University – College of Veterinary Medicine, Pullman, WA.

Arterial thromboembolism (ATE) is a common complication of feline cardiac disease and identification of cats at risk for ATE is important. In human patients traditional markers of coagulation with one or more of the following; platelet factor 4 (PF4), soluble P-selectin (sP-selectin), beta-thromboglobulin (β-TG), or 11-dehydro-thromboxane B2 (11-dehydro-TXB2) have been used to identify patients at risk for thromboembolism.

We hypothesized that human ELISAs for PF4, sP-selectin, β-TG and 11-dehydro-TXB2 could be used to establish normal values for these markers in cats.

The objective of this study was to validate human ELISAs for PF4, sP-selectin, β-TG and 11-dehydro-TXB2 in the feline population.

Twenty-five cats were determined to be free of systemic and cardiac disease via physical examination, echocardiography, electrocardiography, blood pressure, complete blood counts, biochemical profiles, and total T4 levels. Three milliliters of blood was collected from each cat and placed into CTAD tubes. Samples were centrifuged to obtain citrated (sP-selectin) and platelet poor plasma (PF4 and β-TG). Urine samples for 11-dehydro-TXB2 were collected into C&S tubes. Intra-and inter-assay variability was determined for each ELISA.

The β-TG ELISA did not detect β-TG in any of the cats. Intra-assay variability for PF4, sP-selectin and 11-dehyro-TXB2 was 1–33%, 3- > 1000%, and 14–18%, respectively. Inter-assay variability for PF4, sP-selectin and 11-dehyro-TXB2 was 6–110%, 3–89%, and 10–44% respectively.

We conclude that these ELISAs for PF4, sP-selectin, β-TG, and 11-dehydro-TXB2 are not useful in cats. However, the devastating nature of feline ATE warrants further investigation into markers that may identify cats at risk for thromboembolism.

ABSTRACT #63
PLASMA PECAM-1 AND ENDOTHELIN-1 CONCENTRATIONS ARE ELEVATED IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY. KE Jandrey, JW Norris, K Huang, MD Kittleson, F Tablin. University of California, School of Veterinary Medicine, Davis, CA.

The purpose of this study was to determine the plasma concentrations of platelet/endothelial cell adhesion molecule-1 (PECAM-1) and endothelin-1 (ET-1) in normal cats and cats with hypertrophic cardiomyopathy (HCM). Our hypothesis was that cats with HCM have increased concentrations of both variables when compared with normal cats.

Platelet rich plasma and platelet poor plasma (10 ug/ml PGE1, 1% aprotinin, and 2 mg/ml leupeptin) from normal cats and cats from a research colony with familial HCM were prepared from anticoagulated whole blood. All cats were evaluated by echocardiography.

ET-1 concentration was determined in triplicate using a commercially available ET-1 ELISA assay previously validated for use in cats. Recombinant human ET-1 was used to generate a standard curve for assay calibration. Plasma PECAM-1 concentration was measured by Western blot analysis using a polyclonal antibody and densitometry (arbitrary units) was quantified by Image J analysis (NIH Shareware). Paired t-tests were performed to compare groups (Sigma Stat, Systat, San Jose CA) and statistical significance was set at $p<0.05$.

The mean normal cat plasma PECAM-1 concentration (n=5) was 1276+684, and was 2939.9+768.9 (p=0.015) in the group of cats with HCM (n=7). The mean normal cat plasma ET-1 concentration (n=9) was 3.56+0.98 fmol/ml and was 6.55+1.25 fmol/ml in the cats with HCM (n=4; $p<0.001$).

This increase in PECAM-1 and ET-1 likely indicate that cats with HCM have platelet and endothelial up-regulation. An increase in the concentration of these plasma proteins may be an indication of increased risk of thromboembolism.

ABSTRACT #64
ASSOCIATION OF HYPERTROPHIC CARDIO-MYOPATHY PHENOTYPE AND GENOTYPE IN ITALIAN MAINE COON CATS. F Porciello[1], P Ferrari[2], F Birettoni[1], M Rishniw[3], G Pertica[4], M Polli[4], M Longeri[4]. [1]University of Perugia, Italy. [2]Observatory for Feline HCM, Italy. [3]Cornell University, Ithaca, NY. [4]University of Milano, Italy.

The Italian Observatory for Feline Hypertrophic Cardiomyopathy (HCM), established March 2008, aims to monitor the natural history of HCM in Maine Coons via an integrated echocardiographic and genetic database and provide advice to breeder groups. We examined data from 75 Maine Coon cats (3 months to 6 years) enrolled during the first 7 months.

Standard echocardiographic methods were used to define the disease status in all cats: HCM echo-positive or HCM echo-negative (wall thickness $\geq$or$<$6 mm respectively). Any echo-positive cats with concurrent hypertension, nephropathy or hyperthyroidism, were excluded from analysis. All cats were screened for MYBPC3 mutation G91C by sequencing both strands.

Sixty-three cats were echo-negative; 12 were echo-positive for HCM. Genotype frequencies were 64% N/N (Negative/Negative), 28% P/N (Positive/Negative) and 8% P/P (Positive/Positive). Average discrepancy between genetic and echocardiographic results was 35% with most discrepancy (86%) associated with the P/N genotype. In cats >3 years, 50% of echo-negative cats had a P/N genotype and no echo-negative cats had a P/P genotype. However, the incidence of echo-positive cats with N/N genotype increased from 0% to 50% with increasing age.

Our study demonstrates substantial discordance between genotype and phenotype in heterozygous Italian Maine Coons $<$3–4 years of age. Construction of age-specific detection curves for P/N and P/P genotypes is necessary to calculate the probability that asymptomatic subjects will develop the disease. The presence of some echo-positive cats with an N/N genotype indicates that factors other than the MYBPC3 G91C mutation are likely involved in HCM in Maine Coons.

ABSTRACT #65
PULSED TISSUE DOPPLER IDENTIFIES PRECLINICAL LEFT VENTRICULAR MYOCARDIAL DYSFUNCTION IN OBESE DOGS. DS Schwartz, VMC de Oliveira, PRR Melo, AM Mazini, P Claus, GT Goldfeder, AC Ferreira, MHMA Larsson. Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brazil.

Human overweight subjects without overt heart disease show changes on left ventricular (LV) function, detected by tissue Doppler imaging (TDI), even in the absence of coronary heart disease or other comorbidities, but this effect on dogs is not well established.

We sought to investigate preclinical effects of obesity on left ventricular function of dogs, using pulsed TDI.

LV myocardial Doppler derived systolic (Sm), early (Em) and late (Am) diastolic velocities were obtained by transthoracic echocardiography from 16 obese dogs and 30 non-obese, classified by body condition scoring. Other comorbidites were ruled out. Students t test (mean±SD) and Mann-Whitney test (median, P_{25} and P_{75}) were applied (Sigma Stat 3.5), significance was considered if $P<0.05$.

Obese presented significantly lower Em at anular interventricular septum (IVS) (0.0719±0.0229 vs 0.0915±0.0247; P=0.012); Em/Am at IVS (1.114±0.35 vs 1.384±0.370, P=0.020); Em at anular left ventricular free wall (LVFW) (0.103±0.0256 vs 1.20±0.0267, P=0.048); Em/Am at anular LVFW (1.320±0.303 vs 1.576±0.336, P=0.115) and increased Am at mid LVFW (median=0.085, P_{25}=0.07, P_{75}=0.0917 vs median=0.0685, P_{25}=0.06, P_{75}=0.077, P=0.029), a decreased Sm at anular LVFW (0.126±0.0378 vs 0.154±0.0473, P=0.049), and an increased E/Em (10.398±2.915 vs 8.648±2.267, P=0.029), suggesting an increased LV filling pressure. Obese and controls were comparable regarding other longitudinal and radial velocities.

Overweight dogs without overt heart disease present preclinical left myocardial systolic and diastolic function changes detected by pulsed TDI. Even without a predisposition of dogs to ischemic heart disease, obesity has an impact on ventricular function.

ABSTRACT #66
SEROTONIN TRANSPORTER EXPRESSION IS LOCALLY DOWN-REGULATED IN CANINE DEGENERATIVE MITRAL VALVES. SM Scruggs, S Disatian, EC Orton. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Tryptophan hydroxylase 1 (THP1) is the rate limiting enzyme necessary for peripheral serotonin synthesis and the serotonin transporter (SERT) is a key component of serotonin metabolism. SERT transports serotonin across the cell membrane for intracellular metabolism and competes with serotonin receptors for interaction with serotonin. We have recently reported an autocrine serotonin signaling mechanism in canine degenerative mitral valves based on up-regulation of THP1 and down-regulation of SERT in late-stage disease. It is unknown whether SERT down-regulation occurs early or late in degenerative mitral valve disease (DMVD) or whether its down-regulation is a local or systemic phenomenon. SERT expression was determined in canine normal (n=4), early- (n=4), and late-stage (n=4) degenerative mitral valves by immunohistochemical (IHC) and immunoblot (IB) analyses. SERT expression was also evaluated in circulating platelets from normal dogs and dogs with DMVD. SERT was expressed in all layers of normal and early-stage mitral valves based on IHC. SERT was markedly down-regulated in valve interstitial cells, but not valve endothelial cells, of late-stage mitral valves. SERT expression was significantly decreased ($p<0.05$) in late-stage, but not early-stage mitral valves on IB. Up-regulation of TPH1 in late-stage degenerative mitral valves was confirmed. No difference in SERT expression was apparent in circulating platelets from normal and DMVD dogs. We conclude that SERT down-regulation is a local and late-stage phenomenon in canine DMVD. SERT down-regulation likely enhances autocrine serotonin signaling in late-stage DMVD by decreasing local serotonin metabolism and increasing interaction of serotonin with its receptor.

ABSTRACT #67

B-TYPE NATRIURETIC PEPTIDE AND CYCLIC GUANOSINE MONOPHOSPHATE LEVELS INCREASE WHEREAS NITRIC OXIDE BIOAVAILABILITY DECREASES WITH INCREASING DEGREE OF MYXOMATOUS MITRAL VALVE DISEASE.

SG Moesgaard[1], T Falk[1], T Teerlink[2], HH Guðmundsdóttir[1], S Sigurðardóttir[1], CE Rasmussen[1], LH Olsen[1]. [1]University of Copenhagen, Frederiksberg, Denmark, [2]VU University Medical Center, Amsterdam, The Netherlands.

Myxomatous mitral valve disease (MMVD) is a widespread heart valve disease in dogs, and a lot of research has focused on identifying biomarkers in asymptomatic stages which could be beneficial in the future diagnosis, prognosis and treatment of MMVD. Among several potential biomarkers the natriuretic peptides have so far been the only ones showing significant changes in several studies of both asymptomatic and symptomatic dogs. The aim of this study was to measure plasma cyclic guanosine monophosphate (cGMP) concentration in dogs with different degrees of MMVD. cGMP is a second messenger for both natriuretic peptides and nitric oxide (NO). We therefore studied the association between cGMP and both N-terminal pro-B-type natriuretic peptide (BNP) and the L-arginine/asymmetric dimethylarginine (ADMA) ratio (a measure of NO bioavailability).

Five groups consisting of 1) Cavalier King Charles Spaniels (CKCS) with no or minimal mitral regurgitation (MR), 2) CKCS with mild MR 3) CKCS with moderate to severe MR, 4) dogs in heart failure due to MMVD (different small breeds) and 5) Cairn Terriers with no or minimal MR were subjected to a clinical examination, blood sampling and echocardiography.

Plasma BNP increased with increasing MR and it was significantly elevated in the CKCS with moderate to severe asymptomatic MR ($P<0.05$) and further elevated in the heart failure dogs ($P<0.0001$). Plasma cGMP concentration also increased with increasing MR and was highly correlated with BNP ($P<0.0001$). However, cGMP was only significantly increased in the heart failure dogs ($P<0.01$). The cGMP/BNP ratio decreased with increasing MR and the decrease was significant in the heart failure dogs ($P<0.05$). The L-arginine/ADMA ratio decreased with increasing MR which was also significant in the heart failure dogs ($P<0.005$). Furthermore, both cGMP and L-arginine/ADMA ratio levels differed between the CKCS and the Cairn terrier breeds ($P<0.05$).

These results confirm that BNP increases already in the moderate to severe asymptomatic stages of MMVD. The increase in BNP is closely correlated to an increase in cGMP, however, the cGMP/BNP ratio decreases with heart failure, which indicates that the dogs may have developed natriuretic peptide resistance. Finally, the decrease in the L-arginine/ADMA ratio suggests that there is a decrease in NO bioavailability in the heart failure dogs which could then again have a negative influence on the cGMP concentration. These results are interesting considering the promising treatment results with Pimobendan, which is a phosphodiesterase inhibitor that may restore cGMP and improve endothelial function.

ABSTRACT #68

TRANS-THORACIC THREE-DIMENSIONAL ECHOCARDIOGRAPHIC EVALUATION OF NORMAL CANINE HEART VALVES.

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With its unique ability to display and spatially orientate the heart's structures in three dimensions, we sought to determine if three-dimensional echocardiographic (3DE) views of the four canine heart valves (mitral=MV, tricuspid=TV, aortic=AV, pulmonic=PV) could be obtained consistently, and to determine the quality of the 3DE images and whether they provided views and information not obtainable by the standard two-dimensional echocardiographic (2DE) examination.

Both 2DE and 3DE examinations (Philips iE33; 2D: 5 MHz or 8 MHz transducer; 3D: X3 or X7 transducer) were performed in 12 normal dogs which were restrained in lateral recumbency on a table designed to allow ventral transducer placement. A continuous electrocardiogram (ECG) was obtained during all examinations. Standard 2DE views were used to assess the four valves, including a right parasternal long-axis view (MV, TV, AV), a right parasternal short axis view at the level of the mitral valve (MV), a right parasternal short axis view at the level of the heart base (AV, PV), a left apical four chamber and/or five chamber view (MV, TV, AV), and left cranial long axis and short axis views (TV, AV, PV). 3DE datasets included full volume 4 beat acquisitions as well as live 3DE images of the valves. 3DE images of the valves were obtained and then evaluated and manipulated on a separate dedicated workstation. Valves were evaluated in each view and quality was graded subjectively using the following scale: 1=poor; 2=fair; 3=good; 4=excellent. Images of the mitral valve were most consistently obtained (12/12), with a quality grade of 3 or 4 in 10/12 dogs. The tricuspid valve was imaged in most dogs (10/12), with 3 dogs receiving a quality grade>2. The aortic valve was imaged in every dog (12/12), with 5 dogs receiving a quality grade>2. The pulmonic valve was the most difficult to view, with only 3/12 dogs with a valve that could be evaluated, all of which received a grade of 2.

These preliminary results indicate that trans-thoracic 3DE images of the canine heart valves can be obtained and evaluated within the context of the routine cardiology evaluation and provide unique views complimentary to the 2DE examination. Good to excellent images of the MV were consistently recorded. High quality images of TV, AV, and PV were more difficult to obtain.

ABSTRACT #69

CARDIAC TROPONIN-I IS ASSOCIATED WITH SEVERITY OF MYXOMATOUS MITRAL VALVE DISEASE, AGE, HEART RATE AND C-REACTIVE PROTEIN IN DOGS.

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Analysis of cardiac troponin I (cTnI) may increase the knowledge of cardiac remodeling in dogs with myxomatous mitral valve disease (MMVD). An age- and gender dependent variation in cTnI has been shown in rats, dogs and humans, and cTnI is known to be related to systemic inflammatory response. The role of inflammatory mediators in the progression of cardiac remodeling in MMVD is unknown. The aims of the study were to investigate whether plasma levels of cTnI and C-reactive protein (CRP) were associated with severity of MMVD, and to investigate potential associations between cTnI and signalment variables in dogs with MMVD.

Prospective evaluation by physical examination, electrocardiography, blood pressure measurement, and echocardiography was performed in 81 dogs. Plasma cTnI was analysed by a high sensitivity assay (Beckman Coulter AccuTnI) with a lower limit of detection of 0.001 ng/ml (10% CV at 0.014ng/ml), and plasma CRP was analysed by a canine CRP ELISA (TriDelta Development Ltd).

Healthy dogs (n=11) had a median cTnI of <0.001 ng/ml (interquartile range (IQR) <0.001–0.004), dogs with mild MMVD (n=39) had a median cTnI of 0.003 ng/ml (IQR 0.001–0.024), dogs with moderate MMVD (n=13) had a median cTnI of 0.014 ng/ml (IQR 0.008–0.029), and dogs with severe MMVD (n=18) had a median cTnI of 0.043 ng/ml(IQR 0.031–0.087). Cardiac troponin-I concentrations differed significantly between healthy dogs and dogs with moderate MMVD ($P<0.001$), between healthy dogs and dogs with severe MMVD ($P<0.001$), between dogs with mild and severe MMVD ($P<0.001$), and between dogs with moderate and severe MMVD ($p<0.002$). Multiple regression analysis indicated a major effect of age, heart rate, CRP concentration, and left ventricular end diastolic diameter corrected for body weight on cTnI concentration. Age was the variable that contributed most to the model R^2. C-reactive protein levels did not differ significantly between severity groups, but increased significantly with increasing age.

In conclusion, plasma cTnI concentration was found to increase with increasing severity of MMVD, and cTnI concentration increased with increasing age, heart rate, left ventricular end diastolic diameter, and CRP concentration in dogs. C-reactive protein concentration was not associated with severity of MMVD.

ABSTRACT #70

EVALUATION OF A MINIMALLY INVASIVE TREATMENT FOR MITRAL VALVE REGURGITATION.

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Mitral valve regurgitation (MR), due to myxomatous degeneration of the valve leaflets or secondary to dilated cardiomyopathy, is

common in older people and older dogs. Medical management can provide some relief, but mitral valve surgery via cardiopulmonary bypass has been required to improve long term outcome. Percutaneous (transcatheter) beating heart mitral valve repair offers an alternative to open heart surgery. This study was designed to test the feasibility of a novel intracardiac implant system designed to ameliorate MR by enhancing functional valve coaptation (valve-valve or valve-device) during systole.

Following IACUC approval, mature healthy female sheep (65–75 kg; n=6) and pigs (50–65 kg; n=17) were anesthetized to permit fluoroscopic and intracardiac echocardiographic (ICE) guidance of a transcatheter (femoral vein) delivery system. Transseptal atrial puncture was employed to gain access to the left atrium and left ventricle. A steerable 14-French delivery catheter was used to deploy the implant, which consisted of a cylindrical (9×40-mm) collapsible Nitinol wire scaffold enveloped within a silicone polyurethane sheath. The implant was suspended within the mitral annulus by a single Nitinol support wire anchored to the ventricular apical wall using either a screw-in or an umbrella design. Procedural success was recorded and animal health, cardiac function and implant stability were assessed post-implantation using physical exam, echocardiography, fluoroscopy, and necropsy (range 23–328 days post-implantation).

System design modifications and increased operator experience culminated in successful, uncomplicated, and repeatable implantations of the device. When compared with sheep, the pigs' cardiac anatomy and physiology (eg, fewer moderator bands, easier transseptal approach, vigorous recovery) facilitated the implantation procedure. Procedure duration declined from 240 minutes to 45 minutes. Intraoperative and postoperative fluoroscopy and echo-cardiography documented optimal positioning of the implant within the mitral annulus and coaptation with the mitral leaflets with minimal mitral regurgitation. Seven animals succumbed to device perforation of the myocardium, excessive anticoagulation or cardiac failure. Necropsy confirmed accurate device placement in most cases, but post-implantation anchor detachment occurred in 2 animals and metal fatigue resulted in fracture of some umbrella-style anchor tines in 3 animals. A mild focal inflammatory response was noted where the support struts contacted the left ventricular wall and mild myxomatous changes were occasionally noted where the valve leaflets contacted the implant.

This novel implant, documented to achieve functional valve coaptation (valve-valve or valve-device) in systole with minimal regurgitation and cardiac pathology offers a potential percutaneous treatment for human and canine MR patients. Efficacy studies are warranted.

ABSTRACT #71

CAVAL SYNDROME; OUTCOME IN 42 DOGS. SG Gordon, C Bové, AB Saunders, MW. Miller, RM Roland, SE Achen, LT Drourr and MM Boggess. The Michael E. DeBakey Institute, Colleges of Veterinary Medicine and Biomedical Sciences and Science, Texas A&M University, College Station, TX.

The objective of this retrospective study is to report the clinical outcome of canine caval syndrome (CS). Medical records of 42 client owned dogs diagnosed with CS at Texas A&M University were reviewed. Information including signalment, history, physical examination, clinicopathologic testing, diagnostic imaging, treatment and outcome were obtained.

Twenty-one of 42 dogs had transvenous heartworm extraction (TVHWE) attempted. Two of the 21 dogs died during the procedure, 4 died post-operatively, and following anesthesia induction in one dog, worms migrated into the distal pulmonary artery and therefore extraction was not attempted. Fourteen dogs underwent successful TVHWE and were discharged from the hospital. Mean follow up time in these 14 dogs was 24.4±17.7 months with a range of 2–56 months. To date 10 (71.4%) of these dogs have survived at least 18 months, and 7 (50.0%) greater than 24 months. Of the remaining 4 dogs, 3 were lost to follow-up and one was euthanized due to intervertebral disc disease. A parametric proportional hazards model, with a Weibull distribution, was run to see if any of the preoperative clinicopathologic parameters or diagnostic imaging results had an effect on survival time. Dogs with elevated alanine transferase (ALT) or worms documented in the pulmonary artery prior to TVHWE had significantly ($p<0.05$) shorter overall survival.

These results suggest that dogs with CS who undergo TVHWE and survive to discharge have a good mid-term prognosis. Dogs with elevations in ALT or pre-operative evidence of worms in the pulmonary artery may have increased mortality rates.

ABSTRACT #72

MODULATION OF INFLAMMATORY AND OXIDATIVE STRESS MARKERS IN CORONARY ARTERY SMOOTH MUSCLE CELL MODEL. BA Maran[1], GE Snow[2,3], JM Robinson[2,5], RY Au[2], AY Au[2,4], CG Frondoza[1,2,5]. [1]Mississippi State University, Mississippi State, MS. [2]Nutramax Laboratories, Inc., Edgewood, MD. [3]Dartmouth College, Hanover, NH. [4]Syracuse University, Syracuse, NY. [5]Johns Hopkins University, Baltimore, MD.

Dilated cardiomyopathy (DCM) is the leading cause of heart failure in certain breeds of dogs. DCM is associated with inflammation and oxidative stress, but the pathogenesis of the disease is not well understood. Mediators implicated in the pathogenesis of DCM include cytokines, prostaglandins, nitric oxide, and reactive oxygen species. To help clarify the molecular events involved, we evaluated coronary artery smooth muscle cells (CASMCs) cultured *in vitro*. Using this model, we determined whether cytokine-induced inflammation and oxidative stress can be inhibited by silybin phosphatidylcholine complex (SPC) and epigallocatechin gallate (EGCG). These natural products are known to have anti-inflammatory and anti-oxidant properties. SPC has been shown to be bioavailable in dogs and cats and is commercially available (Marin® and Denamarin®). Induction of inflammation was assessed by gene expression analysis of cytokines and COX-2, as well as production of prostaglandin E2 (PGE_2). Induction of oxidative stress was monitored by analysis of superoxide dismutase (SOD) gene expression.

Phenotype expression was monitored by immunostaining for α-actin. CASMCs were pre-incubated with clinically relevant concentrations of SPC (298 ng/ml), EGCG (0.4 μg/ml) or control media alone for 24 hours. Cells were then incubated for 24 hours with the combination of IL-1β (10ng/ml) and TNF-α (1 ng/ml) or control media alone. Expression of cytokines (IL-1β and TNF-α), COX-2, and SOD was determined by semi-quantitative RT-PCR while PGE_2 production was measured by ELISA. Statistical significance was set at $p<0.05$ using one-way ANOVA and the Tukey post-hoc test.

CASMCs proliferated in monolayer culture and maintained their phenotype, as indicated by intense immunostaining for α-actin. Cells responded to cytokine activation by more than a 40% increase in expression of IL-1β, TNF-α, COX-2, and SOD. Activation significantly increased production of PGE_2. Pretreatment of CASMCs with SPC or EGCG reduced gene expression of cytokines, COX-2, and SOD. Pre-treatment also reduced PGE_2 production to near baseline levels. Results from this study demonstrate that SPC and EGCG inhibit the induction of inflammation and oxidative stress markers *in vitro*. The CASMC model serves as a tool to identify agents that may be beneficial in reducing inflammation and associated oxidative stress in veterinary practice.

ABSTRACT #73

HIGH DEFINITION OSCILLOMETRY: A NOVEL, NON-INVASIVE APPROACH FOR PRECISE BLOOD PRESSURE DETERMINATION AND CARDIOVASCULAR ASSESSMENT IN OLD WORLD AND NEW WORLD MONKEYS. Barthel Schmelting[1], Marc Niehoff[1], Beate Egner[2], Gerhard F Weinbauer[1]. [1]Covance Laboratories, Muenster, Germany, [2]Clinical Center for Small Animals, Hoerstein, Germany.

Direct invasive techniques are regarded as gold standard for blood pressure determination. However, these are not feasible for day-by-day routines and large animal numbers as frequently demanded during preclinical safety assessment. Non-invasive cuff methods are used to overcome these limitations with variable success. Small body size and high heart rates (>300 bpm), e.g. in marmosets (*Callithrix jacchus)*, often obviate further cardiovascular investigation of conscious animals. High Definition Oscillometry (HDO) was evaluated as a potential non-invasive approach for blood pressure recordings in cynomolgus monkeys (*Macaca*

fascicularis) and marmoset monkeys. In conscious animals, systolic, diastolic, mean arterial blood pressure (MAP) and pulse/min were determined 15 times within approx. 9 min per individual. This session was performed during three consecutive days. After procedural habituation the MAP (in mmHg, mean±SD) in *C. jacchus* was 94.9±4.3, in *M. fascicularis* males 96.2±3.4 and 86.9±3.0 in females. Importantly, these data correspond to those reported for invasive techniques. Mean intraindividual coefficient of variation for systolic, diastolic, MAP and bpm ranged between 2.6 and 10.1%, demonstrating the high precision and feasibility even in conscious animals. Direct comparison to telemetry revealed that HDO accurately detected drug related cardiovascular changes. Under anaesthesia, a significant decrease in pulse rate and blood pressure could be determined. Due to its accuracy and precision, HDO may be regarded as an alternative approach to invasive surgeries in animal models used for cardiovascular research. HDO is applicable to large number of animals and day-by-day routines.

ABSTRACT #74

PREVALENCE, HERITABILITY AND GENETIC CORRELATIONS OF CONGENITAL SENSORINEURAL DEAFNESS AND PIGMENTATION PHENOTYPES IN 4143 BORDER COLLIES. Luisa De Risio, Julia Freeman, Sarah Blott, Alberta de Stefani, Lara Matiasek, Tom Lewis. The Animal Health Trust, Newmarket, UK.

The objectives of the present study were to estimate prevalence, heritability and genetic correlations of congenital sensorineural deafness (CSD) and pigmentation phenotypes in Border Collies.

Full litters of purebred Border Collies that presented to the Animal Health Trust (1994–2008) for assessment of their hearing status by brain stem auditory evoked response (BAER) at 4–10 weeks of age were included. Heritability and genetic correlations were estimated using restricted estimate maximum likelihood.

4143 puppies (1951 female, 2192 male) met the inclusion criteria. Of these, 4043 (97.59%) had normal hearing status, 84 (2.03%) were unilaterally deaf (UD) and 16 (0.39%) were bilaterally deaf (BD). Iris color was brown bilaterally in 3890 puppies (1.57% UD, 0.18% BD), blue unilaterally in 158 puppies (8.86% UD, 1.27% BD), and blue bilaterally in 95 puppies (9.47% UD, 7.37% BD). White pigmentation of the head was estimated visually at $>50\%$ in 111 dogs (19.82% UD, 7.21% BD) and $<50\%$ in 4032 dogs (1.54% UD, 0.20% BD). 366 puppies (5.19% UD, 2.19% BD) had merle coat color. The sire and dam hearing status was known (based on BAER results) in 3491 and 3801 dogs respectively (3482 and 3776 normal, 5 and 21 UD, 4 and 4 BD respectively). Heritability of deafness (normal/unilateral/bilateral) was estimated as 0.37. The genetic correlations of deafness with iris color, percentage of white pigmentation of the head, and merle coat color were 0.63, 0.12, and 0.24, respectively.

These results indicate that there is significant genetic effect on CSD in border collies, and that some of the genetic regions determining deafness also influence other pigmentation phenotypes.

ABSTRACT #75

INVESTIGATION OF A NEW ELECTROENCEPHALOGRAPHY (EEG) ELECTRODE IN SEDATED AND AWAKE DOGS. FMK James, C Kerr, A Bersenas, J Parent, L Grovum, D Allen, R Poma. Ontario Veterinary College, University of Guelph, Guelph, ON.

The new subdermal wire electrode (SWE) was compared with the traditional gold cup electrode (GCE) and needle electrode (NE), in six sedated and awake dogs.

Eight EEG channels were recorded during twenty-minute video-EEG recording sessions (at 0.5, 2, 4, 6 and 8 hours) in this prospective, randomized, and blinded trial. Non-physiological artifacts were identified. Duration of non-physiological artifact was summed per channel. The number of unaffected channels (NUC) was also noted.

After multivariate analysis of NUC, there were main effects of electrode type and sedation over time, with median SWE (NUC=2.8, 95% confidence interval: 0.84–5.7) significantly different than GCE (7.87, 7.44–7.94) and NE (7.6, 6.61–7.89) medians ($p<0.05$). After 4 hours, and regardless of the electrode type, the NUC decreased in awake dogs, but not in sedated dogs. The duration of non-physiological artifact was significantly different between SWE and the other two electrodes, the medians for SWE, GCE, and NE at the 8-hour recording sessions being 61.55 (95% confidence interval: 21.81–173.65), 1.33 (0.47–3.75), and 21.01 (6.85–64.42) seconds respectively ($p<0.05$).

In conclusion, choices of EEG electrode and level of sedation do not affect the overall recording quality in EEG sessions lasting up to 4 hours. With longer EEG recording sessions, caution should be used in selecting EEG electrodes and sedation state, although the difference between electrodes may not be clinically significant.

ABSTRACT #76

CEREBROSPINAL FLUID NEUROTRANSMITTER CONCENTRATIONS IN DOGS WITH ISCHEMIC INFARCTION OF THE BRAIN. SR Platt[1], R Barber[1], L De Risio[2], M Kent[1], J Eagleson[1], SJ Schatzberg[1]. [1]University of Georgia, College of Veterinary Medicine, Athens, GA, USA. [2]Animal Health Trust, Newmarket, UK.

Cerebrospinal fluid (CSF) concentrations of excitatory (glutamate) and inhibitory (GABA) neurotransmitters may reflect central nervous system excitotoxicity. Excitotoxicity is a component of the purported pathophysiology of cerebrovascular accidents, specifically ischemic infarction. Although CSF glutamate levels have been evaluated in dogs with epilepsy, spinal disease and brain tumors, neurotransmitter concentrations have not been documented in dogs with ischemic infarction (stroke) of the brain. Such information could provide therapeutic targets for clinically affected dogs. The objective of this study was to compare CSF neurotransmitter levels in dogs with clinically confirmed ischemic infarction to those of normal dogs.

Cerebellomedullary cisternal CSF was collected from 10 healthy beagles with normal brain MRI and from 12 various dog breeds which had clinical signs and cerebral MRI consistent with ischemic infarction. The medical records of the clinical dogs were searched to determine duration of disease at time of CSF tap and prior use of anti-inflammatory medications. The CSF samples (20 µl) were analyzed for glutamate, GABA and glycine concentrations with electrochemical high performance liquid chromatography following derivitisation with o-phtaldialdehyde/2-mercaptoethanol. A Student's t-test was used to test for differences between the levels of the 3 neurotransmitters in the following groups: clinical dogs vs. normal; duration of signs $<=5$ days vs. >5 days; anti-inflammatory medicines administered vs. none given. Student's t-test was implemented in PROC GLM in SAS and significance was set at $P\leq0.05$.

There were no significant differences between the mean glutamate concentrations of all clinically affected dogs and normal dogs. However, there were significant differences between the mean CSF glutamate levels for dogs clinically affected for ≤5 days (0.28 µmo/l) when compared to those affected >5 days (0.09 µmol/l) and to normal dogs (0.1 µmol/l) ($P=0.03$). There were also significant differences in the mean glycine concentrations detected between normal dogs (1.64 µmol/l) and those which had experienced ischemic infarction (1.09 µmol/l) $P=0.0099$. There were no differences detected between the GABA concentrations of any of the groups assessed. There was no effect of the use of anti-inflammatory medications on any of CSF neurotransmitters.

The results of this preliminary retrospective study suggest that glutamate –mediated excitotoxicity may be involved in the pathogenesis of canine cerebral ischemic infarction within the first 5 days of disease. The reduction of glycine concentrations may also play an important role. Glutamate elevation is a potential future treatment target for dogs with cerebral ischemic infarction.

ABSTRACT #77

RISK FACTORS FOR POST-MYELOGRAPHIC SEIZURES IN DOGS – 503 CASES. Ronaldo C. da Costa[1], Howard Dobson[2], Aleisha Lusk[2], Joane Parent[3]. [1]The Ohio State University, Columbus, OH, [2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [3]Université de Montréal, St-Hyacinthe, QC, Canada.

The reported incidence of post-myelographic seizures in dogs using iohexol has been highly variable, reaching up to 21.4% on a recent report. Most studies, however, investigated relatively small populations of dogs. Since our experience appeared different, we

retrospectively investigate the incidence and risk factors of post-myelographic seizures in a large patient population.

Medical records of dogs that had a myelogram performed between March 2002 and December 2004 were reviewed. Data relating to breed, sex, age, weight, volume and dose per kilogram of iohexol (240 mg/ml), site and number of injections, location of the lesion, duration of anesthesia, time from injection to recovery, whether or not surgery immediately followed the myelogram, whether acepromazine was used immediately before or after anesthesia, and presence or absence of seizures were collected. Multiple statistical analyses were performed (univariate exact conditional analysis, Fisher's exact tests, and logistic regression with conditional tests).

The files of 503 dogs were evaluated. Only 15 dogs (2.98%) suffered a post-myelographic seizure. Although several factors influenced on seizure occurrence, due to space constraints only a few will be described here. The size of the dog was an important factor. Thirteen out of 140 large (>20 kg) dogs (9.29%) and two out of 124 medium (>9<20 kg) dogs (1.61%) had a seizure. No small (<9 kg) dogs (out of 239) had seizures. Large dogs were 35.35 times more likely to have a post-myelographic seizure (P <0.001) than small dogs. The location of contrast injection was also important. Out of the 424 dogs that were injected into the lumbar cistern, 6 suffered a post-myelographic seizure (1.42%), while this occurred in 6 out of 62 (9.68%) dogs injected into the cerebellomedullary (CBM) cistern. Dogs that were injected into the CBM cistern had a significantly (P=0.001) higher risk of seizures and were 7.46 times more likely to develop seizures than those injected into the lumbar cistern. Multiple injections in the lumbar region increased the seizure risk (P=0.002), but not in the CBM cistern (P=0.36). The total volume of iohexol was highly significant in increasing the seizure risk (P <0.0001). The mean total volume of iohexol for dogs that had a seizure was 11.73±5.52 ml, while for dogs that did not have a seizure it was 4.57±4.13 ml.For each 1 ml increase in iohexol volume, the seizure risk increased by 1.203 (95% CI, 1.11 to 1.32). The location of the spinal lesion was also important. Dogs with cervical lesions had a higher seizure risk (P=0.004) and were 4.65 times more likely to develop seizures than dogs with lesions in other spinal regions.

We conclude that the overall seizure risk is low in small and medium breed dogs with lumbar iohexol injections. Large breed dogs with cervical spinal disease and injection of large volumes of iohexol in the CBM cistern have a significantly higher seizure risk.

ABSTRACT #78

EFFECTS OF PALLIATIVE PHARMACOTHERAPY ON SURVIVAL AND QUALITY OF LIFE IN 50 DOGS WITH PRIMARY INTRACRANIAL NEOPLASMS. JH Rossmeisl, JL Robertson, JC Jones, KL Zimmerman. VA-MD Regional College of Veterinary Medicine, Blacksburg, VA.

This prospective study intended to evaluate effects of palliative therapies on survival and quality of life (QOL) in dogs with brain tumors. We hypothesized that survival and QOL for forebrain tumors would be superior to tumors elsewhere.

All dogs had clinical and CT/MRI evidence of a brain mass, were discharged from the hospital alive, and had histopathologic confirmation of a brain tumor upon death. Animal-, treatment-, and tumor-related factors were examined for survival effects, with treatments standardized based on clinical and tumor-related factors. Therapies included phenobarbital (Group 1), prednisone (Group 2), and phenobarbital and prednisone (Group 3). The impacts of tumors on behavior, physical status, and human-animal interactions were assessed by serial client QOL questionnaires.

Fifty dogs with 19 meningiomas, 10 gliomas, 9 pituitary macroadenomas, 8 choroid plexus tumors, and 4 neuronal tumors were included. Multivariate analysis identified tumor location, clinical signs/severity, and pre-treatment QOL scores as significant prognostic variables. The rates of mortality in Groups 2 (hazard ratio 16.9, 95% CI 1.3–225.1; p=0.03) and 3 (hazard ratio 12.9, 95% CI 1.7–99.3; p=0.01) were significantly greater compared to Group 1, and Group 1 dogs survived longer (median 212 days, 95% CI 178–343 days). No differences existed between groups in QOL scores for days 14–90 post-treatment, although these QOL scores improved in all groups compared to pre-treatment values.

Palliative treatments resulted in short-term QOL improvement in all groups. Dogs with mild neurologic dysfunction due to forebrain tumors can survive for prolonged periods.

ABSTRACT #79

THE PHARMACOKINETICS OF LEVETIRACETAM IN DOGS CONCURRENTLY RECEIVING PHENOBARBITAL. SA Moore, KR Munana, MG Papich, JA Nettifee-Osborne. North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Levetiracetam (LEV) is commonly used as an add-on medication in dogs with refractory epilepsy, although the pharmacokinetics of LEV when given in conjunction with other antiepileptic drugs has not been evaluated in the dog. The objective of this study was to determine if the pharmacokinetics of LEV change when administered in conjunction with phenobarbital (PB). Six healthy dogs were administered a single oral dose of LEV (16.7–27.8 mg/kg). Blood samples were collected at baseline and intermittently for 24 hours. The study was repeated after the same dogs received oral PB (2.0–3.3 mg/kg) twice daily for 21 days. All dogs achieved steady state concentrations of PB. Plasma LEV levels were evaluated by high pressure liquid chromatography. Pharmacokinetic data was analyzed using a compartmental model. Peak concentration (C_{MAX}) for LEV administered alone was 32.39 ug/ml±6.76, compared to18.22 µg/ml±8.97 when given with PB. The elimination $T_{1/2}$ in dogs given LEV alone and dogs given LEV and PB concurrently was 3.43 h±0.47 and 1.73 h±0.22, respectively. Clearance increased from 124.93 ml/hr/kg±26.93 for LEV alone to 252.99 ml/hr/kg+35.43 for LEV and PB in combination. Volume of distribution was 611.59 ml/kg±76.74 for LEV alone compared to 697.75 ml/kg+85.43 for LEV with PB. Concurrent PB administration significantly alters the pharmacokinetics of LEV in the dog, indicating that dosage adjustments may be necessary when he drug is administered to dogs in conjunction with PB.

ABSTRACT #80

METABOLISM AND ACTION OF NELARABINE IN GLIOMA CELL LINES. L Tzipory, PJ Dickinson, CO Rodriguez Jr. University of California-Davis, Veterinary Medical Teaching Hospital, Davis, CA.

Nelarabine, pro-drug of ara-G, is phosphorylated in the initial rate-limiting step by cytosolic deoxycytidine kinase (dCK) and mitochondrial deoxyguanosine kinase (dGK) to ara-GMP. This moiety is then quickly converted to the active 5′-triphosphate, ara-GTP, which competes with the natural endogenous nucleotide, dGTP for incorporation into the replicating DNA strand. Incorporation of ara-GTP into the DNA results in replication block and subsequently inhibition of DNA synthesis leading to apoptosis.

In phase I and II human clinical trials, the most common adverse effects were non-hematological, neurological toxicities, suggesting ara-G penetration the blood brain barrier. These data, combined with the knowledge that tissues from the central nervous system have the highest specific activity of dGK in the body, led us to hypothesize that ara-G would have efficacy in the treatment of tumors of the central nervous system.

The purpose of this study is to evaluate and compare the intracellular pharmacokinetics and pharmacodynamics of Nelarabine in glioma cell lines of canine, murine and human origin.

Short term growth inhibition was assessed with MTT assay. Accumulation of intracellular ara-GTP was quantitated by HPLC. Repeated measure ANOVA and Student' t test were used to assess for significance.

The IC50 for murine (4C8) was 100uM at 72 h. The IC50 for canine (JT3-BG) and human (U87) was >100uM at 72 h. The accumulation of intracellular ara-GTP was similar between the cell lines.

Nelarabine appears to have efficacy against glioma cell lines of canine, murine and human origin. Its efficacy *in vivo* remains to be determined.

ABSTRACT #81

EXPRESSION OF MATRIX METALLOPROTEINASES AND RECK IN MICROGLIAL CELLS IN DIFFERENT INTRACRANIAL DISEASES. V.M. Stein[1], C. Puff[2], W. Baumgärtner[2], A. Tipold[1]. [1]Department of Small Animal Medicine and Surgery, [2]Institute of Pathology, University of Veterinary Medicine, Hannover, Germany.

Microglial cells are the brain-resident tissue macrophages and are closely associated with active central nervous system (CNS) pathology. They are known be activated by a large variety of stimuli

and subsequently show de novo expression of numerous immunologically relevant molecules. As such they can induce the synthesis and secretion of MMPs and TIMPs. The question whether microglial reaction repertoire to a given stimulus is stereotyped or adapted to the underlying pathology is still a matter of debate.

Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in the degradation of the extracellular matrix. They are closely implicated in the pathogenesis of inflammation in the CNS, demyelination and tumor pathogenesis. MMP inhibitors (tissue inhibitors of matrix metalloproteinases, TIMPs) are crucial in the regulation of MMP activities, and are therefore important candidates for the therapy of different diseases. RECK (reversion-inducing cysteine-rich protein with Kazal motifs) is a membrane-anchored glycoprotein that regulates extracellular matrix integrity and angiogenesis. To address the question whether microglia shows a specific repertoire of MMP expression, MMP-2, MMP-9, MMP-12, MMP-13, MMP-14, TIMP-1, TIMP-2, and RECK (reversion-inducing cysteine-rich protein with Kazal motifs) mRNA was evaluated in dogs with different intracranial diseases.

Microglial cells of twenty four dogs were isolated ex vivo with density gradient centrifugation. The dogs suffered from different intracranial diseases which were categorized according to histopathological examination. Disease categories comprised intracranial tumors, intracranial inflammation, idiopathic epilepsy, other intracranial diseases such as trauma and malformations, and extracranial diseases which served as a negative control group. The microglial cell pellets were examined via quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) with SYBR-green using the Mx3005P Quantitative PCR System^{TM} (Stratagene, Amsterdam, The Netherlands). Statistical analysis was conducted using the Kruskal Wallis test and the Wilcoxon rank sum test with the significance level set at $p \leq 0.05$.

MMP-2, MMP-9, MMP-12, MMP-13, MMP-14, TIMP-1, TIMP-2, and RECK mRNA was expressed by microglial cells, although no significant differences could be found between the different intracranial disease categories. However, in single dogs elevated MMP expression was found, such as MMP-2 in a dog with canine distemper virus infection. TIMPs and RECK were elevated in single dogs of each disease category. In conclusion, microglial expression of MMPs and TIMPs reflects their potential to respond to various disease conditions in the CNS rather than to indicate a specific disease etiology. However, the pathogenesis of a given disease might be crucially influenced by microglial MMP and TIMP expression.

ABSTRACT #82

LOW FIELD MAGNETIC RESONANCE IMAGING (MRI) FINDINGS OF THE CAUDAL CERVICAL SPINAL CORD IN CLINICALLY NORMAL DOBERMAN PINSCHERS AND FOXHOUNDS. S. De Decker, IMVL. Gielen, L. Duchateau, I. Van Soens, V. Bavegems, T. Bosmans, H. Van Bree, LML Van Ham. Faculty of Veterinary Medicine, Ghent University, Belgium.

Little is known about the spectrum and incidence of MRI abnormalities in the cervical vertebral column and spinal cord of clinically normal dogs.

Clinically normal Doberman Pinschers (n=20) and Foxhounds (n=17) were studied. The dogs were divided in 2 age categories: ≤ 5 years (n=20) and >5 years (n=17) and were considered clinically normal on the basis of a complete physical, neurological, and blood examination and an echocardiographic examination in all Doberman Pinschers. MRI was performed with a 0.2 Tesla magnet. Sagittal and dorsal T1 and T2 weighted images (WI) of the entire cervical spine and T1 and T2 WI transverse images from C4 to T1 were obtained. On sagittal T2 WI, disc degeneration was classified based on signal intensity as normal, partially or completely degenerated. On sagittal and when available (C4-T1) transverse T2 WI, ventral and dorsal compression were classified as normal, partial or complete subarachnoidal space compression and spinal cord compression. Vertebral body abnormalities were assessed on sagittal T1 WI and were defined as a flattening of the ventrocranial border of the vertebral body. Abnormal intraparenchymal signal intensity (ISI) changes were classified based on the surrounding spinal cord parenchyma on T1 and T2 WI.

Only 1 dog showed no single abnormality on MRI examination. Severe MRI abnormalities were seen in 17 dogs with the occurrence or combination of complete disc degeneration (n=14) and/or spinal cord compression (n=11). Vertebral body abnormalities were seen in 7 dogs and hyperintense ISl changes were seen in 2 dogs. The occurrence of vertebral body abnormalities was significantly higher in the Doberman Pinscher ($P=0.04$). The severity of disc degeneration ($P=0.005$) and ventral compression ($P=0.05$) were significantly higher in the higher age category. There was a significant difference in disc degeneration, ventral and dorsal compression ($P<0.0001$; $P=0.004$; $P<0.0001$, respectively) according to the location of the different intervertebral disc spaces, with increasing abnormalities for the more caudal intervertebral disc spaces ($P<0.0001$; $P=0.0192$; $P<0.0001$, respectively). The correlation coefficient between severity of disc degeneration and spinal cord compression was 0.52 ($P=0.0003$).

The results of this study indicate that MRI abnormalities are commonly seen in the caudal cervical vertebral column and spinal cord of clinically normal Doberman Pinchers and Foxhounds. This suggests that such lesions are part of the normal, or at least, common aging process of spinal degeneration.

ABSTRACT #83

COMPUTED TOMOGRAPHIC FINDINGS IN LARGE AND GIANT BREED DOGS WITH CERVICAL SPONDYLOMYELOPATHY: 58 CASES. Ronaldo C. da Costa, Rita Echandi, Dustin Beauchamp. Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Computed tomography (CT) is routinely used in the diagnosis of cervical spondylomyelopathy (CSM); however, no report presents CT findings in a large population of dogs with CSM. The purpose of this study was to compare the CT findings in large and giant dogs with CSM.

Medical and radiology records were searched for cases of canine CSM between 2003 and 2008. Inclusion criteria included a definitive diagnosis of CSM achieved by myelography and CT myelography (CTM) in large or giant dogs with clinical signs of cervical spinal disease.

Fifty eight dogs met the inclusion criteria. Twenty-three were large breed dogs and 35 giant breed dogs. Among the large breed dogs, 11 were Dobermans, 3 Weimaraners, 3 mixed large breeds, 2 Dalmatians, 2 Labrador retrievers, 1 Greyhound, and 1 German shorthaired pointer. Their median age was 7 years (mean 7.1 years). There were 11 males and 12 females. Among the giant breed dogs, 18 were Great Danes, 8 Rottweilers, 7 Mastiffs, 1 Great Pyrenees, and 1 Bernese mountain dog. Their median age was 2.5 years (mean 4.1 years). There were 21 males and 14 females.

CTM findings in large breed dogs revealed that 11 dogs had a single site of spinal cord compression, while 12 dogs had multiple sites. The main site of compression was at C6-7 in 14 dogs, C5-6 in 7 dogs, and C4-5 and C2-3 with one dog each. The main cause of compression was disk-associated in 19 dogs, and osseous with or without ligamentous hypertrophy or synovial cysts in 4 dogs. The direction of compression was ventral in 19 dogs, dorsolateral in 2 dogs, dorsal in 1 dog and lateral in 1 dog. The severity of the spinal cord compression was graded as severe ($>50\%$ cord diameter), moderate (between 25–50%) and mild ($<25\%$). The main compression was severe in 2 dogs, moderate in 14, and mild in 7 dogs. Four of the 23 dogs had spinal cord atrophy.

CTM findings in giant breed dogs showed that 30 dogs had multiple sites of compression, while 5 dogs had a single site. The main compressive site was at C6-7 in 13 dogs, C5-6 in 8 dogs, C4-5 in 5 dogs, C2-3 in 2 dogs, and C3-4 in 1 dog. Six dogs had 2 or 3 compressive sites of similar severity. Osseous compressions were also observed at C7-T1 in 8 dogs, and T1-T2 or only T2 in 5 dogs. The primary cause of compression was osseous in 27 dogs (2 dogs had concurrent synovial cysts), disk-associated in 4 dogs, and a combination of disk and osseous compression in 4 dogs. The direction of compression was lateralized in 18 dogs, dorsolateral in 5 dogs, dorsal in 3 dogs, ventral in 5 dogs, and a combination in 4 dogs. The main spinal cord compression was graded as severe in 9 dogs, moderate in 19 dogs, and mild in 7 dogs. Seven dogs had spinal cord atrophy.

Results indicate that giant dogs often have multiple compressions, usually caused by malformed or osteoarthritic articular processes that cause lateralized compression. Additionally, an important number of giant dogs had secondary osseous compressions at C7-T1 and T1-2 sites.

ABSTRACT #84

CERVICAL ARTHROPLASTY IN DOGS WITH DISC ASSOCIATED CAUDAL CERVICAL SPONDYLOMYELOPATHY AND CERVICAL DISC HERNIATION: PRELIMINARY STUDY IN TWO CASES. PF Adamo, G Burns. Bay Area Veterinary Specialists, San Leandro, CA.

The purpose of this preliminary study was to determine whether cervical arthroplasty is tolerated in dogs with disc associated Caudal Cervical Spondylomyelopathy (CCSM) or cervical disc herniation.

Two dogs diagnosed with disc associated cervical myelopathy using MR imaging were included in this study. Dog 1: a 4 year old MN Doberman Pincher 31 kg, with acute onset of cervical pain and ataxia. Dog 2: an 8 year old MN mixed breed 23 kg, with a 4 month history of progressive ataxia/tetraparesis. MRI findings showed acute myelopathy secondary to C6-C7 disc herniation in dog 1; and chronic myelopathy secondary to C5-C6 disc disease in dog 2.

Both dogs were surgically treated with standard ventral slot and spinal cord decompression followed by the insertion of the Titanium alloy version (Ti-6AI-4V-ELI) of the canine cervical artificial disc, previously tested by Adamo et al. – *in vitro* study. Post-operatively cervical radiographs were taken, a cast was applied for 6 weeks and serial neurologic examinations with cervical radiographs were taken at 2 and 6 weeks (both dogs) and at 12 weeks and 6 months (dog 1).

Dog 1 had a complete neurologic recovery; dog 2 showed significant improvement at 2 months post-op. On immediate post-operative radiographs the implant was well seated in the slot providing good distraction. In all post-operative cervical radiographs, distraction was maintained with no evidence of ventral bridging spondylosis at the treated site.

Cervical arthroplasty was well tolerated in both dogs, maintained distraction and prevented bridging spondylosis. This procedure warrants further study.

ABSTRACT #85

F RATIO OF THE SCIATIC AND ULNAR NERVE IN DOGS. JM Scarano, C Massicotte. Animal Emergency and Referral Associates, Fairfield, NJ.

We propose a reference value for the F ratio for the sciatic and ulnar nerve of dogs adjusted for the length of their limbs. Eleven healthy dogs with no clinical signs of peripheral nerve or spinal cord diseases were studied thus far.

F ratios were calculated for the sciatic nerve with supramaximal stimulation at the hock, popliteal fossa and hip. F ratios were calculated for the ulnar nerve with supramaximal stimulation at the carpus and the elbow. The formula $^{F\text{-}M\text{-}1}/_{2M}$ was used to calculate the F ratio. Dogs were divided into three groups based on total limb length which was measured from the hip to the fourth digit in hindlimbs and from the proximal scapular spine to the fourth digit in forelimbs. Small dogs had a total limb length less than 400 mm, medium dogs greater than 400 mm and less than 600 mm and large dogs greater than 600 mm.The mean value for each group was calculated and the following reference values proposed (±standard deviation): small hock=4.7±2.6, small stifle=2.7±1.0, small hip=1.7±0.9, medium hock=3.7±0.9, medium stifle=3.7±0.6, medium hip=1.9±1.9, large hock=1.9±0.1, large stifle=0.9±0.1, large hip=0.9, small carpus=5.3±1.2, small elbow=3.3±1.6, medium carpus=4.7±0.8, medium elbow=1.8±0.4, large carpus=4.2±0.4, large elbow 1.6±1.1. These preliminary results suggest the necessity to consider limb length when calculating the F ratio. This study is ongoing and will include one hundred dogs to improve the reliability of these reference ranges.

ABSTRACT #86

PERIPHERAL NERVE PATHOLOGY IN CANINE DEGENERATIVE MYELOPATHY WITH MUTATION IN SUPEROXIDE DISMUTASE 1 GENE. GD Shelton[1], GC Johnson[2], GS Johnson[2], DP O'Brien[2], ML Katz[3], JR Coates[2]. [1]School of Medicine, University of California, San Diego, La Jolla, CA. [2]College of Veterinary Medicine, [3]School of Medicine, University of Missouri, Columbia, MO.

Canine degenerative myelopathy (DM) is a progressive neurodegenerative disease which has recently been shown in several canine breeds to be a result of a missense mutation in the superoxide dismutase 1 (*SOD1*) protein (Awano T et al, PNAS,2009). Based on this mutation and similarities in disease phenotype, canine DM appears to be a spontaneously occurring model for human amyotrophic lateral sclerosis (ALS). In canine DM, spasticity and general proprioceptive pelvic limb ataxia occurs in dogs older than 8 years of age, and if euthanasia is delayed, clinical signs will ascend causing flaccid tetraparesis and other lower motor neuron signs. A similar course of clinical progression has been described in upper motor neuron onset human ALS. To determine if pathologic changes typical of denervation are present in muscle (biceps femoris and gastrocnemius), and nerve (peroneal) of dogs with advanced DM, specimens were collected either as biopsies or following euthanasia from Pembroke Welsh Corgi (5), Boxer (2), German Shepherd Dog (1), Kerry Blue Terrier (1) and Chesapeake Bay Retriever (1) dogs homozygous for the *SOD1* mutation and confirmed as DM with microscopic demonstration of DM-specific lesions in spinal cords. Muscle specimens were evaluated in both frozen and paraffin sections, and peripheral nerve specimens were evaluated in resin sections. Abnormalities were generally classified as denervation atrophy, nerve fiber loss, and axonal degeneration or demyelination, and were scored 0 to +++ (normal to marked, respectively). Similar specimens were collected and processed from age-matched control dogs including Boxer (3), German Shepherd Dog (1) and English Cocker Spaniel (1) shown to be clear or heterozygous for the mutation. The most dramatic and consistent abnormalities were found in the Pembroke Welsh Corgi in which denervation atrophy (+++) was present in all muscle specimens and nerve fiber loss (++ to +++), myelin ovoids (++), myelin splitting and ballooning (++ to +++) and inappropriately thinly myelinated fibers (++ to +++) consistent with mixed axonal degeneration and demyelination was found in peroneal nerves. Similar but milder changes (+ to ++) were present in peripheral nerves in the Boxer, German Shepherd Dog, Kerry Blue Terrier and Chesapeake Bay Retriever breeds with variable muscle atrophy (0 to ++). A longer disease duration in the Pembroke Welsh Corgi (24–48 months) versus other affected breeds (7–12 months) may explain the more extensive disease severity. In conclusion, this study provides pathologic evidence for peripheral nerve involvement in canine DM consistent with the clinical signs of lower motor neuron disease.

ABSTRACT #87

AN SOD1 MUTATION ASSOCIATED WITH DEGENERATIVE MYELOPATHY OCCURS IN MANY DOG BREEDS. JR Coates[1], R Zeng[1], T Awano[1], LA Hansen[1], S Khan[1], GC Johnson[1], JF Taylor[2], SN Long[3], DP O'Brien[1], CM Wade[4], ML Katz[1], K Lindblad-Toh[4,5], GS Johnson[1]. [1]University of Missouri College of Veterinary Medicine and [2]Division of Animal Sciences, Columbia, MO. [3]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA. [4]Broad Institute of Harvard and MIT, Cambridge, MA. [5]Uppsala University, Sweden.

Canine degenerative myelopathy (DM) is a fatal neurodegenerative disease prevalent in several dog breeds. Typically, the initial progressive upper motor neuron spastic and general proprioceptive ataxia in the pelvic limbs occurs at 8 years of age or older. If euthanasia is delayed, the clinical signs will ascend causing flaccid tetraparesis and other lower motor neuron signs. We previously identified a missense mutation in the canine superoxide dismutase 1 (*SOD1*) gene and showed that homozygosity for the A allele of this SOD1:c.118G>A mutation is a risk factor for DM in five dog breeds: Boxer, Chesapeake Bay retriever, German Shepherd dog, Pembroke Welsh corgi and Rhodesian ridgeback (Awano *et al,* PNAS, in press, 2009). We here report the results of genotyping 6407 individual dogs at SOD1:c.118.

The genotyped dogs were from 105 distinct breeds and at least 25 dogs represented each breed. A TaqMan® SNP allele discrimination assay was used for genotyping. The PCR primer sequences were GTGGGCCTGTTGTGGTATCA and CAAACTGATGGACGT GGAATCC and the probe sequences were VIC-CTCGCCTTTA GTCAGC for the mutant allele and FAM-CGCCTTCAGTCAGC for the wild type allele. The frequencies of the *SOD1* A allele ranged from 90% (Wire fox terrier) to zero (54 different breeds). Seventeen breeds had A-allele frequencies over 20%. DM is a well-recognized problem in 6 of these breeds with high A-allele frequencies: Pembroke Welsh corgi (A allele frequency=85%), Boxer (71%), Chesapeake Bay Retriever (45%), Rhodesian ridgeback (33%), German Shepherd dog (32%) and Cardigan Welsh corgi (32%). Clinical histories resembling DM are common among dogs in

7 other breeds with high A-allele frequencies: American water spaniel (46%), Bernese mountain dog (41%), Kerry blue terrier (37%), Canaan dog (33%), Bloodhound (28%), French bulldog (23%) and Kuvasz (22%). Conversely, clinical histories consistent with DM were rare in four breeds with high A-allele frequencies: Wire fox terrier (90%), Tibetan terrier (29%), Welsh terrier (27%), and Chow chow (20%).

The putative existence of dog breeds that have high A-allele frequencies but rarely suffer from DM could be explained by genetic modifiers that alter susceptibility to DM.

ABSTRACT #88

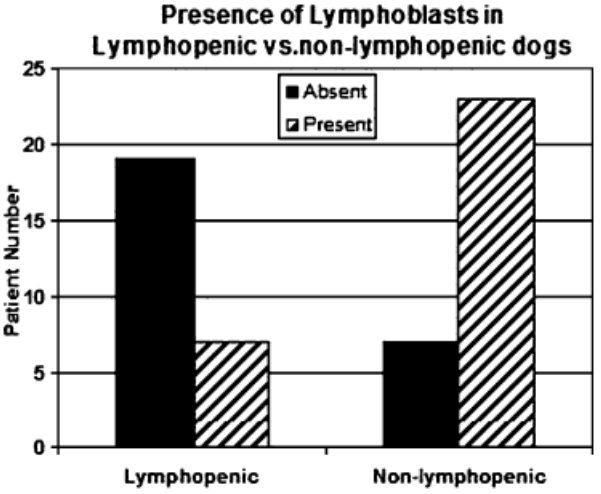
LYMPHOCYTE COUNTS AND CIRCULATING LYMPHOBLASTS IN CANINE LYMPHOMA PATIENTS. Karen V. Jackson[1], Beth Overley[2], Reema Patel[1]. [1]Department of Pathobiology, University of Pennsylvania, Philadelphia, PA. [2]Langhorne, PA.

The purpose of this study was to evaluate the absolute lymphocyte and lymphoblast count in pretreatment complete blood counts (CBCs) from dogs with lymphoma with the suspicion that non-lymphopenic animals likely had circulating lymphoblasts.

Fifty-six dogs diagnosed with lymphoma were prospectively enrolled if they had no previous treatment (including glucocorticoids) and if they had intention-to-treat with a CHOP based protocol. All dogs had pre-treatment CBCs with review by a board-certified clinical pathologist. One hundred lymphoid cells were counted and the percentage lymphoblasts recorded.

The mean absolute lymphocyte count was 2690/μL and mean absolute lymphoblast count was 1350/μL. Twenty-six dogs were lymphopenic (<1000 total lymphocytes/μL) and thirty dogs were non-lymphopenic (range: 1120–26230/μL). The presence of measurable numbers of lymphoblasts (>100/μL) was significantly correlated with a lymphocyte count over 1000/μL ($p<0.001$). The sensitivity and specificity, of a lymphocyte count over 1000/μL predicting the presence of circulating lymphoblasts, are 0.73 and 0.77 respectively.

These results indicate that if the total absolute lymphocyte count is over 1000/μL (i.e. non-lymphopenic) there are likely circulating lymphoblasts. In this situation, a blood smear review by a clinical pathologist is warranted.



ABSTRACT #89

PHARMACOKINETIC PROPERTIES OF TOCERANIB PHOSPHATE (Palladia™, SU11654), A NOVEL TYROSINE KINASE INHIBITOR, IN LABORATORY DOGS AND DOGS WITH MAST CELL TUMORS. MF Yancey, GM Michels, DA Merritt, SP Lesman, JF Boucher. Pfizer Animal Health, Kalamazoo, MI.

Toceranib phosphate (Palladia™, SU11654), an oral multi-kinase inhibitor, is under investigation for treatment of mast cell tumors (MCT) in dogs. Several studies were performed to characterize the pharmacokinetics (PK) of toceranib in dogs. Means of the following PK parameters were estimated following a 1.0 mg (free base equivalents, fbe)/kg IV dose administered to laboratory beagles: plasma clearance, 1.45 L/kg/h; volume of distribution at steady state, 29.7 L/kg; terminal half-life, 17.5 h. Following single oral doses of 3.25 mg (fbe)/kg administered to laboratory beagles, mean Cmax estimates ranged from 67.8–112 ng/mL and occurred between 5.5 and 7.0 h post-dose. Terminal half-life was 31 h. Oral bioavailability was estimated to be 76.9%. There were no statistically significant ($p>0.05$) changes to any PK parameter due to the fed/fasted state or between days 0, 28, and 86 during 13 weeks of every-other-day dosing (3.25 mg/kg) to laboratory beagles. Toceranib concentrations were proportional with dose over the range studied here (2.0 to 6.0 mg/kg). The PK of toceranib in client-owned dogs of a variety of pure and mixed breeds with MCTs was similar to that in healthy laboratory dogs. In summary, toceranib exhibited moderate clearance, high volume of distribution, and a moderate elimination half-life. After a single oral dose at 3.25 mg/kg, the concentration vs. time curve showed broad, sustained exposure with measurable concentrations for more than 48 h. The PK parameters of toceranib described here support every-other-day administration at an initial dose of 3.25 mg/kg for the treatment of MCTs in dogs.

ABSTRACT #90

FELINE INJECTION SITE SARCOMAS IN THE UNITED KINGDOM: ONE HUNDRED AND FIFTY SEVEN CASES. RS Dean[1], VJ Adams[1], DU Pfeiffer[2]. [1]Epidemiology Unit, Centre for Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, UK. [2]Veterinary Epidemiology & Public Health Group, Royal Veterinary College, Hawkshead Lane, Hatfield, Hertfordshire, UK.

Feline injection site sarcomas (FISS) are rare, clinically aggressive tumours of cats. Vaccinations, other injections and local trauma have been implicated in the pathogenesis of the tumour.

A consensus on the histopathological features of FISS was developed in a separate study and specific criteria for diagnosis were identified. These criteria were used to provide a clear case definition. Cases were prospectively recruited and a questionnaire was completed by the owner about the history of each cat.

A total of 259 cases were identified over a 14 month period and questionnaires were returned for 184 cases (71% response rate). When reviewed, 157 (85%) cases met the histopathology consensus. The most common location of the tumour was the interscapular space (148), other sites included the thoracic wall, flank and hindlimb. The mean age of the cats was 10.6 years (sd 3.6) with a mode of 13 and 17% were under 7 years of age. Both sexes were similarly represented with 83 males and 74 females, 99% of cats were neutered and only 8% were pedigree.

The majority of cats (98%) had been vaccinated, 67% in the last 12 months. Of the 154 vaccinated cats 120 (78%) were reported to have received leukaemia vaccinations, 66% (79/120) in the last 12months. Only 3 cats had been vaccinated against rabies. Microchips had been placed in 44% of cats with 11% of these in the last 12 months. Only 12 of the cats were reported to have received an injection of lufenuron, 5 within the last 12 months. Fighting was reported in 43% of cats.

This large case series of FISS did not use previous exposure to vaccination/injection and/or anatomic location as part of the case definition. The tumours were located in places other than the interscapular space. A wide age range was represented in this study including several younger cats. Nearly all the cats had been vaccinated, though rabies vaccines were rarely used and approximately half of the cats had a microchip.

ABSTRACT #91

NONVIRAL GENE TRANSFER OF THE FELINE CYTOKINE GENES IL-2, IFNγ AND GM-CSF AS ADJUVANT IMMUNOTHERAPY OF THE FELINE FIBROSARCOMA. CK Kempf[1], BC Schwarz [1], T Brill [2], C Plank [2], R Köstlin [3], J Hirschberger[1], U Schillinger[2]. [1]Clinic of Small Animal Medicine, Faculty of Veterinary Medicine, Ludwig-Maximilian University Munich, Germany. [2]Institute of Experimental Oncology, Technical University Munich, Germany. [3]Clinic of Small Animal Surgery and Reproduction, Ludwig-Maximilian University Munich, Germany.

This phase I dose-escalating study was performed to determine toxicity and feasibility of a non viral gene-transfer system transmit-

ting a combination of three different feline cytokines. Fifteen cats with primary or recurrent fibrosarcoma were included in the study. All cats had to be healthy based on blood work and physical examination and were staged by ultrasound and radiographs for signs of metastasis. A prior treatment with chemo-, radio- or an immunosuppressive therapy was not allowed. Following a wide surgical resection of the tumor an absorbable collagen sponge was implanted into the wound cavity. Each collagen sponge was loaded with equal amounts of plasmid DNA (pDNA), polyethylenimin and a protecting copolymer (P6YE5C). The plasmids were coding for feIL-2, feIFNγ and feGM-CSF, respectively. The dose of each plasmid was escalated in 4 different treatment groups starting with 75 µg to 600 µg per plasmid. Each cat was evaluated for treatment-related toxicity within the first 90 days after surgery and all occurring adverse events were registered and graded according to the VOCG-CTCAE scale. The most striking adverse event observed during the examination period was a drop in the lymphocyte count. Lymphocytes in cats treated with a dose of 600 µg/plasmid dropped by 75% compared to initial values. This was considered to be therapy-related but not life-threatening. In conclusion, a dose of 600 µg pDNA per cytokine is safe for the patient and feasible for a phase II clinical trial. The collagen sponge proved to be an easy to handle transfer medium for this nonviral gene therapy.

ABSTRACT #92
IMMUNNOHISTOCHEMICAL DIFFERENTIATION OF CANINE PROSTATIC CARCINOMA. M. Fork[1], P. Bock[2], U. Kaim[2], W. Baumgärtner[2], I. Nolte[1]. [1]Small Animal Clinic, University of Veterinary Medicine Hanover, Germany; Department of Pathology, University of Veterinary Medicine, Hanover, Germany.

In distinction from human prostate carcinoma, the disease in the dog is thought to be mostly androgen independent. Therefore basal cells, androgen refractory ductal cells, and transitional cells are a possible origin of canine prostate carcinoma. In human medicine Prostate Specific Antigen (PSA) and Prostatic Acid Phosphatase (PSAP) are markers commonly used for screening for prostate carcinoma.

The aim of this study was to identify the cells of origin of canine prostate carcinoma and to evaluate the use of PSA and PSAP in the diagnosis of the disease.

Nine samples of canine prostate carcinoma, nine samples of benign prostate hyperplasia and thirteen samples of transitional cell carcinoma (TCC) were evaluated using immunohistochemistry for PSA, PSAP, High Molecular Weight Cytokeratin (HMW-CK), staining for basal cells, Cytokeratin 7 (CK7), staining for urothelial cells and ductal cells, and Uroplakin III (UPIII), being specific for urothelial cells. Castration status and in the case of TCC also gender were recorded.

In benign prostate hyperplasia there was a strong positivity for PSA and PSAP, canine prostatic carcinoma was less likely to be positive for PSA and PSAP. In addition the staining was weaker in cells of malignant compared to cells of benign prostatic tissue. No TCC was positive for PSAP, but there was positive staining for PSA in five samples of transitional cell carcinoma. No sample of prostatic origin, benign or malignant, stained positive for UPIII, and only five samples of TCC (38%) showed a positive staining for UPIII. The staining for CK7 was significant more likely to be positive in samples of transitional cell carcinoma than in cells of prostatic origin. There was no significant difference in the staining for HMW-CK between either group.

We conclude that PSAP is a more useful marker for canine prostate carcinoma than PSA. In addition the secretion of PSAP and PSA is reduced in prostate carcinoma of the dog. For differentiation between transitional cell carcinoma and prostate carcinoma, CK7 and PSAP are useful markers. Basal cells do not seem to play a major role in the development of canine prostatic carcinoma. Uroplakin III is not useful for detecting transitional cell carcinoma.

ABSTRACT #93
FEASIBILITY AND SAFETY ASSOICATED WITH SELECTIVE AND SUPERSELECTIVE INTRA-ARTERIAL CARBOPLATIN ± MELOXICAM DELIVERY FOR UROTHELIAL TUMORS IN DOGS. C Weisse, A Berent, Sorenmo K, Todd K, Soulen M, Solomon JA. Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA.

Dogs with transitional cell carcinoma (TCC) are typically diagnosed with locally advanced tumors and up to 50% have distant metastasis at the time of diagnosis. Despite treatment most dogs will die within the first 12 months of diagnosis, often from progression of local disease. More effective local tumor control is necessary in order to improve outcome. Chemotherapeutic agents have activity in dogs with TCC, however these agents are associated with systemic toxicity, therefore preventing significant dose escalation. Intra-arterial delivery of chemotherapy and NSAIDs can provide increased intra-tumoral drug concentration and may therefore improve the anti-tumor activity. The investigators hypothesized that these treatments would be well tolerated and not result in any excessive morbidity or toxicity when compared with intravenous administration.

Twenty-two dogs received a total of 53 treatments (1–6 treatments/dog). All patients were placed under general anesthesia and vascular access to the femoral or carotid artery was obtained. Interventional radiology techniques were used under fluoroscopic guidance to gain selective or superselective access to the internal iliac or prostatic/caudal vesical arteries, respectively. Systemic doses of chemotherapy with or without meloxicam were administered and the animals recovered. The treatments were repeated following standard chemotherapy protocols.

Complications were mostly minor and identified following eleven (11/53) procedures. One dog developed presumptive sepsis after the first procedure and was euthanized one week later. Non-clinical myelotoxicity was identified following five (5/53) procedures.

Selective and superselective intra-arterial administration of chemotherapy with or without meloxicam is safe in dogs with naturally-occurring urothelial tumors.

Previously presented at ACVS, October 2008.

ABSTRACT #94
EFFECT OF INTRACAVITARY CHEMOTHERAPY AS TREATMENT OF RECURRENT PLEURAL EFFUSION FOLLOWING PERICARDIECTOMY: EIGHT CASES. M Ames, EA Rozanski, LM Freeman, L Barber, SM Cunningham, JE Rush. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

Recurrent pleural effusion in dogs following surgical pericardiectomy for idiopathic or malignant pericardial effusion is often a therapeutic challenge. The purpose of this study was to evaluate the survival time and side effects in dogs treated with intracavitary platinum-based chemotherapy after pericardiectomy for treatment of pericardial effusion.

All dogs that developed recurrent pleural effusion following pericardiectomy that were treated with intracavitary cisplatin or carboplatin were retrospectively evaluated. Dogs that had pericardiectomy for treatment of either hemangiosarcoma or chemodectoma were excluded. Data collected from the medical record included underlying diagnosis, survival time from the initial diagnosis of pericardial effusion, survival time from pericardiectomy and evidence of side effects of chemotherapy. Survival times were compared with Kaplan-Meier survival curves and Cox proportional hazards analysis.

Eight dogs met the inclusion criteria. The median age at first diagnosis of pericardial effusion was 8.5 years (range 5.9–10.9 years). Six were large breed dogs (>25 kg) and 2 were small dogs (<10 kg). All dogs had pericardiectomies; 4 via lateral thoracotomy, 2 thoroscopically, and 2 via median sternotomy. The pericardial biopsy was definitive for mesothelioma in 3 dogs and for unclassified neoplasia (likely carcinoma) in 1, while 4 dogs had severe inflammatory pericarditis with notation by the pathologist that neoplasia could not be excluded. No dog had hypoalbuminemia. Six dogs received intracavitary cisplatin (50 mg/m^2) following a therapeutic thoracentesis. Dogs were rolled to distribute the chemotherapy evenly. Dogs received intravenous saline diuresis (18.3 ml/kg/hr$\times$4 hours) in conjunction with cisplatin chemotherapy. Dogs received between 1–7 cisplatin treatments (median=3); treatments were repeated when effusion returned, but no more often than every 4 weeks. Three dogs received intracavitary carboplatin (300 mg/m^2), one of which had first received 4 cycles of cisplatin. The median survival time from the first pericardial effusion was 11.5 months (range 2.5–96.0 months) and the median survival time from pericardiectomy was 9.0 months, (range 1.5–60.0 months). Two dogs developed azotemia during treatment (creatinine values$>$ 3.0 mg/dl) which resulted in discontinuation of chemotherapy. One

dog, treated with carboplatin, developed severe neutropenia (32 PMN/ul) and associated sepsis; this dog recovered with supportive care. There was no difference in survival time from initial diagnosis ($p=0.23$) or from pericardectomy ($p=0.29$) based upon histopathological evidence of cancer.

Intracavitary chemotherapy was associated with few side effects, and represents a viable therapeutic option for dogs that have recurrent pleural effusion after pericardiectomy.

ABSTRACT #95
TECHNIQUE FOR FLUOROSCOPIC NASOJEJUNAL TUBE PLACEMENT IN DOGS. Beal MW, Mehler SJ, Staiger BA, Moore TW, Lam NK. College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Nutritional support contributes to a positive outcome in the management of dogs with critical illness. The advantages of enteral nutritional support when compared to methods of parenteral nutritional support are well documented in humans. Dogs with critical illness are often in need of nutritional support, but are often intolerant of feedings into the stomach. Post-pyloric feeding ay obviate many of these tolerance problems. Surgical jejunostomy techniques are well established, however, the methods are invasive and complications are common. We hypothesized that fluoroscopic nasojejunal feeding tube (NJT) placement is a safe, rapid, and uncomplicated method for achieving sustained post-pyloric access for enteral nutritional support in normal dogs.

Six normal, male, beagle dogs (7–12 Kg) were anesthetized using standard techniques common to those utilized during gastrointestinal endoscopic procedures. Dogs were placed in left lateral recumbency and lidocaine was administered into the right nostril. A 0.035in, 260 cm, standard stiffness, hydrophilic guide wire with a straight, flexible tip (HGW) was advanced through the nostril and into the stomach. A 5F 100 cm berenstein catheter (BC) was advanced over the HGW and into the stomach and used to guide it across the pylorus. The HGW was then advanced as far into the jejunum as possible. The BC was removed and an 8F end-hole and multi fenestrated feeding tube was advanced over the HGW into the jejunum. Gradual NJ feeding escalation was performed over the ensuing 48 hr and NJT location was monitored radiographically (0 hr, 48 hr, 72 hr) to assess for migration. At 72 hr, emesis was induced to determine the effect of emesis on NJT location. Tube migration was determined by measuring the location of the tip of the tube in relation to the caudal duodenal flexure (CDF). Dogs were monitored for gastrointestinal complications, mechanical complications with the tube, and self-removal. A paired t-test was used to compare tube locations at different time points. Statistical significance was set at $p<0.05$.

NJT placement was successful in all dogs. Median time to crossing the pylorus with the HGW was 11.3 min (range 2–28). Median total procedural time was 34.1 min (range 26–49). Median initial location of the tip of the NJT was 20 cm beyond the CDF (range 14–37). Complications of tube placement included mild epistaxis in two dogs. Three dogs removed the NJ tube within the first 48 hr. In the remaining 3 dogs, no significant tube migration occurred between 0 hr and 72 hr. Emesis induction resulted in oral migration of the NJT into the descending duodenum in one dog.

Fluoroscopy guided NJT placement is a safe, rapid, uncomplicated, minimally invasive technique for gaining post-pyloric access for enteral nutritional support in dogs. Further evaluation of NJTs in clinical patients will be necessary to better assess NJT tolerance and tube migration.

ABSTRACT #96
TECHNIQUE FOR PERCUTANEOUS RADIOLOGIC GASTROJEJUNOSTOMY IN THE DOG. Beal MW, Mehler SJ, Staiger BA, Moore TW, Lam NK, Brown AJ. College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Nutritional support contributes to a positive outcome in the management of dogs with critical illness. Post-pyloric feeding may obviate tolerance problems associated with gastric feeds in critically ill dogs. Surgical jejunostomy techniques are invasive and complications are common. We hypothesized that percutaneous radiologic gastrojejunostomy (PRGJ) is a safe and uncomplicated technique for gaining post-pyloric access for enteral nutritional support in dogs.

Six normal, male, beagle dogs (7–12 Kg) were anesthetized using standard techniques common to those utilized during gastrointestinal endoscopic procedures. The dogs were positioned in right lateral recumbency and an orogastric tube allowed for gastric insufflation. Fluoroscopy was utilized to identify a location caudal to the left, 13th rib for placement of 3 gastrointestinal suture anchors (GSA) into the stomach in a triangular pattern, creating a gastropexy. An 18 g puncture needle was advanced through the center of the triangle created by the GSA. A 0.035in, 150 cm, standard stiffness, hydrophilic guide wire with a straight, flexible tip (HGW) was passed through the puncture needle and into the stomach and a 5F angled guiding catheter was then passed over the HGW and used to direct the HGW across the pylorus and duodenum, and into the jejunum. Serial over-the-wire dilation of the body wall facilitated placement of an 18F peel-away introducer through which an 18F/8F 58 cm dual-lumen gastrojejunal feeding tube (GJT) was advanced over the HGW and into the jejunum. Gradual jejunal feeding escalation was performed over 48 hr. Tube location was determined radiographically at 48 hr, 96 hr, after emesis induction at 96 hr, and at the time of tube removal (days 16–18). Location of the distal tip of the jejunal portion of the tube was noted in reference to the caudal duodenal flexure (CDF). A paired t-test was used to compare tube locations at different time points during the study. Statistical significance was set at $p<0.05$, with Bonferroni correction for multiple comparisons. Dogs and GJT were monitored for complications.

PRGJ was successful in all dogs. Median time to traversing the pylorus with the HGW was 23.5 min (range 14–93). Median total procedural time was 53 min (range 42–126). The distal tip of the GJT was located median 35 cm (range 27–40) past the CDF at initial placement (0 hr). No procedural complications were noted. No significant tube migration was noted at any time point or after induction of emesis. 5/6 dogs completed the study period (96 hr). 1/6 dogs removed the tube on day 3. Mild gastrostomy site redness and discharge was noted in all dogs.

PRGJ in the dog is a safe, minimally invasive technique that allows for gastric decompression and jejunal feeding without the need for surgical intervention.

ABSTRACT #97
DIETARY γ-LINOLENIC ACID SUPPORTS ARACHIDONIC ACID ENRICHMENT OF FELINE RED BLOOD CELL MEMBRANES. L Trevizan[1], M Hoernis[1], KE Bigley[1], JE Bauer[1,2]. [1]Companion Animal Nutrition Laboratory, [2]Intercollegiate Faculty of Nutrition, Texas A&M University, College Station, TX.

Conversion of linoleic acid (LA) to arachidonic acid (AA) in cats is limited due to low δ6-desaturase activity. However, it is unknown whether this enzyme can be induced by feeding high dietary concentrations of its LA substrate. Also unknown is whether bypassing the Δ6-desaturase step by providing a dietary source of the enzymatic product, γ-linolenic acid (GLA), will enable AA synthesis. The objective of this study was to evaluate the *in vivo* accumulation of AA after feeding specific fatty acid precursors and measuring the incorporation of their fatty acid metabolites into feline red blood cell (RBC) membranes. It was hypothesized that feeding a GLA enriched diet would bypass the 6-desaturase step resulting in AA synthesis via chain elongation and 5-desaturation, with its subsequent incorporation into RBC membranes. It was also hypothesized that 6 desaturase may be directly induced by providing large dietary amounts of LA precursor. To test these hypotheses, adult female cats ($n=29$) were divided into three groups and fed complete and balanced diets containing high LA (HL, $n=10$), high GLA (GLA, $n=10$), or adequate LA (LL, control, $n=9$). The diets were similar in caloric density, total protein, total fat, carbohydrate, vitamins, and minerals and differed only in their fatty acid compositions. Diets were fed for 56 days according to metabolic body weight ($100\times W_{kg}^{0.67}$) to maintain body condition scores (BCS) of 5/9 with water provided *ad libitum*. Food consumption was recorded daily and body weights (BW) and BCS were determined weekly. After 56 days, blood samples were collected via saphenous vein into EDTA-containing tubes. Red blood cell ghosts were prepared from the samples and total lipids were extracted. Membrane phospholipids were fractionated via thin layer chromatography and fatty acid profiles were determined by gas chromatography. The Shapiro-Wilks test was used to determine normal distribution of fatty acid data and

one-way ANOVA was performed followed by Tukey ($\alpha=0.05$) multiple comparisons. It was found that high dietary LA concentration did not result in RBC AA enrichment vs. control suggesting no induction of -6 desaturase activity. However, chain elongation of LA was observed with the accumulation of 20:2n-6 and the presence of the Δ-5 desaturase product, 20:3n-6 (Δ 5, 11, 14) in the HL group. Most notably, feeding high dietary GLA was successful at bypassing the -6 enzymatic step yielding increased amounts of AA. These findings support the presence of a functionally active -5 desaturase enzyme in cats and that diets containing high amounts of GLA may be suitable for feline species.

ABSTRACT #98

ADIPOKINE EXPRESSION AND SECRETION BY CANINE ADIPOCYTES: STIMULATION OF INFLAMMATORY ADIPOKINE PRODUCTION BY LPS AND TNFα. VH Ryan[1, 2], AJ German[2], IS Wood[1], L Hunter[1], PJ Morris[3], P Trayhurn[1]. [1]Obesity Biology Research Unit, School of Clinical Sciences, University of Liverpool, Liverpool, Merseyside, UK; [2]Department of Veterinary Clinical Sciences, University of Liverpool, Liverpool, Merseyside, UK; [3]WALTHAM Centre for Pet Nutrition, Mars UK, Freeby Lane, Melton Mowbray, Leicestershire UK.

The aim of the current study was to examine the expression of key inflammation-related adipokines in canine adipose tissue and adipokine expression and secretion determined in canine adipocytes.

Adipose tissue samples were collected from adult Staffordshire Bull Terriers of both genders immediately after euthanasia, which had been performed for reasons unrelated to the research. The expression of inflammatory adipokines was assessed in a previously validated canine primary adipocyte culture system. Gene expression was assessed using real-time polymerase chain reaction methodology, whilst ELISA techniques were used to examine adipokine protein secretion into culture medium. The study was performed according to the University of Liverpool Guidelines on Animal Ethics and all tissue used was otherwise to be discarded.

Adiponectin, leptin, IL-6, monocyte chemotactic protein 1 (MCP-1) and TNFα genes were expressed in all the major adipose tissues depots of dogs, but there were no major depot differences in mRNA levels. Each adipokine examined was expressed in adipocytes differentiated in culture and secreted into the medium (leptin was not detected). IL-6, MCP-1 and TNFα were also expressed and secreted by preadipocytes; adiponectin and leptin were, however, only expressed after the induction of adipocyte differentiation. The inflammatory mediators LPS and TNFα had a major stimulatory effect on the expression and secretion of IL-6, MCP-1 and TNFα; there was a >5000-fold increase in IL-6 mRNA level with LPS. IL-6 release into the medium was increased >50-fold over 24 h with both LPS and TNFα, while MCP-1 release was increased 23- and 40-fold by TNFα and LPS, respectively. In contrast, there was no effect or a small reduction in adiponectin and leptin mRNA level with the inflammatory mediators. Dexamethasone stimulated leptin gene expression, had no effect on adiponectin expression, but decreased the expression and secretion of IL-6 and MCP-1. The peroxisome proliferator-activated receptor gamma (PPARγ) agonist rosiglitazone stimulated the expression of both adiponectin and leptin, and inhibited the expression of IL-6, MCP-1 and TNFα; however, only MCP-1 secretion was reduced.

These results demonstrate that canine adipocytes express and secrete key adipokines. It is concluded that canine adipocytes are highly sensitive to inflammatory mediators, these inducing major increases in the production and release of inflammatory adipokines.

ABSTRACT #99

EXERCISE AND ACTIVE CLIENT MOTIVATION IMPROVE RATE OF WEIGHT LOSS IN OBESE DOGS. A Chauvet[1], J Laclair[2], SL Holden[3], D Elliott[4], AJ German[3]. [1]Veterinary Neuro Services, Sarasota, FL, [2]Jim LaClair's Dog-Friendly Dog Training, Sarasota, FL, [3]Department of Veterinary Clinical Sciences, University of Liverpool, Liverpool, Merseyside, UK; [4]Royal Canin USA.

Obesity is a now a major concern in companion animals, and management strategies can either utilize dietary energy restriction or drug therapy. Lifestyle changes, involving increasing activity levels, are usually recommended as an adjunct to weight management, but there is little information on how this impacts success. The hypothesis of the current study was that including an organized exercise program using an underwater treadmill would improve weight loss on a conventional weight management regime.

Eight client-owned obese dogs (condition score 9/9), of various breeds and genders, were enrolled in a weight loss program using a high protein diet and lifestyle changes. An exercise regime was devised for each dog based on regular lead-walking and treadmill exercise. During the 3-month program, dogs were re-examined at 33, 54 and 86 days; at each stage, body weight, thoracic girth, and abdominal girth were recorded. The percentage weight loss over the program was compared with a matched control group enrolled at a weight management referral clinic and using an equivalent diet for weight loss.

Mean (±standard deviation, SD) percentage of starting weight lost was 7.4±2.20%, 10.8±1.88% and 18.9±5.44%, at 33, 54 and 86 days, respectively. The overall mean rate of weight loss for the whole 3-month trial was 1.5±0.43% per week. Both thoracic and abdominal girth also declined significantly over the course of the weight loss program ($P<0.0001$ for both). The median number of treadmill exercise sessions per dog was 13 (range 5 to 17). Median session duration was 30 minutes (range 8 to 37), median speed was 1.2 mph (0.2 to 3.5 mph), and median distance walked was 0.60 miles (range 0.03 miles to 1.70 miles). The session speed ($P=0.0023$), session duration ($P=0.0032$), and distance travelled per session ($P=0.0010$) all increased significantly over the course of the study. Cases lost significantly more weight than their respective controls (56 days: median 11.4% [range 8.1–13.1%] vs. 7.3% [range 3.0–12.8%], $P=0.0043$; 86 days: median 15.5% [range 14.3–25.3%] vs. 9.7% [range 4.6–18.5%], $P=0.0047$).

This study is the first to show that outcome of weight loss is improved when organized exercise sessions are included in conventional canine weight management programs.

ABSTRACT #100

COMPARISON OF DIETARY STRATEGIES ON THE PERCEPTION OF HUNGER DURING A FIELD FELINE WEIGHT LOSS STUDY. T. Bissot[1], E. Servet[1], S. Vidal[1], M. Deboise[1], G. Egron[2], M. Hugonnard[2], S Lardy[2], A.J. German[3], V. Biourge[1]. [1]Royal Canin, Aimargues, France. [2]Ecole Nationale Vétérinaire de Lyon, [3]Veterinary Clinical Sciences, University of Liverpool, UK.

Various dietary strategies exist for inducing weight loss in obese cats, but success in field trials is worse than expected from colony-based studies. The purpose of the current study was to assess the efficacy of different dietary strategies on the perception of hunger by owners and the rate of weight loss in a controlled field weight loss program.

48 overweight (body condition score (BCS) ≥4/5), healthy adult neutered cats completed a 20-wks weight loss trial. Three dietary strategies, intended to promote satiety and weight loss, were evaluated. DietA^a (ME: 2940 Kcal/1000 Kcal as fed, prot.: 116 fat: 31, TDF: 78, starch: 15 g/1000 Kcal, respectively) and C^c (ME: 3060 KCal/kg, prot.: 115 fat: 36, TDF: 81, starch: 62 g/MJ,respectively) were dry diets, whereas dietB was a mixed ration: dietA and a moist diet^b (ME: 550 Kcal/kg as fed, prot.: 137, fat: 36, TDF: 27, starch: 56, water: 1512 g/100 Kcal). On day 0, cats underwent physical examination, BW and BCS measurement. Target body weight (TBW) was estimated from current BCS. Cats were then randomly-allocated to the 3 diet groups, and owners instructed to feed their cat 30 to 35 Kcal/kg TBW/day based on the BCS.. On weeks 4, 12 and 20, cats were weighed; if the rate of weight loss was <0.5 or $>2\%$/week, energy allocation was re-adjusted. Hunger was assessed with a questionnaire, enabling calculation of a begging behaviour score (from 1=no begging to 4=permanent begging), begging manifestation scores (incessant vocalisation, excessive clinginess, food stealing, and aggression; scored as 0 or 1), and a global begging score (sum of both). Data were analysed using a repeated-measure ANOVA design, with cats, weeks and diets as main factors. All results are expressed as mean±sem.

Cats remained healthy and consumed all their food. Mean weight loss (%) over 20 weeks was similar amongst groups (11.0±1.3%, 10.9±1.2% and 11.9±1.7%, respectively). Mean rate of weight loss and energy allocation was not different amongst groups. Begging behaviour, the sum of begging manifestations, and the global begging score were significantly ($p<0.001$) higher with diet C than with diets A and B. At week 12, significantly more owners regarded food

allowance as insufficient on diet C than on diets A and B (64% vs. 31%, 39%, respectively).

Diets A and B performed better than diet C, based on their lower begging scores and owner perception of excess hunger. This confirms that diets formulated to induce satiety can help limit hunger during weight loss program, and are therefore likely to improve chances of success.

[a]Royal Canin, Veterinary Diet, Feline Satiety Dry, Aimargues, France; [b]Royal Canin, Veterinary Diet, Feline Obesity Wet, Aimargues, France; [c] Hill's Prescription Diet, Feline R/D Dry, Topeka, KS, USA.

Previously presented at European Society of Veterinary Comparative Nutrition in Vienna, September 2008.

ABSTRACT #101

INFLUENCE OF DIETARY PROTEIN SOURCE AND CONTENT ON FECAL QUALITY AND GENE EXPRESSION OF WATER AND ELECTROLYTE TRANSPORTERS OF COLON IN MINIATURE POODLES AND GERMAN SHEPHERDS. J Nery[1,2], V Leray[1], C Tournier[3], V Biourge[3], L Martin[1], H Dumon[1] and P Nguyen[1]. [1]Ecole Nationale Vétérinaire de Nantes, France, [2]University of Turin, Italy, [3]Royal Canin, Aimargues, France.

When compared to smaller dogs, large dogs are prone to producing poor quality feces, that could be partially attributed to higher colonic fermentations. Fermentation of undigested proteins could have deleterious effects on the colonic mucosa and affect colonic functions. One of them is water and electrolyte absorption. Highly digestible protein sources, such as wheat gluten, lead to a low protein residue in the colon. The aim of this study was to evaluate the effect of dietary protein source and amount on fecal quality and expression of proteins involved in colonic absorption, as water channel (aquaporin 8; AQP8), and electrolyte channel (Na+/K+ ATPase, as well as cystic fibrosis transmembrane conductance regulator (CFTR)) in the colon of large and small dogs.

Eleven female dogs (5 Miniature poodles: MP, 4.9±1.5 kg BW, and 6 German shepherds: GS, 26.6±2.4 kg BW) were used. Two diets varying in protein source and level were tested in a crossover study. Diets were isoenergetic with similar fat, TDF and ash content. Main protein sources and levels were wheat gluten meal at low protein level (LP; CP=21.4% as fed) and poultry meal at high protein level (HP; CP=34.8% as fed). The study design comprised two 14-d experimental periods and a 24-d washout period. Feces were scored (1=dry feces to 5=liquid diarrhea) daily for 7 days. Biopsies from proximal and distal segments of the colon were performed at the end of each experimental period. mRNA expression of two variants of AQP8 (AQP8v1 and AQP8v2), Na^+/K^+ ATPase and CFTR were measured by RT-real time PCR by comparison with GAPdH expression. Fecal score data were analyzed using Kruskal Wallis test and gene expression data were normalized and analyzed with repeated measures ANOVA.

Fecal score was higher in GS than MP dogs whatever the diet ($p<0.001$ for LP and $p<0.01$ for HP) and in HP fed dogs whatever the breed ($p<0.05$ for MP and $p<0.01$ for GS). A trend for higher mRNA expression of AQP8v1 was found in MP, compared with GS dogs, when fed HP diet ($p<0.09$). A trend for higher expression in the distal colon, associated with a breed effect was also observed ($p=0.15$). Similarly an effect of [breed*biopsy location] was observed in AQP8v2 expression in dogs fed HP ($p<0.09$); expression was higher in the distal colon of MP dogs. mRNA expression of AQP8v1 and AQP8v2 was constant in GS dogs. No effect of diet, breed or biopsy location was found for Na^+/K^+ ATPase nor CFTR expression.

Poorer fecal score in large compared to small dogs could be partially attributed to lower water channels mRNA expression in the distal colon. This divergence between the two breeds appeared to decrease when feeding highly digestible protein at low level. To improve fecal score in large sensitive dogs modulation of protein source and content could therefore be an adequate nutritional strategy.

ABSTRACT #102

INFLUENCE OF EMPIRICAL ANTIMICROBIAL CHOICE ON OUTCOME FOLLOWING SEPTIC PERITONITIS IN DOGS. I Keir, A Mateus, J Wignal, A Boag.

Despite advances in veterinary medical practice, canine sepsis is still associated with a mortality of greater than 50%. With any bacterial infection antimicrobial therapy is the cornerstone of treatment, along with drainage and debridement or removal of infected fluids and tissue. Studies in humans have shown that inappropriate initial empirical antimicrobial therapy is associated with a poorer outcome even when the antimicrobial therapy was subsequently changed based on culture results. Current human recommendations also advise the instigation of antimicrobial therapy within 1 hour of a diagnosis of sepsis.

Medical records of the Queen Mother Hosptial for Animals, Royal Veterinary College were reviewed retrospectively from 2003 to 2008 for dogs with a diagnosis of septic peritonitis. Data collected from the record included choice of empirical antimicrobial therapy, time from admission to first antibiotic therapy, bacterial culture and sensitivity results and any change in antimicrobial therapy made after culture results. Antimicrobial therapy was defined as appropriate if at least one of the drugs included in the empirical antibiotic treatment was effective against the pathogen(s) isolated. Survival was defined as alive at time of discharge. Chi squared analysis was used to compare the survival of patients receiving appropriate antimicrobial therapy with those receiving inappropriate therapy. Fisher's exact test was used to analyze the effect of the time from admission to commencing empirical antimicrobial therapy.

A total of 51 records were available with empirical antimicrobial and culture data available in 38 cases. The overall survival rate was 55%. Dogs that received appropriate empirical antimicrobial therapy had a survival to discharge rate of 61%; dogs that received inappropriate empirical antimicrobial therapy had a survival to discharge rate of 55%. No statistical difference was found between the rate of survival and appropriate empirical antimicrobial selection (p 0.191). The administration of antimicrobials within 1 hour of admission to the hospital did not impact on survival to discharge (p 0.437).

Factors affecting outcome in canine septic peritonitis are likely multifactorial. In our study the use of appropriate empirical antimicrobials and timing of antimicrobial administration were not significant factors, however considering the small number of cases, the possibility of a type II error should be considered.

ABSTRACT #103

DETECTION OF FLUOROQUINOLONE RESISTANCE LEVEL IN CLINICAL CANINE AND FELINE *ESCHERICHIA COLI* PATHOGENS USING RAPID AND EARLY REAL-TIME PCR ASSAY. BW Shaheen[1], DM Boothe[1], C Wang[2], CM Johnson[2], B Kaltenboeck[2]. [1]Department of Anatomy, Physiology and Pharmacology, [2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL.

Fluoroquinolones are used to treat infections caused by *Escherichia (E.) coli* in canine and feline veterinary patients, particularly those infecting the urinary tract. The *gyrA* gene is a primary target causing fluoroquinolone resistance in Gram negative coliforms, with mutations in codon 83 and 87 generally associated with high level of resistance *E. coli* clinical isolates. The purpose of this study was to develop a fluorescence resonance energy transfer (FRET) quantitative PCR that can precisely and specifically identify enrofloxacin-resistance in the canine and feline clinical *E. coli* isolates that carry mutations in codon 83 and 87 of *gyrA*. A total of 70 isolates were randomly selected that represent different levels of resistance to enrofloxacin between May and December 2008. Isolates were collected from 4 different regions: West, South, Midwest, and Northeast. We then tested 5 infected urine specimens to challenge the real-time PCR assay with urine specimens containing the following colony forming unites (CFU): 10^6, 10^5, 10^4, 10^3, 10^2, 10, and 0 CFU/ml. The susceptibility to enrofloxacin was determined by a standard micro-dilution test and according to CLSI guidelines and interpretive standards. Quantitative determination of *E. coli* was performed by real-time FRET-PCR in a LightCycler (Roche Diagnostics). This real-time quantitative PCR assay is rapid, economical, and sensitive compared to cultured antimicrobial susceptibility testing. The assay identified as low as five genome copies per reaction. For the 70 isolates tested, the sensitivity (correctly predicting true positives, that is either intermediate or high level resistance) was 87.5% (95% CI=75% to 95.3%) (n=42/48) whereas specificity (correctly identifying true negatives or susceptible) was 100% (95% CI=87.3% to 100%) (n=22/22). Furthermore, we were able to accurately differentiate between the wild type and

mutants *E. coli* directly from infected canine urine samples (n=5) within 1–2 hrs (as low as 10 CFU/ml corresponded to 19 genome copies). These results were confirmed by sequence alignments of the PCR products and comparison with the susceptibility testing. The FRET-PCR assay appears to have promising clinical application as an early diagnostic tool for rapid and sensitive detection and differentiation of the level of fluoroquinolone resistance among clinical *E. coli* isolates.

ABSTRACT #104
EVALUATION OF POTENTIAL BIOMARKERS FOR CANINE SEPSIS. AM Cruse, JA Yoder, SK Nordone, HS Marr, and AJ Birkenheuer. North Carolina State University, Raleigh, NC.

Sepsis is a complex clinical syndrome defined as the presence of systemic inflammatory response syndrome (SIRS) with documentation or suspicion of concurrent infection. Early recognition and treatment of sepsis has been shown to be crucial for the improvement of patient outcome. The purpose of this study was to evaluate the expression of three potential biomarkers of canine sepsis. Potential biomarkers were selected based upon increased expression in murine and zebrafish models.

Whole blood samples were collected from client-owned dogs presenting to the NCSU-VTH between February-October 2008. The patient populations included dogs with sepsis (n=12), systemic inflammatory response (SIRS without documented infection) (n=10), cancer (n=30), and healthy controls (n=25). Each sample was evaluated for FLT-4, KLF-1, and Tmem150 (TM6P1) mRNA expression using real-time polymerase chain reaction. Relative expression was assessed compared to an internal control gene (β-actin). Differences were compared using a one-way ANOVA.

FLT-4 expression was significantly up-regulated ($p<0.001$) in dogs with sepsis and SIRS compared to dogs with cancer. FLT-4 expression was significantly up-regulated ($p<0.001$) in dogs with sepsis compared to healthy dogs. However there was no difference in FLT-4 expression between dogs with sepsis and SIRS. Tmem150 expression was down regulated ($p<0.05$) in dogs with SIRS compared to healthy dogs. No other significant differences in gene expression were detected between groups.

FLT-4, also known vascular endothelial growth factor receptor 3, is involved in lymphangiogenesis and may be a useful marker for the diagnosis of sepsis and/or SIRS. Further studies with larger numbers of animals are needed to determine if FLT-4 can differentiate between sepsis and SIRS patients.

ABSTRACT #105
PULMONARY MANIFESTATION IN CANINE LEPTOSPIROSIS. K Radeke[1], B Kaser-Hotz[1], G Arndt[2], AD Gruber[3], B Guerra[4], A Jansen[5], F Lotz[2], E Luge[4], K Nöckler[4], B Kohn[1]. [1]Clinic of Small Animals, Faculty of Veterinary Medicine, Freie Universität Berlin; [2]Institute for Biometrics and Data Processing, FU Berlin; [3]Department of Veterinary Pathology, FU Berlin; [4]Federal Institute for Risk Assessment, Berlin; [5]Robert Koch Institute, Berlin, Germany.

Canine leptospirosis is a disease with multi-organ involvement with mostly renal and hepatic manifestations. Severe pulmonary hemorrhage syndrome" with respiratory signs (dyspnea, coughing, hemoptysis), radiological pulmonary changes and mortality rates of over 50% has been described in human medicine. However, thus far lung manifestation in dogs with leptospirosis has been reported infrequently.

Therefore, the medical records of dogs diagnosed with leptospirosis at the Small Animal Clinic, FU Berlin between 2006 and 2008 were reviewed with regard to: 1) clinical signs with special emphasis on respiratory signs and radiological pulmonary changes, 2) comparison of laboratory results among dogs with and without lung manifestation, 3) correlation between severity of pulmonary distress and outcome of disease.

The diagnosis of leptospirosis was based on results of MAT titers, urine PCR, and histopathology. Based on clinical and radiological signs patients were grouped in dogs with (group 1) and without (group 2) lung manifestation. Severity of pulmonary distress was graded as mild to moderate (grade 1) and severe (grade 2). Thoracic radiographs were graded based on pulmonary changes (interstitial, alveolar) and location (dorsal, generalized) in grade 1 (interstitial, dorsal), 2 (alveolar, dorsal), 3 (interstitial, generalized), and 4 (alveolar, generalized). The results of CBC and clinical biochemistry were compared between group 1 and 2.

37 dogs with leptospirosis were included in the study. 22 of 37 dogs (59.5%) suffered from pulmonary distress (15 dogs of grade 1, 7 of grade 2) either at presentation or during course of treatment. Radiological pulmonary changes of grade 1 were detected in 1 dog, of grade 3 in 11 dogs, and of grade 4 in 10 dogs with pulmonary distress. At admission or during course of disease 89% of the dogs were azotemic, 81% had increased liver enzymes and hyperbilirubinemia; no significant differences between groups were detected. There were significant differences between group 1 and 2 with regard to the severity of thrombocytopenia (median platelet count group 1 $68\times10^3/\mu l$, group 2 $151\times10^3/\mu l$, $p=0.01$) and anemia (median Hct group 1 29%, group 2 36%, $p=0.04$). All dogs with dyspnea grade 2 had to be euthanized due to respiratory distress. 33% of the dogs with dyspnea grade 1 and 27% of the dogs without respiratory signs were euthanized due to acute renal or hepatic failure.

A pulmonary form of leptospirosis was detected in 60% of the patients. Lung involvement represents a severe complication causing increased mortality depending on the severity of the respiratory signs.

ABSTRACT #106
SEROPREVALENCE OF FELV AND FIV IN CANADA. Susan Little. Bytown Cat Hospital, Ottawa, Ontario; Dorothee Bienzle, William Sears, University of Guelph, Guelph, Ontario; Jessica Lachtara, IDEXX Laboratories, Westbrook, ME.

FeLV and FIV are retroviruses that represent two of the most common and important infectious diseases of cats worldwide. Several published studies have evaluated the seroprevalence of FeLV and FIV in North American cats, but little of the available data is from Canada. The purposes of this study were to determine the seroprevalence of FeLV and FIV infection among cats in Canada and to identify risk factors for seropositivity.

Veterinary clinics, animal shelters, cat rescue programs, and feral cat programs in Canada participated in the study by testing cats and kittens for FeLV antigen and FIV antibody from August to November 2007. Cats were tested using a commercially available point-of-care ELISA test (IDEXX Snap Combo) or samples were submitted for ELISA testing to a diagnostic laboratory (IDEXX PetChek FIV Antibody, PetChek FeLV Antigen). Confirmatory tests were not performed as part of the study. Seroprevalence was calculated as the percentage of positive tests in the study population for each virus.

A total of 11,144 cats were tested. Seroprevalence for FeLV was 3.4% and for FIV was 4.3%. Of these, 58 cats (0.5%) were co-infected with both viruses. The probability of a positive test for FIV was not significantly higher for cats tested at shelters or rescue programs than at veterinary clinics. The probability of a positive test result for FeLV for cats tested at veterinary clinics was 1.7× higher than for cats tested at shelters or rescue programs ($p=0.023$). Prevalence was significantly higher in sick cats (6.7% FIV, 6.6% FeLV) versus healthy cats (3.2% FIV, 2.0% FeLV), and in adult cats (5.9% FIV, 4.4% FeLV) versus juvenile cats (1.6% FIV, 1.7% FeLV). The probability of a positive test result for FIV was 3.4× higher ($p<0.001$) in cats with access to outdoors when compared to cats with no outdoor access. The probability of a positive test result for FeLV was 1.4× higher ($p=0.027$) in cats with access to outdoors when compared to cats with no outdoor access. The probability of a positive test result for FIV was highest in intact males (7.4%), and for FeLV was highest in intact females (7.3%) and intact males (7.1%).

The seroprevalence for FeLV (3.4%) and FIV (4.3%) reported here is higher than in a recent large study that evaluated samples predominantly from the U.S. (FeLV 2.3%, FIV 2.5%). These findings emphasize variability in prevalence rates, and suggest that despite advances in testing and vaccination, infection with FeLV and FIV remains common in Canada.

ABSTRACT #107
PROGNOSTIC FACTORS IN CATS WITH FELINE PANLEUCOPENIA. D. Kruse[1], S. Unterer[1], K. Horbacher[1], C. Sauter-Louis[2], K. Hartmann[1]. [1]Medizinische Kleintierklinik, [2]Clinic for Ruminants, Ludwig Maximilian University Munich, Germany.

Feline panleucopenia is a highly contagious viral disease characterized by enteritis, panleucopenia, and high mortality. The purpose

of this study was to identify prognostic factors for the survival of cats with panleucopenia.

Medical records of 244 cats diagnosed with panleucopenia between 1990 and 2007 were evaluated retrospectively with regard to history, signalment, as well as clinical and laboratory parameters. Cats that tested positive for feline panleucopenia virus (FPV) *via* electron microscopy, polymerase chain reaction (blood, feces), antigen ELISA (feces), or that had histopathological lesions consistent with panleucopenia at necropsy were included.

Survival rate was 51.1%. No significant correlation was found between outcome and living conditions (indoor *versus* outdoor cat), age, vaccination status (vaccinated *versus* unvaccinated cats) or severity of clinical signs. Cats that died from the disease had significantly lower leucocyte and thrombocyte counts at time of presentation when compared to surviving cats. The relative risk of death for patients with a leucocyte count of $<1000/\mu l$ was 1.77 times as high as in patients with a leucocyte count of 1000–2500/µl ($p=0.038$), and 1.85 times as high as in patients with a leucocyte count of $>2500/\mu l$ ($p=0.001$). Furthermore, the likelihood of a fatal outcome was higher when the serum albumin concentration was <30 g/l or the serum potassium concentration was <4 mmol/l.

In conclusion, leucocyte and thrombocyte counts as well as albumin and potassium serum concentrations at time of presentation can be used as prognostic markers in cats with panleucopenia.

ABSTRACT #108

PREVALENCE OF PROTECTIVE ANTIBODY TITERS FOR DISTEMPER VIRUS AND PARVOVIRUS IN DOGS ENTERING A FLORIDA ANIMAL SHELTER. ES Lechner[1], PC Crawford[2], CH Edinboro[3], EJ Dubovi[4], R Caligiuri[5], and JK Levy[2]. [1]University of Florida College of Veterinary Medicine, Gainesville, FL. [2]University of Florida Maddie's Shelter Medicine Program, Gainesville, FL. [3]Exponent® Inc. Health Sciences Group, Menlo Park, CA. [4]the Animal Health Diagnostic Center, College of Veterinary Medicine, Cornell University, Ithaca, NY. [5]Alachua County Animal Services, Gainesville, FL.

Outbreaks of canine distemper virus (CDV) and canine parvovirus (CPV) are common in animal shelters, resulting in death of thousands of dogs each year. The purpose of this study was to determine the proportion of dogs entering an animal shelter with protective antibody titers (PAT) for CDV and CPV and to identify predictive factors associated with these titers.

CDV and CPV antibody titers were measured in 431 dogs on admission into a Florida animal shelter. Overall, 65% had insufficient CDV and/or CPV titers at admission. Only 35% of the dogs had PATs for both CDV and CPV, 8% had PATs for CDV but not CPV, 32% had PATs for CPV but not CDV, and 25% lacked PATs for either virus. In univariate analyses, dogs 1 year of age or older, neutered dogs, and dogs reclaimed by owners were more likely to have CDV and CPV PATs. In multivariate analyses, age and neutered status were significant predictors for CDV PAT, while age was a significant predictor for CPV PAT. Dogs with PATs for one virus were more likely to have PATs for the other virus.

Crowding, stress, and random co-mingling of dogs from different sources are common at shelters and contribute to risk for disease outbreaks. In this study, most dogs did not have protective antibody titers to CDV and/or CPV at the time of admission to the shelter. This supports current guidelines to vaccinate all dogs immediately upon admission to shelters, regardless of source, age, or physical condition.

ABSTRACT #109

DETECTION OF CANINE PARVOVIRUS TYPE 2C BY A COMMERCIALLY AVAILABLE IN-HOUSE RAPID TEST. Nicola Decaro[1], Costantina Desario[1], Melissa J. Beall[2], Marco Campolo[1], Maria Loredana Colaianni[1], Anthony A. Dimarco[2], Canio Buonavoglia[1]. [1]Department of Public Health and Animal Sciences, Faculty of Veterinary Medicine, Valenzano (Bari), Italy, [2]IDEXX Laboratories, Inc., Westbrook, Maine, USA.

Canine parvovirus (CPV) is one of the main pathogens causing haemorrhagic gastroenteritis and mortality in pups. In addition to the recognized variants CPV-2a and CPV-2b, a third variant, CPV-2c, emerged in Italy in 2000 and quickly spread to the canine population worldwide. Recently, concerns have been expressed about the ability of the in-house tests to detect the new variant 2c at the same extent of CPV-2a/2b. In this study, we have evaluated for the first time the detection rates of the different CPV variants by using a commercially available in-house test. A total of 201 specimens, containing CPV DNA loads higher than 10^5 DNA copies mg^{-1} faeces, were tested with the SNAP® Canine Parvovirus Test kit (IDEXX Laboratories, Inc.). The sample distribution according to the virus type was determined by minor groove binder (MGB) probe PCR assays. The samples originated from Italy ($n=125$), United Kingdom ($n=56$), Spain ($n=5$), and Greece ($n=15$). Performance was assessed by calculating the confidence intervals about the means using exact binomial limits with an alpha of 5%. The in-house assay was able to detect 41/51 types 2a, 39/50 types 2b and 77/100 types 2c CPVs, and percent positives was 80.4%, 78.0% and 77.0% for CPV types 2a, 2b and 2c, relative to real-time PCR. The in-house test gave positive results for specimens ranging from 3.92×10^5 to 1.5×10^{11} DNA copies mg^{-1} faeces. The detection rate for CPV-2c was not significantly different from those of the other two CPV types, thus dissipating any previous concerns about the hypothesised but never demonstrated less efficiency of the test in detecting the new variant. At the same time, some important mutations identified more recently were proven not to affect test sensitivity.

ABSTRACT #110

PREVALENCE OF, RISK FACTORS FOR, AND ASSEMBLAGE TYPES OF *GIARDIA* INFECTION IN CATS HOUSED IN AN ANIMAL SHELTER. SD Janeczko, B Griffin, SC Barr, JM Scarlett. Cornell University College of Veterinary Medicine, Ithaca, NY.

Giardia spp. infection in cats housed in animal shelters is presumably common, with the reported prevalence ranging from 1–80%. Infected cats may suffer from small bowel diarrhea but are frequently asymptomatic, and risk factors for infection are not well understood. Data regarding the frequency of zoonotic transmission is lacking and is generally based on circumstantial rather than experimental evidence, making it difficult to assess the public health risk associated with *Giardia* in cats. Both host adapted and potentially zoonotic assemblages have been identified in the small number of feline *Giardia* isolates that have been genotyped to date. Thus, confusion exists regarding the significance of *Giardia* in cats and the need for surveillance and treatment in animal shelters. The purpose of this study was to estimate the prevalence of *Giardia* in cats available for adoption at an animal shelter in central New York, assess zoonotic risk by determining the assemblage type(s) found in cats shedding *Giardia* cysts, and determine risk factors for infection.

A single fecal sample was collected monthly for 5 months from all cats and kittens available for adoption. Samples were collected from cats housed in cages and in colony rooms. Cats consumed food coloring or glitter once daily in canned food during the sample collection. These served as fecal markers, allowing identification of each cat's feces in the litter box. Each cat was individually examined in order to determine age, sex, body weight, and body condition score. Additional information collected for each cat included: origin (stray vs. relinquished), length of time in the shelter, type of housing, number in the housing unit, square footage per cat, and total number of cats in the shelter at the time of sample collection. A fecal sample was identified for each cat, scored using a standardized fecal scoring system (Ralston Purina, St. Louis, MO), and tested for the presence of *Giardia* antigen using a commercially available ELISA test (SNAP Giardia, IDEXX Laboratories, Westbrook, ME). Magnesium sulfate centrifugation was performed on all positive samples to concentrate the cysts and assemblage type was determined. Risk factors for infection were analyzed.

Of 554 fecal samples from 302 different cats, 60 positive samples were obtained from 49 different cats during the 5 month study period. The monthly prevalence as determined by the SNAP ELISA ranged from 6.6% to 14.8%, with increasing rates of infection seen during months with higher population densities and correspondingly lower square footage per cat. Cats housed in group settings were more likely to be shedding *Giardia* cysts than those cats housed individually. Preliminary sequencing results have identified both the zoonotic assemblage A and host adapted assemblage F, suggesting the potential for zoonotic transmission from some of the shelter cats.

ABSTRACT #111
ASSESSMENT OF REPRODUCTIVE TRACT DISEASE IN CATS AT RISK FOR ENTERIC *TRITRICHOMONAS FOETUS* INFECTION. SG Gray, SA Hunter, MR Stone, JL Gookin. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

The purpose of this study was to determine if *T. foetus* infection can be demonstrated in reproductive tract tissue from cats housed under conditions of intense breeding and for which a high prevalence of intestinal *T. foetus* infection has been identified.

The sample population consisted of purebred, cattery-housed cats undergoing elective ovariohysterectomy or castration and for which reproductive tract tissue, feces, and a reproductive history were submitted. Reproductive tract tissue was examined for *T. foetus* by light microscopy, immunohistochemistry, and PCR. History of reproductive tract disease was evaluated for statistical association with identified or reported exposure to intestinal *T. foetus* infection.

Sixty-one cats from 36 catteries met the inclusion criteria. 25% (15/61) of cats and 67% (22/33) of catteries were identified with active or reported *T. foetus* infection. Light microscopic, immunohistochemical, or molecular evidence of *T. foetus* infection of the reproductive tract was not found in any cats including; 15 with intestinal *T. foetus* infection, 29 residing in a cattery in which *T. foetus* infected cats were identified, and 8 for which gross or light microscopic evidence of reproductive tract disease was identified. Despite intense breeding and a high prevalence of reported reproductive problems, there were no differences in total number of litters, number of litters per breeding, kitten mortality, or birth defects between cats or catteries infected with *T. foetus* and those for which *T. foetus* infection was not identified.

In this study, there was no evidence that the feline reproductive tract is colonized by *T. foetus*. Accordingly, it is unlikely that reproductive tract infection with *T. foetus* plays a significant role in overall disease transmission or response to treatment.

ABSTRACT #112
USE OF SNAP®4Dx® ASSAY TO MONITOR TICK-BORNE INFECTION IN MINNESOTA: A 4–YEAR STUDY. B. Thatcher[1], M. Beall[1], K. Cyr[1], B. Stillman[1], M. Eberts[2], R. Chandrashekar[1]. [1]IDEXX Laboratories, Inc, Westbrook, ME. [2]Lakeland Veterinary Hospital, Baxter, MN.

In an effort to study the prevalence as well as efficacy of treatment and prevention of canine tick-borne diseases, we have been conducting a prospective study in Baxter, Minnesota. A total of 1809 dogs were tested by SNAP® 4Dx® between July 2004 and June 2008. The majority of the dogs were sampled as part of routine wellness exams (1630, 90.1%), while 179 (9.9%) were suspected of anaplasmosis and/or borreliosis at time of presentation. Serological trends were assessed by calculating the percentage of positive results for each condition by year. Confidence intervals about the means were calculated using normal approximation with an alpha of 5%. Overall, there appeared to be a decline in the number of dogs seroreactive for *Borrelia burgdorferi* since 2005 (39.4% in 2005 vs. 30.3% in 2008), coincident with the adoption of rigorous vaccination and treatment protocols. Similar to earlier findings (Beall et al. 2008), dogs suspected of clinical anaplasmosis and/or borreliosis were more likely to be seroreactive to both organisms (40.6%) compared to healthy dogs (21.1%). However, the number of dogs seroreactive to both *A. phagocytophilum* and *B. burgdorferi* appeared to be on the decline from 30.7% in 2005 to 21.3% in 2008 with an overall decrease in the number of clinically ill dogs from 17.4% in 2005 to 3.1% in 2008. Twenty-four dogs suspected of anaplasmosis and/or borreliosis from the previous study were followed from 2005 to 2008 to assess health, risk of re-infection, and serologic reactivity. None of the dogs reported relapses that would be consistent with chronic anaplasmosis or borreliosis; 42% became seronegative by SNAP® 4Dx® for at least one of the infections. Data from this canine field population suggests that the awareness of tick-borne diseases has encouraged the use of rigorous preventive and treatment strategies resulting in fewer seroreactive dogs as well as fewer dogs with clinical disease.

ABSTRACT #113
PREVALENCE OF HEMOPLASMA DNA IN FIELD-CAUGHT MOSQUITOES IN COLORADO. PC Lin, JR Hawley, BG Bolling, LM Eisen, MR Lappin. College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Mycoplasma haemofelis, '*Candidatus* M. haemominutum', and '*Candidatus* M. turicensis' (haemoplasmas) are common blood-borne organisms in cats. In dogs, *M. haemocanis* and '*Candidatus* M. haematoparvum' also exist. Other mammalians also harbor hemoplasmas including *M. wenyonii* in cattle. Transmission of hemoplasmas by arthropods has been suspected in cats, however, results of flea transmission studies have been inconclusive. The purpose of this study was to determine whether canine or feline hemoplasma DNA could be amplified from mosquitoes.

Aedes vexans mosquitoes caught in 2006 and 2007 as part of a West Nile Virus surveillance study were used. 100 sample pools containing approximately 50 mosquitoes each were selected from one of four field sites in Fort Collins, Colorado that were chosen based on probable close proximity to dogs and cats. Mosquitoes were pulverized and DNA extracted using a modified Qiagen protocol. Polymerase chain reaction for hemoplasma DNA was performed using a previously published assay and positive amplicons were sequenced.

Of the 100 mosquito pools, 5 pools (5%) had a band on gel electrophoresis consistent with a haemoplasma and adequate DNA for sequencing. *Spiroplasma* spp. DNA (a bacterium that infects insects) were amplified from four pools and DNA most consistent with *M. wenyonii* were amplified from the fifth pool.

Amplification of *M. wenyonii* DNA suggests that the positive mosquitoes had ingested blood from a large animal, likely cattle. Further study is needed to determine whether mosquitoes could be a mechanical vector for feline or canine hemoplasmas.

ABSTRACT #114
TOPICAL IMIDACLOPRID AND MOXIDECTIN PREVENTS FLEA TRANSMISSION OF *BARTONELLA HENSELAE* IN CATS. CA Bradbury, MR Lappin. Colorado State University, School of Veterinary Medicine, Department of Clinical Sciences, Fort Collins, CO.

Bartonella species are common pathogens in cats and people and are considered significant zoonotic agents especially in immunocompromised people. The most common species isolated from cats are *Bartonella henselae* and *B. clarridgeae* which are both transmitted by fleas. The purpose of this study was to determine whether monthly topical administration of imidacloprid and moxidectin would lessen flea transmission of *B. henselae* among cats.

Eighteen specific pathogen free cats were housed in three groups of six. The three enclosures were separated by mesh so as to allow fleas to pass among groups yet prevent cats from contacting one another. One group was inoculated intravenously with *B. henselae* and after infection was confirmed in all cats based on positive polymerase chain reaction (PCR) assay results, the cats were housed in the middle enclosure. The *B. henselae* infected cat group was flanked by a group that was administered topical imidacloprid and moxidectin monthly for three months and by a group that was not treated. On days 0, 30, and 60, 100 fleas per cat were placed on each of the six cats in the *B. henselae* infected group. Blood was collected from all cats weekly for *Bartonella* spp. PCR, serology and culture.

While *B. henselae* infection was ultimately confirmed in all of the untreated cats by blood culture and PCR assay results, none of the treated cats became infected.

In this setting, monthly topical imidacloprid and moxidectin prevents flea transmission of *B. henselae* in cats.

ABSTRACT #115
OUTCOME AND PROGNOSTIC FACTORS IN DOGS WITH CANINE LEISHMANIASIS IN A NON-ENDEMIC COUNTRY. K. Hartmann[1], K. Geisweid, C. Sauter-Louis[2]. [1]Medizinische Kleintierklinik, [2]Clinic for Ruminants, Ludwig Maximilian University Munich, Germany.

Due to increasing numbers of dogs that are imported from or have been visiting Mediterranean countries, canine leishmaniasis has become a very important infectious disease in Germany. So far,

the disease is not yet endemic, and autochthonic infections are rare. The aim of this study was to find out, (1) which clinical and laboratory parameters can be used as prognostic factors, (2) whether height of antibody titers is a prognostic factor in a country in which re-infection is extremely unlikely, and (3) whether treatment of asymptomatic dogs leads to a better prognosis.

Data of 93 dogs infected with *Leishmania infantum*, that had been presented to the Veterinary Teaching Hospital of the University of Munich and whose owners answered a detailed questionnaire, were evaluated retrospectively.

Most of the dogs had been imported from endemic countries (78%) and were mixed-breed (34%) with a median age of 7.5 years. Most common problems were lethargy (69%), skin lesions (63%), proteinuria (55%), lymphadenopathy (52%), and hypoalbuminemia (43%). There was no correlation between height of antibody titers and severity of clinical signs at presentation or survival time. However, there was a significant correlation between survival time and presence of proteinuria ($p<0.001$) and presence of lymphopenia ($p=0.016$). Also, treatment was correlated with a longer survival time ($p=0.017$), even in asymptomatic dogs, and treated dogs lived about two years longer then non-treated.

In conclusion, height of antibody titers does not correlate with prognosis. More useful parameters to predict shorter survival are proteinuria and lymphopenia.

ABSTRACT #116

THE USE OF COMPUTER TOMOGRAPHY IN THE DIAGNOSIS OF SEPTIC ARTHRITIS/OSTEOMYELITIS IN THE NEONATAL FOAL. Mary Rose Paradis, Mauricio Solano, Amy Tidwell. Louise Maranda Cummings School of Veterinary Medicine at Tufts University, N. Grafton, MA.

Septic arthritis/osteomyelitis is a recognized sequela to bacteremia in the neonatal foal. It manifests as lameness with increase joint effusion. Historically diagnosis of osteomyelitis has been made by radiographic examination of suspected joint or growth plate but radiographic evidence of osteomyelitis often lags behind the clinical signs. The hypothesis of this study was that computer tomography (CT) would detect bone lesions earlier and more reliably than radiography in the neonatal foal. Twenty foals between the ages of 8 hours to 36 days who were presented to the hospital with lameness secondary to septic arthritis/osteomyelitis during the foaling seasons of 2002–2006. Clinical information was collected for each foal. A total of 54 joints (34 clinically affected and 20 normal) were evaluated by radiographs and CT. Foals enrolled in the study had radiographs and CT of the affected joint and contralateral normal joint within 5 days of each procedure (mean=1 day). The images were evaluated by 2 board certified radiologists (individually and blinded) for the presence of osteomyelitis. Descriptions of the lesions were noted. It was found that investigators were more likely to agree on the presence of osteomyelitis from CT on normal and abnormal joints (Kappa 1 and 0.86 respectively) than normal and abnormal radiographs (Kappa 0.714 and 0.476.). CT was 1.7 times more likely to detect osteomyelitis than radiography. Our conclusion was that osteomyelitis lesions in foals with septic arthritis are more likely to be seen earlier with the use of CT than radiographs. This could have an effect on both the treatment choices and prognosis for affected foals.

ABSTRACT #117

ENDOCRINE ENERGY RESPONSE IN SEPTIC FOALS: INSULIN, LEPTIN AND ADIPONECTIN. RJIM Barsnick[1], SDA Hurcombe[1], NM Slovis[2], KA Sprayberry[2], PA Smith[1], RE Toribio[1]. [1]The Ohio State University College of Veterinary Medicine, Columbus, OH. [2]Hagyard Equine Medical Institute, Lexington, KY.

Anorexia and hypoglycemia are common clinical findings in critically ill septic foals. The therapeutic plan for hypoglycemic foals often requires the administration of parenteral glucose or parenteral nutrition. A number of septic foals develop glucose intolerance (hyperglycemia, glycosuria), and insulin is frequently administered to affected foals. These complications, we believe are the result of sepsis-mediated dysregulation of hormones involved in energy homeostasis (insulin, leptin and adiponectin).

The goal of this study was to investigate the association of insulin, leptin and adiponectin with sepsis, severity of disease, glycemia, triglyceridemia and mortality in foals.

We measured the plasma concentrations of aforementioned hormones using validated immunoassays in 44 septic, 62 sick non-septic (hospitalized for other diseases) and 19 healthy neonatal foals. Sepsis was defined as a sepsis score >11.

Insulin concentrations were higher in healthy foals than in sick non-septic and septic ($p<0.01$) foals. Septic foals had lower serum glucose and higher triglyceride concentrations than healthy foals ($p<0.01$). Among the septic foals, non-survivors had lower glucose and higher triglyceride values than survivors ($p<0.05$), but no difference was found with insulin concentrations. A number of septic foals had a poor insulin response to hyperglycemia (normal or low insulin concentrations).

Leptin and adiponectin concentrations were not different comparing septic, sick non-septic and healthy foals. However, plasma leptin and adiponectin concentrations were significantly lower in septic foals that died ($p<0.05$).

These results indicate that endocrine dysregulation of the energy metabolism in septic foals is frequent and likely important in the pathogenesis of sepsis. The lack of insulin response to hyperglycemia indicates sepsis-mediated impairment of insulin release. The low leptin and adiponectin concentrations in septic foals support energy impairment and are potential prognosticators of survival.

Our findings are clinically applicable in the evaluation, prognosis and treatment of critically ill foals.

ABSTRACT #118

OPSONIZATION OF *RHODOCOCCUS EQUI* WITH HIGH ANTIBODY PLASMA DECREASES BACTERIAL VIABILITY AND PROMOTES PHAGOCYTE ACTIVATION. DR Dawson[1], MJ Flaminio[1], D Nydam[1], JE Graham[2], M Cynamon[3], TJ Divers[1]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY. [2]University of Louisville School of Medicine, Louisville, KY. [3]Department of Veterans Affairs Medical Center, Syracuse, NY.

Susceptibility to *Rhodococcus equi* pneumonia in foals is exclusive to the first few months of life. Once infection is established, virulent *R. equi* survive and replicate inside macrophages. There are conflicting published data about the actual effect of intravenous high antibody plasma transfusion in the prophylaxis of *R. equi* disease in foals. In this study, we evaluate the effect of commercially available high antibody plasma on bacterial viability and phagocyte activation *in vitro*. We hypothesized that opsonization of virulent *R. equi* would decrease bacterium viability inside macrophages and increase phagocyte activation. We used a virulent *R. equi* (strain 103S) construct that expressed luciferase (*R. equi*-LUX). Luciferase activity in live *R. equi* reflects both the number of viable organisms, and the metabolic activity of the bacteria. Opsonization of *R. equi*-LUX with plasma decreased bacterial luciferase activity in comparison to non-opsonized bacteria ($p<0.0001$). A similar suppression was also observed in bacteria phagocytized by monocyte-derived macrophages after 24 hr culture ($p=0.006$). The opsonization of *R. equi*-LUX increased oxidative burst activity in phagocytes ($p<0.0001$). TNFα production increased in macrophages infected with opsonized versus non-opsonized bacteria ($p<0.0001$). The effect of opsonization in *R. equi*-LUX was associated with antibody and heat-resistant factors. Low opsonization (10%) significantly decreased bacterial viability and increased phagocytic activation, and greater levels of opsonization (40%) revealed further advantages. It is possible that passively transferred antibodies against *R. equi* affect bacterial viability before macrophage invasion *in vivo*. These data suggest a possible role of colostrum and plasma antibody in protection of foals.

ABSTRACT #119

PLASMA ENDOTHELIN CONCENTRATIONS IN SEPTIC AND NON-SEPTIC NEONATAL FOALS. L.R.R. Costa[1]*, S.E. Eades[1], A. Polkes[2], R.J. MacKay[2], R.M. Moore[1]. [1]Equine Health Studies Program, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA. [2]Large Animal Clinical Sciences, School of Veterinary Medicine, University of Florida, Gainesville, FL. *Currently at Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA.

Endothelins are endogenous peptides known by their potent spasmogenic action of smooth muscles, but shown to have diverse

roles in physiological and pathophysiological processes. The best studied isoform, endothelin (ET)-1, has been implicated in the pathophysiology of vascular, cardiac, respiratory and inflammatory conditions.

The purpose of this study was to compare plasma ET-1 concentration among neonatal foals considered healthy, sick but unlikely septic and sick and likely septic.

Plasma ET-1 concentrations were determined in samples collected at the University of Florida and Louisiana State University from a total of 137 foals including 119 ill foals (divided into two age groups: $<$7-d-old and $\geq$7-d-old) and 18 normal foals. Foals were divided into 3 disease groups: Group 1=healthy, Group 2=sick but unlikely septic (i.e., sepsis score less than 11), and Group 3=sick and likely septic (sepsis score greater or equal 11). Differences among groups were tested for significance using a Kruskal-Wallis test, followed by Dunn test to separate group medians.

Plasma ET-1 levels were significantly higher in Group 3 foals than in foals from Groups 1 or 2 ($P<0.001$). Overall, younger foals ($<$ 7-d-old) had significantly higher plasma ET-1 levels than older foals ($\geq$7-d-old) ($P<0.05$). Within Group 3, the median and interquartile range for ET-1 in plasma from foals $<$7-d-old was 5.58 pg/ml (IQR=3.52 to 8.96, n=39) and was 4.74 pg/ml (IQR=1.67 to 8.77, n=10) for those $\geq$7-d-old. Within Group 2, the median and interquartile range for ET-1 in plasma from foals $<$7-d-old was 2.16 pg/ml (IQR=0.48 to 5.26, n=47) and 0.33 pg/ml (IQR=0.10 to 0.43, n=23) for those $\geq$7-d-old. The median for ET-1 in plasma from foals in Group 1 was 1.48 pg/ml (IQR=0.75 to 2.78; n=18). Moreover, plasma ET-1 concentrations appeared to be affected by the presence of enteritis, hypoxic-ischemic encephalopathy, and maturity (premature birth or term foal). These results suggest a pathophysiological role of ET-1 in equine neonatal sepsis.

Previously presented at Dorothy Havermeyer Foundation Workshop in Neonatal Septicemia, November 2008.

ABSTRACT #120

THROMBOELASTOGRAPHY (TEG): AN INNOVATIVE TECHNIQUE FOR EARLY DETECTION OF HEMOSTATIC ABNORMALITIES IN FOALS WITH SEPTICEMIA. JL Mendez, P Vilar, MC Mudge, CG Couto. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Hemostatic abnormalities are common in neonatal foals with severe septicemia, and frequently result in secondary complications such as disseminated intravascular coagulation and multiple organ dysfunction syndrome. Newborn foals with severe septicemia have recently been shown to have widespread intravascular fibrin deposits with evidence of hypercoagulability (Cotovio 2008). Conventional hemostasis profiles do not detect hypercoagulability reliably. Thromboelastography provides data about the entire hemostatic system, from clot formation to fibrinolysis. Early detection of hypercoagulability by TEG should allow for interventional therapy and may change the outcome in hypercoagulable septic foals. The purpose of this study was to evaluate and compare TEG parameters in sick septic and non-septic neonatal foals.

Blood samples were collected from 18 foals ($<$2 weeks) upon admission to the Emergency Service into citrated tubes; they were placed in the thrombelastograph (TEG) cup and recalcified by adding $CaCl_2$ 30–90 minutes after sampling. Group 1 included septic foals (n=9) with a positive blood culture and/or sepsis score $>$11 and a focus of infection, and Group 2 included sick non-septic foals (n=9) with sepsis score $<$10 and no evidence of systemic inflammation. CBC, fibrinogen, prothrombin time and activated partial thromboplastin time were performed. Specific TEG parameters including R-time, K-time, angle "α", maximum amplitude "MA" and G-value were compared between groups.

There were significant differences ($p<0.05$) in TEG parameters; foals with septicemia had shorter K-time, wider "α", increased "MA" and G-value, changes consistent with hypercoagulability.

In conclusion, TEG can be used to accurately detect early hemostatic changes such as hypercoagulability in septic foals.

ABSTRACT #121

EFFECTS OF DOBUTAMINE AND ATROPINE AND ACETYLPROMAZINE ON AORTIC VALVE FUNCTION IN THE HORSE. GD Hallowell[1], TJ Potter[2] and IM Bowen[1]. [1]School of Veterinary Medicine and Science, University of Nottingham, UK. [2]Royal Veterinary College, North Mymms, UK.

Echocardiograms are performed on horses after drug administration for stress echocardiography and sedation and therefore an understanding of the effects of these agents on valve function is important for image interpretation. The objective of this study was to evaluate the effects on aortic valve function of administration of the sedative acetylpromazine (ACP) and atropine and dobutamine in horses subjected to stress echocardiography, to ascertain if these agents induced or masked the echocardiographic appearance of aortic valve prolapse (AVP).

For evaluation of ACP, echocardiography was performed on eight healthy Thoroughbred (TB) research horses that were sedated for routine dental work. For evaluation of atropine and dobutamine, images from eight TB or TB cross horses that were presented for investigation of poor performance were examined. Following a detailed clinical investigation none of the horses with poor performance were subsequently found to have underlying cardiac dysfunction.

Two dimensional and Doppler echocardiographic views were obtained before and after acetylpromazine and atropine-dobutamine stress echocardiography. Changes in internal cardiac dimensions, presence and extent of AVP (and physiological aortic regurgitation (PAR)) were recorded.

Acetylpromazine administration did not affect the occurrence of AVP (25% to 33%; p=0.46). There was no effect upon occurrence of PAR (p=0.75). However although there were no significant differences in jet length or width, there was an increase in jet area (p=0.02) identified.

The occurrence of AVP increased from 25 to 75% with atropine and dobutamine administration (p=0.02). PAR was noted in 75% of horses prior to drug administration, in 63% post-atropine administration and in 100% post-dobutamine administration (p=0.26). There was no significant difference in the length (p=0.73), width (p=0.75) or area (p=0.21) of the regurgitant jet following drug administration.

In conclusion it appears that the administration of dobutamine and atropine changes aortic valve competency leading to an increased occurrence of AVP and PAR. Acetylpromazine may increase the degree of PAR. Changes due to the direct effects of these agents should be taken into account on interpretation of echocardiographic results.

ABSTRACT #122

INTRAVENOUS 99 m-TECHNETIUM LIPOSOMES IN HORSES: A SAFETY AND BIODISTRIBUTION STUDY. C. Underwood[1], A.W. van Eps[1], P. Laverman[2], G. Storm[3], L. van Bloois[3], S.R. van Tomme[3], M.W. Ross[1], T.P. Schaer[1]. [1]University of Pennsylvania, New Bolton Center, Kennett Square, PA. [2]Radboud University Nijmegan Medical Center, Nijmegen, NL. [3]Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Utrecht, NL.

Liposomes are phospholipid nanoparticles that can extravasate at sites of increased vascular permeability. They can be used for targeted drug delivery and diagnostic imaging. This study is the first to describe the intravenous administration of ^{99m}Tc labeled polyethylene glycol (PEG) coated liposomes in normal horses.

The liposomes were prepared via the film hydration method. Labeling of the glutathione liposomes was performed using ^{99m}Tc-hexamethyl-propylene-amine-oxime (^{99m}Tc-HMPAO). An assay was developed to establish the 50% serum hemolytic complement activity (CH_{50}) in horses, as complement-mediated reactions are a common adverse effect in other species. *In vitro* CH_{50} assays were performed prior to liposome injection. Seven horses were administered 0.4 μmol/kg ^{99m}Tc-HMPAO PEG-liposomes and 2.5 μmol/kg unlabelled PEG-liposomes intravenously. Clinical parameters,

hematology, plasma biochemistry and serum complement activity were monitored serially. Scintigraphic imaging was performed at 1, 12, and 21 hours post-infusion. Three of the horses were euthanized at 23 hours. The percentage of injected dose per kilogram of tissue (%ID/kg) was calculated for multiple organs. Results were analyzed using ANOVA and Friedman tests.

Complement assays required the addition of C3 depleted human serum to the standard hemolytic assay to obtain a CH_{50} value. No significant complement activation was detected. No significant clinical, hematological or biochemical changes occurred following liposome administration, although one horse developed mild transient urticaria and mild agitation during the infusion. Scintigraphic studies revealed a prolonged vascular phase that lasted to 21 hours, with increased radiopharmaceutical uptake in the liver, lungs, spleen and kidney. There was a repeatable pattern of organ distribution. Evaluation of tissue samples revealed the highest concentrations within the lung, kidney, liver and spleen, with significantly increased ^{99m}Tc activity in the lung and spleen compared with blood. The mean circulatory half life of the ^{99m}Tc PEG liposomes was 11.51±2.2 hours.

There were minimal adverse effects associated with intravenous liposome administration in normal horses. This study establishes normal scintigraphic findings after administration of ^{99m}Tc PEG liposomes. Liposomes have significant potential in horses, as a diagnostic tool and for targeted drug delivery, particularly in the management of refractory infections and neoplasia.

ABSTRACT #123
PREVALENCE AND RISK FACTORS FOR HYPERINSULINEMIA IN CLINICALLY NORMAL HORSES IN CENTRAL OHIO. J Muno[1], L Gallatin[1], RJ Geor[2], GA Anderson[3], KW Hinchcliff[3]. [1]The Ohio State University, Columbus, OH. [2]Virginia Tech, Blacksburg, VA. [3]University of Melbourne, Australia.

Obesity is common in horses and is a risk factor for insulin resistance. However, other risk factors for insulin resistance in horses have not been well documented. The aim of this study was to determine the prevalence and risk factors for hyperinsulinemia in clinically normal, adult horses.

A convenience sample of 300 horses (138 mares, 143 geldings and 19 stallions; 4 to 30 years) was drawn from 18 farms in central Ohio. Plasma insulin and glucose were measured after a 10–12 hour period of grain and grass withholding. Body condition score, height, weight tape, neck circumference and management practices were recorded. A univariable logistic regression and a multivariable logistic regression with a random effect of farm were used to estimate odds ratios for hyperinsulinemia.

The prevalence of hyperinsulinemia, ($>15\,\mu$U/L) was 22.3% (67/300). A number of factors including breed, age, body condition score, girth circumference, not feeding grain and lack of access to pasture were associated with hyperinsulinemia in the univariable analysis. Multivariable analysis revealed that increasing age (odds ratio per year of increasing age 1.1, 95% CI 1.03–1.17) and body condition score (odds ratio 2.43, 95% CI 1.6–3.7) increased the risk for hyperinsulinemia while access to pasture (odds ratio 0.34, 95% CI 0.14–0.86) decreased the risk. Breed, sex, exercise, and measures of neck and girth circumference were not associated with risk of hyperinsulinemia.

This study demonstrates that when accounting for common management factors, only indirect measures of obesity and increasing age are independently associated with increased risk of hyperinsulinemia.

ABSTRACT #124
SEASONAL VARIATION IN PLASMA INSULIN AND GLUCOSE CONCENTRATION IN NORMAL HORSES IN CENTRAL OHIO. J Muno[1], L Gallatin[1], RJ Geor[2], GA Anderson[3], KW Hinchcliff[3]. [1]The Ohio State University, Columbus, OH. [2]Virginia Tech, Blacksburg, VA. [3]University of Melbourne, Australia.

Some tests of endocrine function in horses, including the dexamethasone suppression test and plasma adrenocorticotrophin concentration have seasonal variation. The purpose of this study was to determine if plasma insulin and glucose concentrations vary with season in healthy horses in central Ohio.

Twenty-nine healthy horses, as determined by physical examination, were evaluated at three-month intervals over a one year period. Plasma insulin and glucose concentrations were measured after a 10–12 hour period of grain and grass withholding. Body condition score, height, weight tape and neck circumference were measured at the same time. Friedman's test and Wilcoxon's signed rank test were used to compare the overall and individual differences between the four seasons in the values of insulin and glucose. The type 1 error rate was 5%.

There was an effect of season on the concentration of both insulin and glucose ($P<0.001$). The concentration of insulin in summer was lower (median 4.6 μU/L, range 1.2 to 22.0) than any of the other three months ($P<0.001$). There was no significant difference between any of the other seasons (autumn 13.4, range 4.3 to 271.7; winter 13.6, range 5.3 to 401.7; spring 12.5, range 2.0 to 412.4). The concentration of glucose in autumn was less than in any of the other three seasons ($P<0.001$), and the concentration in summer was less than in spring ($p=0.041$).

This study demonstrates that an apparent seasonal variation exists in the plasma concentrations of both insulin and glucose in normal horses. This information may modify the interpretation of laboratory findings indicative of insulin resistance.

ABSTRACT #125
EFFECTS OF A SUPPLEMENT CONTAINING CHROMIUM AND MAGNESIUM ON INSULIN SENSITIVITY IN HORSES WITH EQUINE METABOLIC SYNDROME. K Chameroy, N Frank, SB Elliott, RC Boston. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN (Chameroy, Frank, Elliott); and the Department of Clinical Studies, University of Pennsylvania, Kennett Square, PA (Boston).

Chromium and magnesium are commonly recommended as supplements for horses with equine metabolic syndrome (EMS), which is a clinical syndrome comprised of obesity, regional adiposity, insulin resistance, and laminitis. The aim of this study was to determine whether a combination supplement containing chromium, magnesium, and herbs would alter physical measurements and/or insulin sensitivity in horses affected by EMS. Fourteen horses with resting hyperinsulinemia ($>30\,\mu$U/mL) and/or abnormal combined intravenous glucose-insulin tolerance test results were randomly allocated to treatment (n=8) and control (n=6) groups. Treated horses received the supplement once daily in 0.5 lb oats for a total of 16 weeks. The supplement evaluated contained 5 mg chromium as yeast and 8.8 g magnesium oxide/proteinate. Frequently sampled intravenous glucose tolerance tests and physical measurements were performed at 0, 8, and 16 weeks.

Body weight and neck circumference measurements remained unaffected by treatment in this study. Glucose and insulin data from one horse in the treatment group were excluded because pain associated with recurrent laminitis affected results. Mean±SD (n=13) insulin sensitivity was $0.64\pm0.62\times10^{-4}$ L•min^{-1}•mU^{-1} for horses included in this study and no effect of treatment was detected over time. Other measures of glucose and insulin dynamics and resting blood glucose, insulin, and triglyceride concentrations also remained unaffected by treatment.

We conclude that the specific formulation evaluated, given once daily in the feed for 16 weeks did not alter physical measurements or glucose and insulin dynamics in horses with EMS.

ABSTRACT #126
SEASONAL CHANGES IN THE COMBINED GLUCOSE INSULIN TOLERANCE TEST IN NORMAL AGED HORSES. RA Funk, AJ Stewart, AA Wooldridge, RJ Kemppainen, EN Behrend, AK Johnson. Auburn University College of Veterinary Medicine, Auburn, AL.

Insulin resistance (IR) is increasingly recognized in adult horses. Insulin resistant horses are often obese and predisposed to developing laminitis, especially in spring and summer. Insulin resistance can also occur secondary to pituitary pars intermedia dysfunction (PPID). Diagnostic testing for PPID is challenging in the fall due

to seasonal increases in hormones in normal horses. The purpose of this study was to evaluate seasonal changes in the combined glucose insulin tolerance test (CGIT) for IR. Ten healthy aged horses with normal dexamethasone suppression tests (indicating absence of PPID) were administered CGIT in February, May, June, August, September, and November. Horses were administered dextrose (150 mg/kg) and insulin (0.1 U/kg) intravenously. Glucose concentrations were measured at 0, 1, 5, 15, 30, 45, 60, 75, 90 and 150 minutes. Insulin concentrations were analyzed at 0, 5, and 75 minutes. Mean glucose and insulin concentrations for each time point and mean area under the curve for positive and negative glucose peaks were compared between months using 2-way analysis of variance. Significance was set at $p < 0.05$. Glucose returned to baseline levels by 45 minutes in all horses, except for two individuals in September. No significant differences in mean glucose and insulin concentrations at each time point nor in mean area under the curve for positive and negative glucose peaks between months were identified. Therefore, the majority of horses were considered insulin sensitive throughout the study. Based on this initial analysis, seasonal changes do not appear to affect the results of the CGIT in the majority of normal aged horses.

ABSTRACT #127
EFFECTS OF SAMPLING TIME AND HAY FEEDING ON BLOOD GLUCOSE, INSULIN, AND ADRENOCORTICOTROPIN HORMONE (ACTH) CONCENTRATIONS IN HORSES. NS Chumbler, Tüth F, Elliott SB, N Frank. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN.

Ideal sampling conditions for glucose, insulin, and adrenocorticotropin hormone (ACTH) measurements have not been established for horses, so this study was undertaken to measure interday and intraday variability, and to determine whether horses should be deprived of feed or fed hay prior to sampling.

Six healthy crossbred mares were compared with 6 horses with hyperinsulinemia ($>30\,\mu U/mL$). Blood samples were collected at 0800 for 4 days and then every hour from 0800 to 2000 on the fourth day. Sampling took place after feed deprivation the first week and after hay feeding the next week according to a crossover design.

Serum insulin concentrations remained below $30\,\mu U/mL$ for 5 of 6 mares in the control group, but hyperinsulinemia (maximum: $43.3\,\mu U/mL$) was detected in the remaining mare when hay was fed. Overall mean interday coefficients of variation were 6%, 43%, and 20% for glucose, insulin, and ACTH, respectively. Hay feeding was associated with significantly ($P < 0.05$) higher serum insulin concentrations in both healthy (8.2 versus $2.2\,\mu U/mL$) and insulin resistant (56 versus $39\,\mu U/mL$) horses across the 4-day testing period. Significantly higher mean serum insulin concentrations were also detected when hay was fed to healthy (9.5 versus $2.3\,\mu U/mL$) and insulin resistant (78.8 versus $19.1\,\mu U/mL$) horses across 12 h.

Feeding hay raised serum insulin concentrations, which suggests that horses should be deprived of feed before blood samples are collected. However, hyperinsulinemia developed after hay feeding in one mare, so a provocative test may be useful in these cases.

ABSTRACT #128
EFFECTS OF ENDOTOXEMIA AND CARBOHYDRATE OVERLOAD ON GLUCOSE AND INSULIN DYNAMICS AND THE DEVELOPMENT OF LAMINITIS IN HORSES. F Tüth, N Frank, RC Boston. Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN (Tüth, Frank); and the Department of Clinical Studies, University of Pennsylvania, Kennett Square, PA (Boston).

Endotoxemia lowers insulin sensitivity and has been anecdotally associated with laminitis in horses, but a direct cause-and-effect relationship between endotoxemia and laminitis has not been established. The aims of this study were therefore to examine the effects of endotoxemia and alimentary carbohydrate overload on glucose and insulin dynamics and to determine whether administration of both treatments sequentially affected the incidence or severity of laminitis.

Horses (n=24) were randomly allocated to three treatment groups, each containing 8 horses. A lipopolysaccharide (LPS) group received LPS intravenously for 8 h, an oligofructose (OF) group received 5g/kg OF via nasogastric intubation, and a LPS/OF group received LPS for 8 h followed by OF the next day. Glucose and insulin dynamics were assessed before and after treatment by frequently sampled intravenous glucose tolerance testing and minimal model analysis. Physical examinations and complete blood counts were performed and the severity of laminitis was determined using the Obel grading scale.

Horses receiving LPS exhibited clinical signs of a systemic inflammatory response and leucopenia. Mean insulin sensitivity decreased by 66%, 74%, and 49% in LPS, OF, and LPS/OF groups, but responses did not differ significantly among groups. In contrast, development of Obel grade ≥ 2 laminitis was significantly associated with treatment, with the highest incidence detected for the LPS/OF group.

Insulin sensitivity decreases after endotoxin and/or OF administration in horses. Endotoxemia predisposes horses to carbohydrate overload-induced laminitis and increases the severity of disease. Medical management of endotoxemia is warranted to prevent laminitis in horses with intestinal disease.

ABSTRACT #129
PRO-INFLAMMATORY CYTOKINE AND CHEMOKINE EXPRESSION PROFILES OF VARIOUS ADIPOSE TISSUE DEPOTS OF INSULIN-RESISTANT AND INSULIN-SENSITIVE LIGHT BREED HORSES. Burns TA[1], Geor RJ[2], Mudge MC[1], McCutcheon LJ[2], Belknap JK[1]. [1]The Ohio State University, Columbus, OH. [2]Michigan State University, East Lansing, MI.

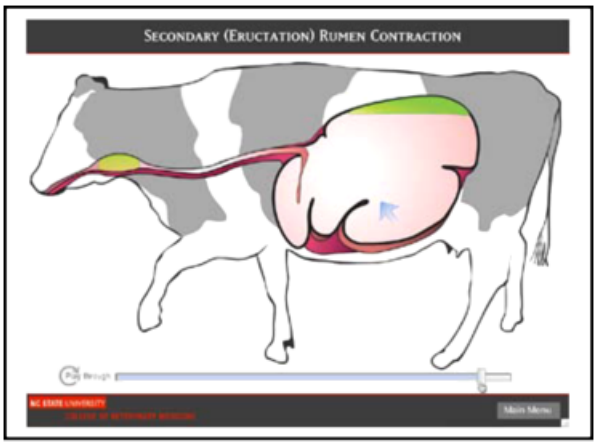
Insulin resistance has been associated with predisposition to laminitis in horses and ponies. In humans and rodents, omental adipose tissue (AT) expresses pro-inflammatory cytokines and adipokines at significantly higher levels than subcutaneous or retroperitoneal AT, in correlation with the degree of insulin resistance. While this has been postulated to also be a factor contributing to insulin resistance in horses, no published data currently support or refute this hypothesis. This study characterized the expression of pro-inflammatory cytokines and chemokines in several AT depots of insulin-resistant (IR) and insulin-sensitive (IS) horses. Eleven mares (8 Quarter horses, 2 Thoroughbreds, and 1 Standardbred), categorized as IR (insulin sensitivity [SI]$=0.58\pm0.31\ L\bullet min^{-1}\bullet mU^{-1}$; n=5) or IS (SI$=2.59\pm1.21\ L\bullet min^{-1}\bullet mU^{-1}$; n=6) based on results of an insulin-modified frequently-sampled intravenous glucose tolerance test were studied. No statistically significant differences in weight, body condition score, neck circumference, girth, or ultrasonographic retroperitoneal fat thickness were noted between groups. Omental, retroperitoneal, and mesocolonic AT was collected concurrently via ventral midline celiotomy; nuchal ligament and tail head AT biopsies were collected via skin incision. All tissues were snap-frozen and stored at $-80\,^{\circ}C$. For each depot, total RNA was extracted and cDNA analyzed via real-time quantitative PCR to quantify expression of TNF-α, IL-1β, IL-6, plasminogen activator inhibitor-1 (PAI-1), and monocyte chemoattractant protein-1 (MCP-1). Data were analyzed using a Kruskal-Wallis test with a Dunnett's post test (p=0.05). No differences in AT expression of TNF-α, IL-1β, IL-6, PAI-1, or MCP-1 were noted between IR and IS groups for each depot. However, when data from IR and IS groups were combined for each depot, the expression of IL-1β (p=0.009) and IL-6 (p=0.023) was significantly higher in nuchal ligament AT than in other depots, suggesting that this AT depot has different biological behavior in the horse and is more likely to adopt an inflammatory phenotype than other depots examined. Importantly, these data indicate that omental adipose tissue (and other visceral fat depots) may not be as important to the pathophysiology of obesity in the horse as in other species.

ABSTRACT #130
AN ANIMATED MODEL OF RETICULORUMEN MOTILITY. JL Gookin, DM Foster, AM Harvey. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Akin to the heart, faithful contraction of the ruminant forestomach is required to sustain life. Reticulorumen motility mediates the mixing of fiber with fermentative bacteria, regulates the rate of nutrient intake and delivery, permits offloading of ~60 liters of fermentative gases per hour, and enables these animals to eat in

haste and re-masticate their food at leisure and in greater safety. An understanding of reticulorumen motility is important to the assessment of ruminant health, optimal production, and in recognition, diagnosis and treatment of disease. Teaching reticulorumen motility can be challenging because real-time images of the contraction events are largely unavailable. Didactic material is based mostly on written descriptions, line drawings, or pressure tracings obtained during a contraction sequence. These approaches make learning reticulorumen motility dry and tedious and fails to leave the student with an appreciation for the integrated nature and periodicity of the contraction events. Our goal was to develop an animated model of reticulorumen motility. We hypothesize that first-year veterinary students will prefer use of the animated model over traditional instructional methods for learning.

An animated model of the reticulorumen was created using Adobe® Flash. Illustration components were created in Adobe® Illustrator and exported into Flash; 4 different sequences of motility were then created by manipulating the Illustrator paths frame by frame within Flash. Each sequence was saved as a scripted movie clip and placed in an interactive interface with a main menu for user access and control of each animation. An interactive overview of the associated anatomy with labels that can be turned on/off is accessible for reference and self testing. The program contains 4 animated learning modules: primary reticulorumen contraction, secondary reticulorumen contraction with eructation, rumination, and rumination superimposed on primary and secondary contraction sequences. Contraction events are depicted with real-time periodicity or can be rate-controlled using a slider bar. Written description of the contraction events accompany each module. This project is the first to use animation as an alternative to traditional instructional methods for teaching reticulorumen motility. Use of this model may translate into an improved understanding of reticulorumen motility and, subsequently, improved problem-solving skills.



ABSTRACT #131

A RETROSPECTIVE STUDY OF 106 CASES OF BOVINE LYMPHOSARCOMA. AJ Burton[1], DV Nydam[1] and TJ Divers[2]. [1]Dept of Population Medicine and Diagnostic Sciences and [2]Dept of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Prevalence of infection with the Bovine Leukemia Virus (BLV) in the USA is high, with 83.9% of dairy operations testing positive for BLV (NAHMS, 2007). Less than 5% of cattle infected with BLV go on to develop neoplasia (lymphosarcoma). There are many publications discussing the transmission, epidemiology, testing and control of BLV. However, there are no peer-reviewed publications reporting on large numbers of clinical cases of cattle affected with lymphosarcoma. The objective of this study was to describe the initial clinical presentation, ante mortem diagnostic tests and tumor location in cattle diagnosed with lymphosarcoma.

A retrospective study of all cattle admitted to the Cornell University Farm Animal Hospital between January 1980 and December 2008, that had a definitive diagnosis of lymphosarcoma was conducted. Data on signalment, presenting complaint, clinical pathology, ante mortem diagnostic tests and necropsy findings was collected. Categorical data were analyzed using and Chi-square tests with calculation of Exact binomial 95% confidence intervals for proportions. Alpha was set at 0.05.

Of 106 total animals, 94% were cows and 6% were bulls. The median age (n=95) was 5 years**.** Enlarged peripheral lymph nodes (PLNs) were detected in 54% of animals (n=102). Median lymphocyte count (n=96) was 4,900 cells/L with 10.4% having circulating lymphoblasts. Ante mortem aspirate/biopsy sites were based upon localization of clinical disease. The proportions of false negatives for ante mortem diagnostic tests were as follows: PLN aspirate, 57% (C.I. 34–78), PLN wedge biopsy, 0% (C.I. 0–29), surgical abdominal exploration and biopsy, 0% (0–23), abdominocentesis, 64% (C.I. 35–87), pleurocentesis, 20% (C.I. 1–72), pericardiocentesis, 33% (C.I. 4–78) and cerebral spinal fluid tap, 81% (C.I. 54–96). In cows that had a full necropsy (n=83) the most frequently identified locations of a lymphosarcoma tumor were the heart (66%), abomasum (59%), uterus (37%), kidney (31%) and epidural space (27%). Retrobulbar tumors were identified in only 10%.

This study has identified more kidney tumors and fewer retrobulbar tumors, in contrast to the frequently cited 5 'classical' predilection sites (abomasum, heart, uterus, epidural space and retrobular). A higher percentage of cows exhibited PLN enlargement (54%) than previously estimates (≈25%). Abdominocentesis, PLN aspirate and cerebral spinal fluid taps were found to be fairly insensitive tests while PLN biopsy and surgical exploration and biopsy found to be sensitive tests for lymphosarcoma.

ABSTRACT #132

DETERMINATION OF DAIRY COWS HEMATOLOGICAL REFERENCE INTERVAL BY 3 METHODS AND EVALUATION OF CONFOUNDING FACTORS. D Francoz, SM Buczinski, M Moreau, C Bédard. Faculté de Médecine Vétérinaire, Université de Montréal, Montréal, Canada.

The purpose of this study was to establish hematological reference intervals in dairy cows in Québec to compare them to those established by Schalm in 1965, and to evaluate the effect of herds and physiological stage on hematological values.

Blood samples were obtained from 45 healthy Holstein cows between 1 to 60 days in milk (DIM), 21 between 61 to 180 DIM, 21 between 181 and 300 DIM and 19 dry cows, randomly selected in 4 different farms. White blood cells counts were obtained using an Advia 120 analyzer. Fibrinogen concentration was determined by heat precipitation technique. Serologic testing for bovine leukosis virus (BLV) was performed by ELISA. A bootstrap resampling method (BRM) was performed yielding 1000 samples from the study cohort to validate reference intervals (95%) estimated by parametric (PM) and nonparametric (NPM) methods. Statistical inferences for between class comparisons were done under a probability value of 5%.

Farms of origin and DMI categories had significant effect on monocyte and fibrinogen levels, respectively. However, values remain within the interval references. BLV status had a significant effect on leukocyte ($p=0.003$), lymphocyte ($p<0.001$) and basophil ($p=0.003$) counts.

	PM		NPM		BRM	
	Mean		Percentile		percentile	
Blood parameters [Schalm's reference interval]	**−1.96SD**	**+1.96SD**	**2.5**	**97.5**	**2.5**	**97.5**
Reference interval for all selected cows (n=106)						
Fibrinogen g/L [2.0–6.0]	0.9	5.5	1.6	5.0	1.6	5.0
Leukocyte 10^9/L [4.0–12.0]	0.5	15.7	4.2	16.8	4.2	16.8
Neutrophil segmented 10^9/L [0.6–4.0]	1.0	4.6	1.3	4.6	1.3	4.6
Lymphocyte 10^9/L [2.5–7.5]	0.0	11.2	1.9	12.3	1.9	12.3
Monocyte 10^9/L [0.0–0.8]	0.0	0.9	0.1	1.1	0.1	1.1
Eosinophil 10^9/L [0.0–2.4]	0.0	1.0	0.0	0.9	0.0	0.9
Basophil 10^9/L [0.0–2.2]	0.0	0.3	0.0	0.3	0.0	0.2
Reference interval for BLV-negative cows (n=75)						
Leukocyte 10^9/L [4.0–12.0]	3.6	10.7	4.2	11.3	4.2	11.3
Lymphocyte 10^9/L [2.5–7.5]	1.0	5.9	1.9	6.9	1.9	6.8
Basophil 10^9/L [0.0–2.2]	0.0	0.3	0.0	0.1	0.0	0.1

Based on BRM, NPM appears more adequate for the determination of reference intervals. On the opposite to BLV status, farms of origin and DMI categories do not seem important confounding factors when establishing reference interval. As recently reported, reference intervals have changed over the time when comparing to those established in 1965.

ABSTRACT #133
CORRELATION OF SERUM CARDIAC TROPONIN I AND MYOCARDIAL DAMAGE IN CATTLE WITH MONENSIN TOXICOSIS. A Varga[1,2], KE Schober[2], CH Holloman[2], PC Stromberg[2], J Lakritz[2], DM Rings[2]. [1]William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA. [2]The Ohio State University, Columbus, OH.

Cardiac troponin I (cTnI) is a sensitive and specific biomarker of myocardial injury in people and small animals. Little is known about the diagnostic use of cTnI in cattle. We hypothesized that serum cTnI correlates to myocardial function and histopathologic lesions in cattle with monensin-induced myocardial injury.

Ten cows received one dose of monensin orally; 30 mg/kg ($n=1$) or 40 mg/kg BW ($n=1$) (Group A) or 50 mg/kg monensin ($n=8$, Group B). Repeated measurements of serum cTnI and echocardiographic variables were performed until study termination at 80 (Group A) and 144 hours (Group B) post dosing. Semi-quantitative histopathologic examinations of the heart (90 fields in 9 target areas of the myocardium) were performed in each cow. A scoring system with regard to the magnitude of myocardial injury was established and the total heart score per cow was compared to maximum cTnI concentration measured after monensin administration. Five hearts from healthy cows served as controls.

Increased cTnI concentration (>0.07 ng/ml) was found in 9/10 cows. cTnI was significantly associated with left ventricular shortening fraction ($r^2=0.51$, $p=0.02$) and myocardial histopathologic lesion score ($r^2=0.49$; $p=0.021$). All cows (n=7) with evidence of myocardial necrosis had a cTnI concentration ≥ 1.04 ng/ml.

cTnI is related to myocardial necrosis and severity of myocardial damage in cattle with monensin toxicosis. cTnI may become a useful diagnostic tool in the non-invasive assessment of myocardial injury in cattle with naturally-occurring cardiac disease.

ABSTRACT #134
VALIDATION OF THE I-STAT® STALLSIDE IMMUNOASSAY FOR THE MEASUREMENT OF BOVINE CARDIAC TROPONIN I AND ITS CLINICAL RELEVANCE. A Varga, JA Angelos, JM Cornish, M Chigerwe. William R Pritchard Veterinary Medical Teaching Hospital, University of California, Davis, CA.

Measurement of cardiac troponin I (cTnI) is the reference method for non-invasive diagnosis of myocardial injury in humans and small animals. Increased circulating cTnI concentrations also have been associated with non-cardiac diseases. The objective of this study was to validate the I-STAT analyzer for the detection of bovine serum cTnI and to measure cTnI levels in cattle with cardiac and non –cardiac diseases.

Purified bovine cTnI was diluted in cTnI-free serum from a healthy cow. Precision, linearity and recovery were assessed using the I-STAT (HeskaCorporation,CO) immunoassay. At concentrations 0.5, 1, 2, 8 and 32 ng/ml the mean intra-assay precision was 11±6.1% and mean inter-assay precision 13±4.9% (coefficient of variation). The assay demonstrated good linearity of serial dilutions from a concentration of 2 to 32 ng/ml and mean cTnI recovery was 100.5±26.7%. Thirty healthy cattle had serum cTnI concentrations between 0 and 0.01 ng/ml.

In our study cows with non-cardiac primary diseases such as left displaced abomasum, had cTnI concentrations$\leq$0.29 ng/ml, while cows with cardiac diseases such as endocarditis, had significantly higher cTnI concentrations.

These preliminary data suggest that the I-STAT immunoassay has sufficient test performance to detect bovine serum cTnI concentrations. Cattle with myocardial diseases have higher cTnI concentrations as compared to cows without primary cardiac diseases, but those concentrations are still significantly higher than in healthy cattle. Further studies are required to evaluate the relationship between cTnI concentration and clinical outcomes in cattle.

ABSTRACT #135
RGD-CONJUGATED HELICAL ROSETTE NANOTUBES INHIBIT CHEMOTAXIS AND CELL SIGNALING OF CATTLE NEUTROPHILS. LMH Anh[1], H Fenniri[2], SS Suri[1] and B Singh[1]. [1]Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada. [2]National Institute for Nanotechnology, Edmonton, Canada.

Bovine respiratory disease complex is the most common disease that causes significant economic loss, typically in feedlot cattle. Current treatment methods are focused on reducing inflammatory responses, control of airway reactivity and improvement of pulmonary functions without potential side effects. Neutrophils are the key contributors in acute lung inflammation. However, activated neutrophils live longer and cause excessive tissue damage upon migration into lungs. Therefore, modulation of their migration and lifespan are attractive approaches in treatment strategies of bovine respiratory disease. Nanotechnology holds significant potential to design new compounds by our ability to manipulate at the nanoscale. We used helical rosette nanotubes, which are a class of novel, biologically inspired, water soluble and metal-free tubes. We used helical rosette nanotubes (RNT) conjugated to arginine-glycine-aspartic acid (RGD-RNT) to study their effects on neutrophil chemotaxis and cell signaling. Bovine neutrophils exposed to 5% RGD-RNT reduced their migration in response to fMLP ((formyl-Methionyl-Leucyl-Phenylalanine), compared to the non-treated group ($P<0.001$). This inhibitory effect was the same as that of groups treated with ERK1/2 inhibitor (UO126) and p38 mitogen-activated protein kinase (p38 MAPK) inhibitor (SB239063). In addition, the phosphorylated ERK1/2 and p38 MAPK for the first time were quantified by sandwich ELISA to elucidate the mechanism of neutrophil migration. The phosphorylation of both the ERK1/2 and p38 was inhibited at 5 minutes by RGD-rosette nanotubes ($P<0.05$). Furthermore, integrin $\alpha v\beta 3$ is possibly involved in migration of bovine neutrophils. These experiments provide the first evidence that RGD-rosette nanotubes suppress phosphorylation of ERK1/2 and p38 MAPK and inhibit chemotaxis of bovine neutrophils.

ABSTRACT #136
EXPERIMENTAL INFECTION OF COLOSTRUM-DEPRIVED CALVES WITH BOVINE VIRAL DIARRHEA VIRUS TYPE-1 ISOLATED FROM FREE-RANGING WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*). EA Raizman, RM Pogranichniy, M Lévy, M Negron, WG Van Alstine. School of Veterinary Medicine, Purdue University, West Lafayette, IN.

Bovine viral diarrhea virus (BVDV) is a pestivirus associated with important economic losses to the cattle industry in the United States. The role of wildlife as a reservoir for the virus is unclear. The objective of this study was to determine if calves could be experimentally infected with a BVD type 1 virus isolated from free ranging White-tailed deer (WTD), develop lesions and shed the virus.

Twelve colostrum-deprived male Holstein calves (8 infected and 4 controls) were used in this study. Both groups were housed in an isolation unit. After a 7 day period of acclimation, the experimental group was challenged intranasally with a 2 ml inoculum containing $10^6 TCID_{50}$/ml BVDV type 1 isolated from free ranging WTD in Indiana, while the control group was inoculated with a virus-free culture medium. Daily physical examinations were performed. Serology for BVD (virus neutralization and ELISA) was performed on days −5, 0, 3, 7, 10, 14 and 21 post inoculation (DPI). Whole blood, saliva, nasal and rectal swabs were collected on days 0, 3, 7, 10, 14 and 21 DPI for virus isolation, virus neutralization, ELISA and Real Time PCR (RT-PCR) assays. On day 14 DPI, 4 calves from the infected group and 2 controls were humanely euthanized; multiple tissue samples were collected for histopathology. The same protocol was used on day 21 for the remaining 4 infected and 2 control calves. The experimental protocol was approved by the Purdue Animal Care and Use Committee.

Several calves develop intermittent diarrhea before inoculation with BVDV. The most important microscopic lesions were thymic atrophy and lymphoid depletion of the Peyer's patches in all 8 infected calves. No significant lesions were identified in the control calves. RT-PCR was positive in the buffy coat of 8/8, in the nasal samples of 7/8 and in the saliva samples of 2/8 infected calves. The rectal samples were negative at all times for the 12 calves. On virus

neutralization assay, 4/8 animals developed antibodies against BVDV and on ELISA assay 3/8 animals were positive for BVDV. All samples from the control calves remained negative.

These results support our hypothesis that colostrum-deprived calves can be successfully infected with a BVDV type 1 isolated from WTD and that infection results in the development of typical lesions in lymphoid tissues. Furthermore, positive identification of BVDV in nasal and saliva samples suggests that shedding of the virus in the environment is possible.

ABSTRACT #137
EVIDENCE FOR TRANSPLACENTAL PASSAGE OF BLUTONGUE VIRUS SEROTYPE 8 IN SHEEP. Kirschvink N.[1], Bolkaerts B.[1], Baricalla C.[1], Wiggers L.[1], de Leeuw I.[2], Vandenbussche F.[2], De Clercq K.,[2] Saegerman C.[3] [1]Animal Physiology, Veterinary Department, University of Namur, Belgium; [2]Veterinary and Agrochemical Research Centre, Department of Virology, Brussels, Belgium; [3]Epidemiology and Risk Analysis applied to Veterinary Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium.

Serotype 8 of bluetongue virus (BTV8) emerged in summer 2006 in Northern Europe and strongly spread in 2007. Transplacental passage of the BTV8 has recently been documented in naturally infected cattle, but not yet in sheep. This study aimed at assessing within one sheep flock whether a natural BTV8 infection passes the placental barrier.

309 pregnant ewes belonging to the flock of Namur University were followed since summer 2007 and pregnancy diagnosis, which was performed by ultrasonography 6–8 weeks after conception. 185 ewes had developed clinical signs of BTV8 during or shortly prior to gestation in summer 2007. Lambing occurred at 4 periods: November 2007 (70 ewes, 121 living lambs, 16 aborted fetuses), January 2008 (40 ewes, 65 living lambs, 0 aborted fetuses), March 2008 (141 ewes, 215 living lambs, 8 aborted fetuses) and May 2008 (54 ewes, 74 living lambs, 2 aborted fetuses). At lambing, blood was sampled and serum was prepared from all ewes and all lambs prior colostrum intake in order to perform BTV antibody detection (ELISA, Id-Vet France). At the age of 2–4 weeks red blood cells (RBC) were sampled from lambs when they were BTV-antibody positive at birth. Abortions occurring during the last 3 weeks of gestation were recorded. Aborted lambs underwent necropsy and if possible, their spleen was sampled. RBC and spleen were analyzed by RTqPCR for BTV8 detection.

All ewes were BTV-positive (ELISA) at lambing and 9 lambs had BTV antibodies at birth before colostrum intake. The RBC of 7 of those lambs were also positive for BTV8 by RTqPCR. The seven ELISA and RTqPCR positive lambs were all born in November 2007, 1 lamb born in January 2008 and 1 lamb born in March 2008 had BTV antibodies but were RTqPCR negative. Lambs born in May 2008 were all negative for BTV antibodies. Among the 26 aborted animals, the fetuses were most often in an advanced state of decomposition. Only 10 spleens could be analyzed by RTqPCR. 4 samples from fetuses aborted in November 2007 and March 2008 were positive for BTV8, the only 2 samples from abortions in May 2008 were negative, other samples did not provide conclusive results.

These data provide evidence that BTV8 passes the placental barrier in sheep. It has to be determined to what extent this way of virus transmission contributes to the overwintering phenomenon of BTV8.

ABSTRACT #138
LONG TERM FOLLOW-UP OF RAMS' SEMEN QUALITY AFTER NATURAL INFECTION WITH BLUETONGUE VIRUS SEROTYPE 8. Saegerman C.[1], Bolkaerts B.[2], Raes M.[2], Kirschvink N.[2] [1]Epidemiology and Risk Analysis applied to Veterinary Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium; [2]Animal Physiology, Veterinary Department, University of Namur, Belgium.

Bluetongue virus (BTV) is reported to induce transient infertility in ruminants. Serotype 8 of BTV emerged in 2006 in Northern Europe and infected ~90% of the Belgian sheep population in 2007. Whilst recovery of normal semen quality in BTV8-infected rams occurs within 2–4 months after infection (Kirschvink et al., 2007; Vet. J), long term follow up of naturally infected rams has not yet been described. The aim of this field study was to assess in 2008 the semen quality of rams that underwent a natural BTV8 infection in 2007 and whose semen quality had been monitored in 2007 and 2008.

11 healthy rams tested prior to the BTV8 outbreak in Belgium were used as controls (C) and 20 rams with natural infection by BTV8 in 2007 were included in this study. BTV-infected rams were tested in 2007 between 5 days and 2 months after development of clinical signs of disease (Clinical BTV8) as well as 6 to 12 months later (BTV8 recovery). Semen was evaluated for motility (score ranging from 0–5), spermatozoa concentration and viability (% of normal living [NL]; normal death [ND]; abnormal death [AD] spermatozoa).

Results are shown as mean±SD.

	Controls (n=11)	Clinical BTV8 in 2007 (n=20)	BTV8 recovery in 2008 (n=20)
Motility score	4.5±0.2	1.2±1.5*	4.3±0.4$
Conc (10^9 sperm/ml)	3.2±0.7	1.2±1.1*	3.2±0.6$
% NL spermatozoa	75±6	21±27 *	75±9$
% ND spermatozoa	21±7	49±20*	23±11$
% AD spermatozoa	4±3	30±18*	4±4$

*significantly different from respective control-value;

$significantly different from respective Clinical BTV8-value; $p<0.05$

These results show (1) an important impact of serotype 8 BTV on rams' semen quality within 5 days and 3 months after development of clinical disease and (2) that BTV8-infected rams show a fully recovered normal semen quality within 6 to 12 month after infection.

ABSTRACT #139
PREVALENCE OF BVDV ON ALPACA FARMS IN EASTERN AND MIDDLE TENNESSEE. TM Buchheit, SR Van Amstel, KV Thomas, M Abd-Eldaim. The University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

The purpose of this study was to survey the seroprevalence to BVDV and possible presence of persistently infected (PI) animals in alpaca herds. Blood was collected from 244 alpacas in 5 herds with a negative BVDV vaccination history. All animals in each herd were tested for the presence of antibodies to BVDV by use of immunofluorescence assay (IFA) and for the presence of viral RNA by conventional polymerase chain reaction assay using reverse transcriptase RT-PCR. Overall, antibodies to BVDV were detected in 1.6% (4/244) of the samples which is similar to most other BVDV prevalence studies. Two herds had at least 1 BVDV-positive animal with a titer $\geq$1:80, with 3 of the 4 seropositive alpacas from the same herd (Farm 1). All 244 samples were found virus-negative by PCR. No animals younger than 2 years of age were found to be seropositive and none of the BVDV-positive animals were pregnant at the time of testing. Only one of the herds tested had cattle on the farm; however, none of the alpacas in this herd tested BVDV-positive. No suspected PI animals were identified. The presence of 3 seropositive animals on Farm 1 may be due to unrestricted comingling of animals. In conclusion, although no animals with viremia were identified in the area, BVDV should still be considered as a cause of recurring illness, ill-thrift, low birth-weight or premature crias and abortion.

ABSTRACT #140
SEROPREVALENCE OF BOVINE VIRAL DIARRHEA IN NEW WORLD CAMELIDS IN SWITZERLAND AND IMPLICATIONS FOR THE ERADICATION CAMPAIGN IN THE BOVINE POPULATION. M. Mudry[1], M. Meylan[1], R. Zanoni[1], G. Schüpbach Regula[2], P. Zanolari[1]. [1]Vetsuisse Faculty, University of Berne. [2]Swiss Veterinary Office, Berne, Switzerland.

Infection with the Bovine Viral Diarrhea Virus (BVDV) is common in the Swiss cattle population, with seroprevalence rates of 60–90% and 0.6% of persistently infected (PI) animals. The economic

losses resulting from BVDV infection (estimated at 4–6 Mio US$/year for a cattle population of 1.5 Mio) prompted the decision to eradicate the disease in Switzerland. The entire Swiss bovine population has been tested since the fall of 2008 and all calves are currently tested immediately after birth, PI animals are culled. The susceptibility of New World Camelids (NWC) to BVDV infection and their role in the epidemiology of the disease have not been clearly defined to date, seroprevalence rates of 2 to 11% in NWC can be found in the literature. As no data were available for Switzerland prior to the start of the eradication campaign, a study was undertaken to evaluate the seroprevalence of BVD in the Swiss NWC population.

Blood specimens were collected during the summer of 2008 from 349 NWC from 40 herds, a representative sample of the Swiss population. Information was obtained, among others, about possible contact of the NWC with other ruminants, especially with cattle. The serum samples were analyzed for antibodies to BVDV using an ELISA method (Institute of Veterinary Virology, University of Berne). For comparative purposes, 250 serum samples collected in 40 herds in 2000 and representative of the Swiss NWC population at that time were analyzed with the same serological method.

The individual seroprevalence rates were 3.6% (9 animals out of 250) in 2000 and 5.7% (20 animals out of 349) in 2008. Similarly, herd prevalence was higher in 2008 (30%) than in 2000 (20%). Seropositive animals were found in all regions of the country. Contact with cattle was possible in 42% of the herds with at least one seropositive animal whereas this was the case in 24% of herds without seropositive NWC.

The seroprevalence of BVD in the Swiss NWC population was higher than the 2–3% expected based on the fact that cattle and NWC are rarely kept in close contact to each other in Switzerland, even in herds where both species are present. The apparent seroprevalence rate increased from 3.6% to 5.7% between 2000 and 2008. Whether NWC only seroconvert after contact with the virus or if they serve as a reservoir by circulating the virus within the camelid population or through PI animals needs now to be determined. The possible persistence of BVDV in the NWC population is highly relevant in view of the efforts undertaken to eradicate the infection in cattle.

ABSTRACT #141

TUBERCULOSIS CAUSED BY *MYCOBACTERIUM MICROTI* IN NEW WORLD CAMELIDS

P. Zanolari[1], N. Robert[1], G. Pfyffer[2], K. Lyashchenko[3], M. Meylan[1]. [1]Vetsuisse Faculty, University of Berne, Berne, Switzerland. [2]Department of Medical Microbiology, Luzern, Switzerland. [3]Chembio Diagnostic Systems, Medford, NY.

Tuberculosis is one of the world's leading infectious causes of death among humans, with one third of the world's population currently being infected with *Mycobacterium tuberculosis*. In ruminants, tuberculosis is mainly caused by *M. bovis* and rarely by *M. tuberculosis*. The disease is significant not only because of economic losses but also because of its zoonotic potential. Infection with *Mycobacterium microti,* a member of the *M. tuberculosis* complex, has been reported in several species, including humans. The goal of the present study was to describe the clinical and pathological findings in 11 New World Camelids (NWC) infected with *Mycobacterium microti*.

The records of 11 animals (10 llamas and 1 alpaca, aged 4 to 18 years) from 6 different herds with a history of wasting and weakness admitted to the Clinic for Ruminants and/or the Institute for Animal Pathology within a timeframe of 7 years (2001–2008) were reviewed, and the clinical findings, clinicopathologic abnormalities, diagnostic imaging findings and necropsy results were described.

Clinical signs were limited to weight loss, recumbency and anorexia in the late stages of the disease. Two thirds of the animals showed increased breathing effort or respiratory distress. Palpable lymph nodes were enlarged in 4 animals. No consistent hematologic abnormalities were identified. The comparative intradermal tuberculin test with bovine protein purified derivate (PPD) and avian PPD was negative in all animals. Abdominal and/or thoracic ultrasound and thorax radiology revealed abnormal findings such as enlarged mediastinal, mesenteric and/or hepatic lymph nodes only in advanced cases. The presence of granulomatous lesions in various lymph nodes, in the lungs, pleura, mediastinum, peritoneum and mesenterium, liver, spleen, pancreas and/or kidneys was confirmed at necropsy in all animals. Infection with *M. microti* was confirmed by bacteriological culture and/or spoligotyping.

An infection caused by *M. microti* should be considered as a differential diagnosis in chronic debilitating diseases with or without respiratory signs in NWC. However, the clinical diagnosis remains challenging, particularly in the early stages of the infection. As cases of *M. microti* infection have been reported in immunocompromized human patients, the zoonotic potential of the organism should be kept in mind when dealing with the disease in NWC and the owners of affected animals should be informed about the potential health hazard related to clinical and subclinical infection in NWC.

Previously presented at World Buiatrics Congress, Budapest 2008.

ABSTRACT #142

PHARMACOKINETICS OF INTRAVENOUS ENROFLOXACIN AND ITS ACTIVE METABOLITE CIPROFLOXACIN IN HOSPITALIZED HORSES

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Enrofloxacin is a fluoroquinolone antibiotic with excellent activity against gram-negative enteric bacteria, as well as some staphylococci, and *Mycoplasma, Chlamydia,* and *Rickettsia* spp. Enrofloxacin is metabolized to ciprofloxacin, which is believed to produce an additive effect on antimicrobial activity. The pharmacokinetics of enrofloxacin administered via various routes of administration have been reported in multiple studies using healthy adult horses. However, there are no reports of the pharmacokinetics of this drug when used in sick, hospitalized horses that are frequently on multiple medications and may have hepatic or renal compromise. The purpose of this study is to examine the pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in hospitalized horses using a population sampling method.

Data from nineteen horses was available for analysis. Horses were being treated for post-operative colic (n=5), other gastrointestinal diseases (n=5), liver disease (n=4), musculoskeletal diseases (n=4), and pneumonia (n=1). Heparinized plasma samples were collected from horses after 3 or more doses of 5 mg/kg intravenous (IV) enrofloxacin once daily, to approximate steady state concentrations. Horses were assigned to a sampling schedule so that each horse had blood drawn at 5 time points for analysis. Samples were frozen at −70 °C until analysis by high pressure liquid chromatography with fluorescence detection. Data was analyzed using a naïve pooled approach with noncompartmental modelling.

Following intravenous administration, enrofloxacin had a mean±SD clearance of 2.14±1.62 mL/kg/min, and a plasma half-life ($t_{1/2}$) of 9.55±5.14 hr. This represents a slower clearance and a longer half-life compared to other reports of IV enrofloxacin in horses. The volume of distribution was 1.25±0.34 L/kg, which is lower than other reported values. The combined total area under the curve (AUC) for enrofloxacin and ciprofloxacin was 71.2±48.5 hr*ug/mL and ciprofloxacin represented 12.4±8.3% of the total AUC. In the subset of horses with liver disease, t 1/2 and clearance of enrofloxacin was 12.3±6.4 hr and 1.5±1.3 mL/kg/min, respectively, compared to 8.7±4.7 hr and 2.3±1.7 mL/kg/min, respectively, in horses without liver disease. These differences were not statistically significant ($P > 0.05$).

The pharmacokinetic-pharmacodynamic indicator of success for fluoroquinolone antibiotics is based on a ratio of the plasma AUC to the minimum inhibitory concentration (MIC) of the bacteria of ≥100–125. Based on the results of this study and this AUC:MIC ratio, enrofloxacin administered at 5 mg/kg IV q24 h in hospitalized horses should be successful in treating bacteria with a MIC of≤0.5 μg/mL.

ABSTRACT #143

LAMINAR LEUKOCYTE ACCUMULATION AND EPITHELIAL STRESS IN HORSES WITH CARBOHYDRATE OVERLOAD-INDUCED LAMINITIS

RR Faleiros[1,2], GJ Nuovo[1], PJ Johnson[3], SJ Black[4], JK Belknap[1]. [1]Ohio State University, Columbus, Ohio.[2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil. [3]University of Missouri, Columbia, MO. [4]University of Massachusetts, Amherst, MA.

Laminar failure in laminitis results from a dysadhesion of the basal laminar epithelium from the underlying basement membrane.

We recently reported prominent laminar leukocyte infiltration prior to the onset of laminar epithelial stress in the developmental stage in the Black Walnut Extract (BWE) laminitis model. The purpose of this study was to characterize laminar leukocyte populations and the temporal relationship of leukocyte infiltration to laminar epithelial stress in the CHO model of laminitis, a model that more commonly results in laminar failure observed in clinical laminitis cases. Immunohistochemistry for calprotectin (CP, marker for neutrophils, classically activated macrophages, and stressed [not normal] keratinocytes) and CD163 (antibody specific for resident macrophages and monocytes in alternatively activated phenotype) was performed on laminar paraffin sections obtained: 24 h following water administration (CON, n=8), and at onset of fever (10–20 hours, DTP group, n=6) and at the onset of lameness (20–46 hours, LAM group, n=6) following CHO administration. Minor histologic changes (slightly abnormal PAS staining of basement membrane [BM]) were present in 3/6 horses in the LAM group. Similar to the BWE model, laminar epithelial stress (increased CP signal) occurred at the onset of lameness. Increases ($P<0.05$) in laminar CP+ leukocytes and CD163+ monocytes/macrophages occurred in the LAM group (not DTP group) compared to CON group. The temporal pattern of laminar leukocyte infiltration and epithelial stress is markedly different in the two models, with leukocyte infiltration preceding epithelial stress in the BWE model, and both events occurring simultaneously in the CHO model. Importantly, the marked leukocyte infiltration in the CHO model prior to severe histologic changes in this model indicates that, in contradistinction to previous reports, leukocyte infiltration may be a cause of and not a reaction to BM degradation and structural failure.

ABSTRACT #144

THE EFFECT OF TRACHEAL LAVAGE ADMINISTRATION ON LUNG FUNCTION IN HEALTHY KITTENS. EA Rozanski, G Buckley, MR Mazan, D Bedenice, AH Hoffman. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

The purpose of this study was to evaluate the effect of tracheal lavage on lung function in healthy kittens. While tracheal wash sampling for evaluation of lung cytology and bacterial culture is considered routine, there are reports of respiratory embarrassment or failure and even death following this procedure in cats. Seven healthy kittens were recruited from consenting owners for study enrollment. The study was performed prior to routine castration or ovariohysterectomy. All kittens were healthy on examination. Cats were pre-medicated with ketamine (7 mg/kg mg), butorphanol (0.75 mg/kg), and diazepam (0.3 mg/kg) intramuscularly. Anesthesia was induced with propofol 2–4 mg/kg intravenously to effect. Cats were intubated with a standard 3.5 mm ID sterile endotracheal tube and ventilated with a 10 ml/kg tidal volume at 15 breaths/min and 30% inspired oxygen, using a Puritan Bennett 840 critical care ventilator. A standard volume history was created by 2 breaths to total lung capacity (25 cmH20) prior to testing. Using commercial software, static compliance (Cstat) and plateau pressure (Pplat) were measured. Additionally, cats' oxygenation was assessed via pulse oximetry. All animals underwent a standard tracheal lavage with 3 aliquots of 2 ml/kg sterile saline, followed by measurements. Repeated measures ANOVA was used to compared the effect of lavage. Tracheal lavage caused a significant decrease in Cstat, significant increase in Pplat and desaturation.

	Cstat (mean +/SD)	Pplat	Pulse oximetry
Baseline	6±1.1 ml/cmH20	6.1±0.8 cmH20	99±1 %
Post lavage	2.9±0.9 ml/cmH20 [a]	9.6±2.1 cmH20[a]	93±4%[b]

p=0.001 compared to baseline, b=p=0.01 compared to baseline.

The results of this study demonstrated that tracheal lavage in healthy cats is associated with an acute 'restrictive effect' on lung function most likely associated with airway closure or derecruitment (atelectasis) as the likely causes of mechanical dysfunction and hypoxemia.

ABSTRACT #145

UTILITY OF BIOMARKERS IN BRONCHOALVEOLAR LAVAGE FLUID FOR DISCRIMINATION OF ASTHMA AND CHRONIC BRONCHITIS IN CATS. LA Nafe, AE DeClue, TM Lee-Fowler, JE Eberhardt, CR Reinero. University of Missouri, College of Veterinary Medicine, Columbia, MO.

Feline allergic asthma and chronic bronchitis are lower airway diseases that are often grouped together as one syndrome. We postulate that as in humans, the molecular mechanisms which trigger and sustain these diseases are not identical and that differences in biomarker signatures in bronchoalveolar lavage fluid (BALF) exist. The study objective was to compare IL-4, IFN-γ, TNF-α and total nitric oxide (NO) in BALF from cats with naturally developing asthma (CLIN ASM) and chronic bronchitis (CLIN CB) and further compare them to research cats with experimentally induced asthma (EXP ASM), non-septic suppurative inflammation (RES SUPP) and healthy controls (RES NORM).

Ninety-seven cats were divided into five groups [CLIN ASM (n=13), CLIN CB (n=8), EXP ASM (n=23), RES SUPP (n=33), RES NORM (n=20)] based on predefined BALF cytological criteria. Unconcentrated BALF supernatant was assayed for IL-4 and IFN-γ concentrations, TNF-α bioactivity and total NO. Statistical analysis was performed using a one-way ANOVA and *post-hoc* Tukey test, with a $p<0.05$ considered significant.

Concentrations of BALF IL-4 and IFN-γ were below the limits of detection for most cats, precluding statistical analysis. No significant differences were detected between groups for TNF-α bioactivity. The group mean±SEM of BALF NO was significantly higher in CLIN CB (7.9±0.6 μM) compared to RES SUPP (5.0±0.4 μM; $P=0.002$) and EXP ASM cats (4.6±0.2 μM; $P=0.001$).

Using the currently available relatively insensitive methodologies, none of the measured cytokines or NO are useful for discriminating local immune changes in the airways of pet cats with naturally developing asthma versus chronic bronchitis.

Previously presented at ECVIM in Belgium, September 2008.

ABSTRACT #146

COMPARISON OF INTRADERMAL SKIN TESTING AND ALLERGEN-SPECIFIC SERUM IMMUNOGLOBULIN E (IgE) IN EXPERIMENTAL FELINE ASTHMA. TM Lee-Fowler[1], LA Cohn[1], AE DeClue[1], CM Spinka[2], RD Ellebracht[2], CR Reinero[1]. [1]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, [2]Department of Statistics, College of Arts and Sciences, University of Missouri, Columbia, MO.

Reliably identifying clinically relevant allergen(s) for allergen-specific immunotherapy as a treatment for feline allergic asthma may be challenging. Poor correlation has been found between intradermal skin testing (IDST) and serum allergen-specific IgE; however, these studies evaluated atopic pet cats with unknown type(s), duration, timing and quantity of allergen exposure. We proposed to control these factors by using a feline model of allergic asthma induced by specific allergens to compare IDST and serum IgE tests. We hypothesized that IDST results would more closely correlate with the known allergen exposure than would allergen-specific serum IgE determination.

Thirteen cats were randomly assigned to saline (placebo), bermuda grass allergen (BGA), or house dust mite allergen (HDMA) groups for sensitization and challenge. On day 0 (prior to sensitization) and days 28 and 50 (post-allergen sensitization) IDST was performed and serum collected. Serum from each cat at each time point as well as pooled serum samples (BGA or HDMA) had determination of allergen-specific IgE using an Fcepsilon receptor based ELISA (Heska, CO); pooled samples were also analyzed using an enzymoimmunometric assay (VARL, CA). Half of each pooled sample was heat inactivated (HI) to selectively destroy IgE. Sensitivity (SE), specificity (SP), and positive and negative predictive values (PPV and NPV, respectively) were calculated. Prausnitz-Kustner (PK) testing using the pooled and pooled HI samples was performed.

Combined results for IDST found SE=90.9%, SP=86.7%, PPV=83.3%, and NPV=92.9%. For ELISA-based serum IgE testing, the SE=22.7%, SP=100%, PPV=100% and NPV=63.8%. The enzymoimmunometric assay did not detect sensitizing IgE, but

did detect IgE reactivity to a variety of irrelevant allergens (even in HI samples). Results from the heat inactivated samples (ELISA) and PK test suggest either incomplete destruction of IgE or the presence of homocytotropic IgG. In conclusion, both IDST and allergen-specific IgE determination via ELISA were specific; either test can be used to guide selection of allergens for immunotherapy. The enzymoimmunometric assay was unreliable and can not be recommended.

Previously presented at ECVIM in Belgium, September 2008.

ABSTRACT #147

CHRONIC ADMINISTRATION OF feG-COOH IN EXPERIMENTAL FELINE ASTHMA. JM Eberhardt, AE DeClue, CR Reinero. University of Missouri, College of Veterinary Medicine, Columbia, MO.

Previously we demonstrated that a single dose of the immunomodulating tripeptide, feG-COOH, administered *prior* to allergen challenge partially dampens eosinophilic airway inflammation in experimental feline asthma. We hypothesized that chronic (2 weeks) administration of feG-COOH would decrease clinical signs and airway inflammation in experimentally asthmatic cats. Asthma was induced in 9 cats using Bermuda grass allergen (BGA). Cats were randomized to receive oral feG-COOH (1 mg/kg, q 24 h) or placebo (saline q 24 h) for 2 weeks followed by a 2 week washout before crossover to the other treatment. A clinical scoring system was employed to grade clinical signs after allergen challenge. Bronchoalveolar lavage fluid (BALF) and blood were collected 24 hours after allergen challenge. Differential cell counts were performed on BALF. Peripheral blood mononuclear cells (PBMCs) were cultured in vitro with BGA. Supernatant from BALF and PBMCs and plasma was harvested for determination of IL-4 and IFN-γ concentrations using commercial ELISAs or TNF-α bioactivity using a cell killing bioassay. Repeated measures ANOVA was used to compare the treatment groups with a $p<0.05$ considered significant.

There was no significant difference between treatments in clinical scores (mean$\pm$SD: feG 1.0$\pm$1.1; placebo 1.2$\pm$1.1, p=0.386), % BALF eosinophils (feG 33$\pm$18%, placebo 29$\pm$16%, p=0.479) or neutrophils (feG, 4.7$\pm$4.7%, placebo, 5.0$\pm$4.5%; p=0.504). No differences in cytokine concentrations were detected between treatments; however many samples were below the assay's lower limit of detection. In conclusion, chronic administration of feG did not significantly decrease clinical signs or airway inflammation in cats with experimentally-induced asthma.

ABSTRACT #148

PROGRESSION OF CIRCULATING ANTIGEN AND ANTIBODY RESPONSES IN CATS EXPERIMENTALLY INFECTED WITH *DIROFILARIA IMMITIS*. K Curtis[1], L Lorentzen[1] R Chandrashekar[1], J McCall[2], DM Tillson[3], AR Dillon[3]. [1]IDEXX Laboratories, Westbrook, ME. [2]TRS Labs, Athens, GA. [3]College of Veterinary Medicine, Auburn University, Auburn, AL.

Cats can suffer complications from adult heartworm infections (*Dirofilaria immitis*) and emerging evidence demonstrates that immature adult heartworms, reaching the pulmonary arteries at approximately 2.5–4 months post-infection, can also cause significant lung pathology (Heartworm Associated respiratory Disease or HARD). To better understand the humoral immune response to *D. immitis*, 12 cats were infected by SC injection of 100 third stage (L_3) larvae per cat. Pre- and post-infection serum samples were collected from each cat at selected time points (days -1, 7, 14, 21, 28, 42, 56, 68, 75, 84, 112, 140, 168, 189, 217, 245 and terminal). The cats were necropsied 278–299 days post-infection and adult worms were recovered in 11 of 12 cats. Other imaging and histopathology data were collected.

Temporal serum samples obtained from each cat were tested for antibodies to two *D. immitis* recombinant antigens, HWAg-1 and HWAg-2. In addition, samples were tested for circulating antigenemia (polycolonal assay) using PetChek® HTWM PF Test Kit (IDEXX Laboratories). Eleven of 12 cats were HWAg-1 antibody positive 68 days post-infection and the remaining cat became HWAg-1 antibody positive on day 140 post-infection. 11 of 12 cats remained HWAg-1 antibody positive through end of the study. All but one cat was positive for HWAg-2 antibody, with the first positive tests occurring between 42–84 days post-infection. Ten of the 11 HWAg-2 antibody positive cats remained positive during the study except one cat that became negative by day 271. Both antibody assays became positive during the pre-cardiac stages of the heartworm life cycle. Circulating antigen was detected in 10 of the 12 cats and 1 cat died at day 140 with heartworms. Of the remaining 9 positive cats, 2 were first positive on day 140 (4.7 months) and 5 first positive on day 168 (5.6 months). One cat first became antigen positive on day 217. Using SoloStep® CH Heartworm Test (Heska), antigen was detected in 3 of the cats at day 168.

This study demonstrates that in cats with experimental infections, parasite antigenmia can be detected prior to heartworms reaching sexually maturity. Historically, the antigen has been detected only in the circulation of dogs and cats harboring mature female heartworms, but target antigens have been demonstrated in the cuticle of heartworms. These data suggest that disintegration of immature adult heartworms can result in detectible levels of antigen utilizing a PetChek® HTWM PF Test Kit, and less commonly using SoloStep® CH Heartworm Test. In summary, in this experimental model, *D. immitis* antigen was detectable in some cats at time points associated with dying immature adult heartworms and the initiation of HARD (days 140 and 168).

ABSTRACT #149

ISOMETRIC RESPONSES OF ISOLATED INTRAPULMONARY BRONCHIOLES FROM CATS WITH AND WITHOUT ADULT HEARTWORM INFECTION. AA Wooldridge, AR Dillon, DM Tillson, Q Zhong, S Barney. Auburn University College of Veterinary Medicine, Auburn, AL.

Adult heartworm (HW) infections (*Dirofilaria immitis*) in cats cause bronchiolar pathology resulting in Heartworm Associated Respiratory Disease (HARD). Peribronchiolar smooth muscle (SM) proliferation and increased bronchial wall to lumen ratios are induced by HW infections in cats, but the contribution of bronchiolar SM reactivity to the pathophysiology of HARD is unknown.

To better understand the bronchiolar response to *D. immitis*, 13 purpose-bred cats were utilized. Heartworm infected cats (n=7) received 100 third stage larvae subcutaneously, and 6 cats served as age matched controls. The left caudal lung lobe was collected at necropsy 278–299 days post-infection in heartworm cats. Surrounding lung tissue was removed, and four 2 mm rings of 4th generation intrapulmonary bronchioles (IPB) were mounted on a wire myograph (Danish Myotechnologies) interfaced with a physiograph. Three cycles of pre-contractions to 1μM acetylcholine (ACh) were performed, followed by concentration-response curves to the contractile agonists ACh, histamine (HIS), and 5-hydroxytryptamine (5-HT). To evaluate relaxation, IPB were constricted with 1 μM 5-HT and concentration-response curves were generated to sodium nitroprusside (SNP) and isoproterenol (ISO). Data were analyzed by 2-way analysis of variance, and $p<0.05$ was significant.

In HW cats compared to control cats, relaxation to ISO was significantly reduced at 2 concentrations. HW cats demonstrated significantly reduced responses to ACh for 2 cycles of pre-contractions and at the highest concentration of ACh in the curve. There were no differences between control and HW cats in responses to 5-HT, HIS, or SNP.

The slightly reduced response of IPB in HW cats to ISO may indicate some level of refractoriness to bronchiolar relaxation. These results suggest that despite peribronchiolar SM proliferation and increased bronchial wall to lumen ratios in cats with heartworms, a direct hyper-reactive response of the bronchiolar SM cannot be demonstrated as the major mechanism of HARD.

ABSTRACT #150

MATRIX METALLOPROTEINASE -2 AND -9 IN BRONCHOALVEOLAR LAVAGE FLUID OF DOGS WITH IDIOPATHIC PULMONARY FIBROSIS. HP Heikkilä, MM Rajamäki. Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, interstitial lung disease of unknown etiology that mainly affects West Highland white terriers (WHWTs). Matrix metalloproteinases (MMPs) are proteases participating in pathological processes of different pulmonary diseases. The aim of this study was to investi-

gate the role of gelatinolytic MMP-2 and -9 in bronchoalveolar lavage fluid of WHWTs with IPF.

Seven WHWTs with IPF (mean age 12 years, range 10–13) and 13 clinically healthy beagles (mean age 9 years, range 7–13) were included in the study. IPF was verified by clinical examinations and additionally by histopathology in 5 WHWTs. MMP-2 and -9 activities were analyzed by gelatinase SDS-page zymography, and the zymograms were evaluated for MMP activity with AlphaEaseFC software.

ProMMP-2 and -9 activities were significantly elevated in WHWTs compared with controls. Median proMMP2 was 0.72 (interquartile range 0.64–1.05) in WHWTs vs. 0.23 (0.11–0.43) in controls ($P < .01$). The corresponding figures for median proMMP-9 were 0.39 (0.20–0.48) and 0.05 (0.03–0.06) ($P < .01$). Mean arterial PaO2 was significantly lower in WHWTs than in controls (69.0 ± 15.2 mmHg vs. 92.5 ± 7.6 mmHg, $P < .05$). A trend towards a negative correlation between proMMP2 and PaO2 was detected.

Our results suggest that upregulated MMP-2 and -9 participate in the pathogenesis of canine IPF and that the level of MMP-2 increases during disease progression.

ABSTRACT #151

RECRUITMENT OF PULMONARY INTRAVASCULAR MACROPHAGES IN IMMUNE-MEDIATED HEMOLYTIC ANEMIA IN DOGS. P Hodgson, A Carr and B Singh. Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Canada.

Immune-mediated hemolytic anemia (IMHA) is a devastating disease in which macrophages, complement and antibodies attack the circulating red blood cells leading to their premature destruction. Dogs with IMHA have an increased incidence of pulmonary thromboembolism. The mechanisms of IMHA and the contributions of pulmonary pathophysiology to IMHA-induced mortality are poorly understood and consequently there is no effective treatment and a high mortality rate of 20% to 80%. Pulmonary intravascular macrophages (PIMs) are not normally found in the dog and rat lungs but can be recruited in sepsis in rats. Recruited PIMs promote systemic pathophysiology and mortality. The objective of the experiments was to examine the recruitment of PIMs in dogs with IMHA and in a mouse model of IMHA.

First, we examined lung tissues from dogs ($N=16$) that had died of IMHA and the normal dogs ($N=4$). Lung sections stained with H&E showed large macrophage-like cells in pulmonary microvessels. Lung sections were stained with antibodies against macrophages (MAC387), platelet-endothelial cell adhesion molecule-1 (PECAM-1) and Toll-like receptor-4 (TLR4). While MAC-387 antigen is observed in granulocytes, monocytes and tissue macrophages while PECAM-1 is expressed on the monocytes, neutrophils, platelets and a subpopulation of T-cells and TLR4 is found in peripheral blood leukocytes, macrophages and endothelial cells. Our data showed increased numbers of PIMs along with increased expression of PECAM-1 in airway epithelium in the lungs of IMHA dogs compared to the lungs from normal dogs. The expression of TLR4, which regulates sensitivity to endotoxin, was also increased in macrophages and vascular endothelium in the lungs from the IMHA dogs. Second, we examined recruitment of PIMs in a mouse model of IMHA. The mice were treated with Ter-119, an antimurine red blood cell antibody, which induced significant anemia, splenomegaly and recruitment of PIMs compared to saline-treated mice. These data show recruitment of PIMs and increased expression of inflammatory molecules PECAM-1 and TLR-4 in lungs of IMHA dogs. Furthermore, evidence of recruitment of PIMs in the mice provides us with a model to study the role of PIMs in IMHA-associated pulmonary pathophysiology in dogs.

ABSTRACT #152

CYCLIC AMP ATTENUATES FATTY ACID INDUCED APOPTOSIS IN AN IN VITRO MODEL OF HEPATIC LIPOTOXICITY. K Ponzetti, CRL Webster. Tufts Cummings School of Veterinary Medicine, N Grafton, MA.

In the last several years, it is becoming increasingly evident that lipid accumulation within hepatocytes can lead to hepatic failure. In humans, lipidosis (or steatosis) contributes to the hepatic pathology seen in alcoholic hepatitis and nonalcoholic steatohepatitis (NASH). In cats, a syndrome of acute hepatic failure is accompanied by widespread lipidosis. Free fatty acids such as palpitate and oleate, which are increased in the liver of cats with the hepatic lipidosis syndrome, are important mediators of lipotoxicity. In rodent models, fatty acid sensitizes hepatocytes to apoptotic cell death. The aim of this study was to establish an in vitro model of fatty acid hepatotoxicity and to initiate investigations into potential therapeutic options. Rat hepatocytes were isolated by standard collagenase perfusion and placed in culture. Twenty-four hrs later they were treated with 200 uM palmitate or 300 uM oleate. After 5–6 hrs the cells were fixed and stained with Hoescht for morphologic determination of apoptosis by fluorescence microscopy. Whole cell lysates were prepared from treated hepatocytes and used for immunoblotting for the cleavage fragment of the effector caspase, caspase 3. Some cultures were pre-treated for 1 hr with one of two cAMP analogues either 100 uM chlorophenyl thio-cAMP (CPT-cAMP) or 20 uM of the protein kinase A (PKA) independent cAMP analogue, 4-(4-chloro-phenylthio)-2′-O-methyladenosine-3′-5′-cyclic monophosphate (2-Me-CPT-cAMP) and then processed for morphologic or biochemical assessment of apoptosis. There was a significant increase in apoptosis in palmitate and oleate treated hepatocytes, 2.51 ± 0.38 and 3.31 ± 0.56 fold increase over control, respectively ($p < 0.05$). In addition, immunoblotting revealed an increase in the amount of the cleavage fragment of caspase 3 in palmitate treated cells. Pre-treatment with CPT-cAMP or 2-Me-CPT-cAMP reduced the morphologic evidence of palmitate induced apoptosis by $57 \pm 10\%$ and $57 \pm 8.6\%$ ($p < 0.05$), respectively and ameliorated biochemical evidence of apoptosis. In conclusion, exposure of cultured rat hepatocytes to palmitate or oleate induces apoptosis. Increases in intracellular cAMP prevent fatty acid lipotoxicity in a PKA independent manner.

ABSTRACT #153

CULTURE-INDEPENDENT DETECTION OF BACTERIA IN FELINE INFLAMMATORY LIVER DISEASE. D.C. Twedt[1], S.D. Janeczko[2], K.W. McCord[1], J.L. Dudak[1], J.M. Cullen[3], P. Fisher[3], K.W. Simpson[3]. [1]Colorado State University, Fort Collins, CO, [2]Cornell University, Ithaca, NY, [3]North Carolina State University, Raleigh, NC.

The etiopathogenesis of feline inflammatory liver disease (ILD) is unclear. We sought to determine the presence and distribution of bacteria within the livers of cats with ILD by use of culture-independent methods.

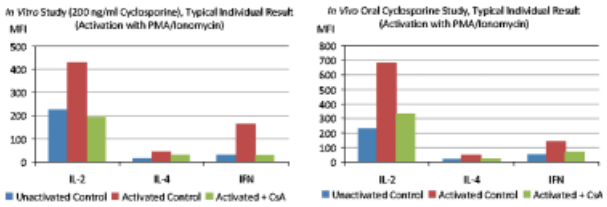
40 archived samples from cats with inflammatory hepatic changes and 14 histologically normal livers were evaluated. Histopathology was classified according to WSAVA guidelines. Unstained hepatic tissue sections were subjected to fluorescence in situ hybridization (FISH) with a 16S rDNA probe that recognizes bacteria in general (EUB338) and a non-EUB probe used as a control. Antibodies against cytokeratins (clones AE1, AE3) and factor VIIIa were used to enable unequivocal distinction between bile ducts and vascular structures respectively. Tissues were examined with a fluorescence microscope for the presence and location of bacteria. Histopathology was classified as non-specific reactive hepatitis (RH,12), neutrophilic cholangitis (NC,10), lymphocytic cholangitis (LC,7), cholestasis/obstruction (CO,6), probable lymphoma (LSA, 3), and neutrophilic hepatitis (NH, 2). Bacteria (EUB338) were observed in sections from 22/40 diseased and 3/14 normal livers (N) ($P < 0.05$). In 11 sections bacteria were restricted to the outer surface of the liver capsule (4RH, 3LC, 2N, 1NC, 1CO) and may represent contaminants. Intrahepatic bacteria were visualized within portal vessels or venous sinusoids of 9 (4NC, 3RH, 1NH, 1LSA, 1N), the bile duct of 1 (NC), and within the hepatic parenchyma near the capsule in 4 (1LC, 1NC, 1NH, 1RH). Bacterial colonization was highest in 3 sections with NC and 1 cat with RH. Concurrent disease, predominately pancreatic and GI disease, was present in 12 of 14 cats with intrahepatic bacteria. Bacterial culture was positive in 12/24 samples (5 *E.coli*, 3 *Enterococcus*, 2 *Staphylococcus*, 1 *Actinomyces*, 1 *Streptococcus*). FISH and culture concurred in 15/24 cases. FISH and culture revealed *E.coli* in 3/3 NC with invasive bacteria. In 3/6 sections with capsular bacteria FISH and culture concurred, and 3 FISH positive samples were culture negative.

The results of this study suggest that bacteria may play a role in the etiology of feline inflammatory liver disease. The localization of intrahepatic bacteria suggests translocation of enteric bacteria is a likely source of infection.

ABSTRACT #154
DEVELOPMENT OF A FLOW CYTOMETRIC PANEL OF T-LYMPHOCYTE BIOMARKERS TO EVALUATE THE IMMUNOSUPPRESSIVE EFFECTS OF CYCLOSPORINE IN DOGS. T Archer, K Lunsford, A Mackin, C Fellman, J Stokes, L Pinchuk, S Pruett, C Langston. Mississippi State University College of Veterinary Medicine, Starkville, MS.

Cyclosporine is a potent immunosuppressive agent used to treat many canine diseases. Optimal dosing regimens remain unclear, primarily because standard methods that monitor effectiveness of immunosuppression have not been established. Our study was designed to establish a comprehensive panel of biomarkers in the dog that could be used to monitor the immunosuppressive effects of drugs such as cyclosporine. For our panel, we used flow cytometry to measure expression of cytokines (IL-2, IL-4, & INF-γ) and surface antigens (CD25 & CD95) on T-lymphocytes activated with concanavalin A and PMA/Ionomycin.

In an initial *in vitro* study, lymphocytes from 3 healthy dogs were incubated for different time periods in varying concentrations of activator and cyclosporine. This study demonstrated significant cyclosporine-associated reduction in biomarker expression: IL-2 expression was reduced by 54%, IL-4 by 34%, and IFN-γ by 81% (maximal suppression 12 hours after exposure to activator), and CD25 was reduced by 25% and CD95 by 18% (2 days after activator exposure). In a subsequent *in vivo* study, biomarkers were evaluated in 2 healthy dogs after one week of cyclosporine PO BID, with dose adjusted to attain published target blood levels, compared to a control dog. Treated animals had decreased expression of IL-2 (reduced by 51% & 42%), IL-4 (33% & 26%), and IFN-γ (51% & 38%), with more variable results for CD25 & CD95.



Our results confirm consistent cyclosporine-induced suppression of selected biomarkers of immunosuppression. Further investigation of the effects of cyclosporine on these biomarkers in both normal dogs and clinic patients is warranted.

ABSTRACT #155
EVALUATION OF SERUM CONCENTRATIONS OF IMMUNOGLOBULIN A AND IMMUNOGLOBULIN M IN CHINESE SHAR PEIS WITH COBALAMIN DEFICIENCY. N Grützner, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Chronic small intestinal disease in Chinese Shar Peis (Shar Peis) has been associated with cobalamin deficiency. In humans, immunoglobulin A (IgA) has been suggested to play a role in the pathogenesis of cobalamin deficiency and may involve an anti-intrinsic factor-like activity or other mechanisms that interfere with normal cobalamin absorption. IgA producing cells in the small intestine belong to the B cell lineage. After an isoform switch from immunoglobulin M (IgM) to immunoglobulin A (IgA) expression, which is mediated through Th2 cytokine activity in the Peyer's patches of the lamina propria and the mesenteric lymph nodes, these cells return to the lamina propria of the intestinal tract by through the cardiovascular system (i.e., homing). Thus, serum IgA and IgM concentrations are the result of secretory function of the plasma cells. The aim of this study was to evaluate the serum concentrations of IgA and IgM in cobalamin deficient Shar Peis and Shar Peis with a normal serum cobalamin concentration.

Serum samples were collected from a total of 71 Shar Peis. Owners were asked to complete a questionnaire regarding the current health status of each dog. Serum cobalamin, folate, and canine trypsin-like immunoreactivity concentrations (reference ranges: 252–908 ng/L, 7.7–24.4 μg/L, and 5.7–45.2 μg/L, respectively) were measured by routine laboratory assays. Serum IgA and IgM concentrations were quantified by enzyme-linked immunosorbent assays using commercial kits (Bethyl Laboratories, Montgomery, TX). A Mann-Whitney test was used to compare the non-parametric data sets. Significance was set at $p < 0.05$.

Undetectable serum cobalamin concentrations (<150 ng/L) were observed in 23 of 71 Shar Peis (32.4%), and these Shar Peis were considered to be severely cobalamin deficient. Serum cobalamin concentrations were within the reference range in the remaining 48 dogs. Serum trypsin-like immunoreactivity concentrations were within the reference range in all the dogs. Serum folate concentrations were within the reference range in all the control dogs. Three of the cobalamin deficient Shar Peis had slightly decreased serum folate concentrations (6.1, 6.4, and 7.2 μg/L). The median serum IgA concentration was significantly higher in cobalamin deficient Shar Peis (1.995 g/L) than in Shar Peis with a normal serum cobalamin concentration (0.812 g/L; $p < 0.0001$). The median serum IgM concentration was significantly lower in cobalamin deficient Shar Peis (1.037 g/L) than in Shar Peis with a normal serum cobalamin concentration (2.071 g/L; $p < 0.0001$).

In conclusion, cobalamin deficient Shar Peis had significantly higher median serum IgA and lower serum IgM concentrations compared to Shar Peis with a normal serum cobalamin concentration. These results warrant further investigation of gastrointestinal immunoglobulin secretion in Shar Peis with cobalamin deficiency.

ABSTRACT #156
GENETICAL ANALYSIS OF TOLL-LIKE RECEPTOR GENES IN GERMAN SHEPHERD DOGS REVEALS POTENTIAL POLYMORPHISM ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE. A Kathrani[1], A House[1], D Werling[2], B Catchpole[2], A Murphy[3], H Trojer[3], N Gruetzner[4], K Allenspach[1]. [1]Department of Veterinary Clinical Sciences, [2]Department of Pathology and Infectious Diseases and [3]Department of Veterinary Basic Sciences,[4]Royal Veterinary College, University of London, UK and the Gastrointestinal Laboratory, Department of Small Animal Clinical Science, College of Veterinary Medicine, Texas A&M University, College Station, TX.

The identification of associations between specific polymorphisms in pattern recognition receptors such as Toll-like receptors (TLR) and human Inflammatory Bowel Disease (IBD) has provided data that demonstrates the importance of these genes and their role in innate immune function in the pathogenesis of this disease. To date no studies have been performed to evaluate polymorphisms in pattern recognition receptors in canine IBD. The aim of this study was to investigate whether polymorphisms in canine Toll-like receptor (TLR) 2, 4 and 5 genes are associated with IBD in German Shepherd dogs (GSDs).

Mutational analysis of TLR2, TLR4 and TLR5 was performed in 10 GSDs with IBD. Genomic DNA was extracted from blood stored in EDTA and the coding region of TLR2, TLR4 and TLR5 amplified using a polymerase chain reaction (PCR). PCR products were sequenced (Geneservice, Cambridge, UK) and full-length sequences obtained from each dog. Sequence data was compared to the canine genome (www.ensembl.org/Canis_familiaris). No non-synonymous single nucleotide polymorphisms (SNPs) were identified in the canine TLR2 gene. Four non-synonymous SNPs (T23C, G1039A, A1572T and G1807A) were identified in the TLR4 gene and three non-synonymous SNPs (G22A, C100T and T1844C) were identified in the TLR5 gene.

The three non-synonymous SNPs in TLR5 were evaluated further in a case-control study using a SNapSHOT multiplex reaction. Sequencing information from 21 GSDs with IBD from the UK were compared to two control groups consisting of 92 healthy GSDs recruited from the USA and 83 GSDs from patients treated for non-inflammatory disease at the Queen Mother Hospital for Animals referral hospital (UK). All three SNPs were found to be in Hardy-Weinberg equilibrium. When the case population was compared to the healthy controls from the USA the G22A SNP was found to be significantly associated with IBD ($p < 0.001$). When the case population was compared to the GSDs with non-inflammatory disease from the UK the G22A SNP was found to be tending towards significance ($p = 0.058$).

Our study suggests that the TLR5 SNP G22A could play a role in the pathogenesis of IBD in GSDs. Further studies are required to confirm the functional importance of this polymorphism in the pathogenesis of this disease.

ABSTRACT #157

URINARY LEUKOTRIENE E4 EXCRETION IS INCREASED IN DOGS WITH CHRONIC ENTEROPATHIES. M Im Hof[1], M Schnyder[1], S Hartnack[2], F Stanke-Labesque[3], N Luckschander[1], IA Burgener[1]. [1]Division of Small Animal Internal Medicine and [2]Department of Clinical Research and Veterinary Public Health, Vetsuisse Faculty of the University of Bern, Switzerland, and [3]Grenoble University Hospital, Grenoble, France.

Inflammatory bowel disease (IBD) and food-responsive diarrhea (FRD) are canine chronic enteropathies (CCE) that can only be differentiated by their response to treatment after exclusion of other diseases. Their severity can be scored with the canine IBD (CIBDAI) or the CCE clinical activity index (CCECAI). Cysteinyl leukotrienes (CysLTs) are proinflammatory 5-lipoxygenase-derived products that play a major role in the immune and inflammatory response. Leukotriene E4 (LTE4), the end product of CysLTs metabolism, is excreted in urine and is stable in this fluid. Its urinary quantification is therefore considered the best marker of in vivo CysLTs production in humans. The urinary LTE4 excretion is increased in human patients with IBD, and LTE4 levels are higher in patients with active IBD than in remission. The aims of this study were to evaluate the urinary LTE4 excretion in dogs with IBD, FRD and healthy controls, and to compare the LTE4 excretion with the activity of the disease using the CIBDAI scoring system.

Urine and CIBDAI were taken at initial presentation in dogs suffering from CCE. Due to their response to treatment, they were divided in FRD (responding to elimination diet alone) and IBD (need for supplementary immunosuppressive treatment). Quantification of LTE4 was performed by liquid chromatography-tandem mass spectrometry. The levels of LTE4 were corrected to the urinary level of creatinine measured with a Jaffe kinetic-based assay. Comparison between dogs suffering from CCE and healthy controls was performed with a Mann-Whitney U test, whereas comparison between IBD, FRD and control dogs were made with a Kruskal-Wallis one-way ANOVA with Dunn's test. Correlation between urinary LTE4 excretion and CIBDAI were analyzed using a Spearmann rank test. $P < 0.05$ was considered significant.

15 IBD and 18 FRD patients were enrolled in the study together with 23 healthy control dogs. IBD dogs showed the highest urinary LTE4 excretion [median 79.9 pg/mg creatinine (10^{th}–90^{th} percentiles 9.8–665.0)] followed by FRD [median 31.7 pg/mg creatinine (10^{th}–90^{th} percentiles 6.2–169.4)] and control dogs [median 21.1 pg/mg creatinine (10^{th}–90^{th} percentiles 9.1–86.5)]. The urinary LTE4 excretion was increased when comparing CCE (IBD+FRD) and control dogs ($P=0.024$) as well as IBD and control dogs ($P=0.018$). A ROC-analysis revealed a specificity of 95.7% for a LTE4 excretion of 90.6 pg/mg creatinine. No correlation was detected between LTE4 excretion and the clinical activity of the disease as measured with the CIBDAI.

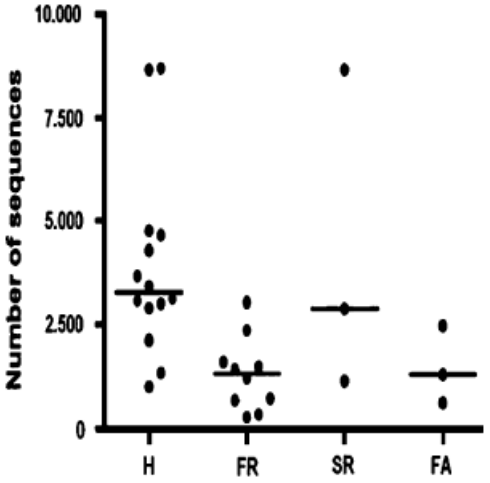
In conclusion, dogs suffering from CCE have an increased urinary LTE4 excretion. Cysteinyl leukotriene pathway activation could contribute to the inflammation associated with IBD in dogs. The comparison of urinary LTE4 excretion before and after treatment is currently performed.

ABSTRACT #158

HIGH THROUGHPUT PYROSEQUENCING REVEALS REDUCED BACTERIAL DIVERSITY IN THE DUODENAL MUCOSA OF DOGS WITH IBD. M Craven, *SE Dowd, S McDonough, KW Simpson. College of Veterinary Medicine, Cornell University, Ithaca, NY and *Research and Testing Laboratory, Lubbock, Texas.

The enteric microflora is increasingly implicated in the etiopathogenesis of inflammatory bowel disease (IBD). Recent work has revealed reduced bacterial diversity and a shift towards a Gram -ve flora in the inflamed intestine across species, but the relationship of individual components of the enteric flora to different disease syndromes and clinical response is unclear. We sought to determine if the duodenal mucosal flora of dogs with IBD differs from healthy dogs, and to gain insight into the relationship of the mucosal flora to clinical response to diet, antibiotics, or immunosuppression.

A culture-independent bacterial tag-encoded FLX 16S rDNA amplicon pyrosequencing (bTEFAP) approach was used to evaluate the microbiome of endoscopic duodenal biopsies from 16 dogs with a histopathological diagnosis of IBD (7MN 7FN 2M, median age 5 yrs) and 14 clinically healthy (H) dogs (5FN, 6MN, 3F, median age 7.5 yrs). 10 dogs were food responsive (FR), 3 steroid responsive (SR), and 3 were partially responsive to a combination of food and antibiotics (FA). A total of 85,065 sequences (54776 from H, 30289 from IBD) were analyzed. The number of sequences per individual (median) was higher in H (3,912) than IBD (1,893) ($P=0.007$). Dogs with IBD had lower overall species diversity than H at all primary levels of taxonomy based upon rarefaction ($p<0.05$). When evaluated by Genus, we observed marked differences in the proportions of sequences in 16S rDNA libraries from H vs. IBD (**$p<0.001$, *$p<0.01$) for: Actinomycetales,* Bacteroidales,* Burkholderiales,* Campylobacteriales,* Clostridiales,* Coriobacteriales,* Enterobacteriales,**Fusobacteriales,* Lactobacillales* and Mycoplasmatales.**Analysis by clinical response indicated differences in numbers of sequence between groups (H, FR, SR and SA: Kruskal Wallis, $P<0.003$), and lower numbers in FR than H ($p=0.007$, see graph). Comparison of H and FR revealed most marked differences in proportions of Bacillales, **Erysipelotrichales,**Flavobacteriales,**Pasteurellales, *Pseudomonadales,** and Sphingobacteriales.



We conclude that duodenal inflammation is associated with decreased diversity of the mucosal flora, and altered relative proportions of dominant floral groups. Further work is required to determine whether differences in the mucosal flora impact the clinical response.

ABSTRACT #159

***E. COLI* ASSOCIATED WITH GRANULOMATOUS COLITIS OF BOXER DOGS FREQUENTLY MANIFEST RESISTANCE TO ANTIBIOTICS**. M Craven, B Dogan, A Schukken, M Volkman, A Chandler, PL McDonough, KW Simpson. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Granulomatous colitis of Boxer dogs (GCB) is associated with the presence of *E. coli* within colonic mucosa and macrophages. A pivotal role for *E. coli* in the pathogenesis of GCB is supported by clinical and histological remission following the eradication of invasive *E. coli* with enrofloxacin. To optimize therapy against GCB associated *E. coli* we sought to determine antibiotic resistance profiles of *E. coli* strains isolated from GCB, and compared them with those from healthy dogs.

E. coli were isolated from colonic biopsies of 14 Boxers with a histological diagnosis of GC and mucosally invasive *E. coli* (8M, 6F, median age 12 m), and from rectal mucosal swabs of 17 clinically healthy dogs (H, various breeds, 6M, 11F, median age 60 m). 10 to 15 isolates from each sample were evaluated for differences in overall genotype and phylogroup (A, B1, B2, and D) by RAPD- and triplex PCR respectively. The antimicrobial resistance profile of representative strains from each sample was determined by broth microdilution MIC (NARMS 2004 Annual Report).

Culture yielded 23 different *E. coli* strains from GCB (1–3/dog, median 2) and 34 strains from healthy dogs (1–3/dog, median 2).

The distribution of phylogroups was not significantly different (P=0.18) between GCB (5A, 7B1, 5B2, 6D) and H (2A, 10B1, 15B2, 7D), Resistance to antibiotics is shown in the table below (Fisher Exact, CGB vs H: *=P<0.05, **<0.01, ***P<0.001).

	Resistant (%)					Resistant (%)			
	strains		patients			Strains		patients	
Antibiotic	GCB	H	GCB	H	Antibiotic	GCB	H	GCB	H
Clavamox	35*	8	57*	12	Trimethoprim-sulfa	44***	6	57**	7
Ampicillin	49**	15	64**	18	Ciprofloxacin	35***	0	43**	0
Cefoxitin	30***	0	50***	0	Gentamicin	13	18	14	36
Tetracycline	48*	18	64*	24	Chloramphenicol	17*	0	21	0

A high proportion of Boxers with GC harbored *E. coli* strains that were resistant to macrophage penetrating antibiotics ciprofloxacin (43%), trimethoprim (57%) and tetracycline (64%). Empirical treatment of GCB before colonic biopsy was associated with resistance against the antibiotic used in 6/6 Boxers receiving enrofloxacin, 3/8 receiving TMPS, 3/9 receiving Clavamox, 2/10 receiving ampicillin and 1/10 receiving tetracycline.

We conclude that antibiotic resistance is common in *E. coli* from GCB and suggest that antibiotic selection should be guided by sensitivity testing rather than empirical wisdom.

ABSTRACT #160
DESCRIPTION OF PROTEIN-LOSING ENTEROPATHY IN YORKSHIRE TERRIER DOGS USING THE W.S.A.V.A. GASTROINTESTINAL CLASSIFICATION SYSTEM. SM Simmerson, A Wunschmann, L Crews, PJ Armstrong, M Fee, and R Washabau. University of Minnesota, College of Veterinary Medicine, St. Paul, MN.

Yorkshire terrier dogs are reported to be at increased risk for the development of protein-losing enteropathy (PLE). Few reports have been published that document clinical features, histopathologic lesions, or outcome in this disorder, and the few cases that have been reported have been evaluated with differing non-standardized classification systems.

The study objectives were (1) to describe historical, physical examination, clinical pathologic, imaging, and histopathologic findings in a large group of Yorkshire terrier dogs with a diagnosis of PLE and (2) to use a standardized classification system for the evaluation of gut pathology.

Retrospective study. The diagnosis of PLE was made on the basis of gastrointestinal clinical signs, hypoalbuminemia and intestinal histopathology in Yorkshire terrier dogs evaluated between 2002–2007 in which this information was available. Dogs that were proteinuric, had abnormal bile acids, or received glucocorticoids within two weeks prior to diagnosis were excluded from the study. Intestinal biopsies were interpreted according to standards for cytoarchitectural change and cellularity using the W.S.A.V.A. G.I. Histopathology Classification system.

30 dogs met the inclusion criteria (mean age 6.7 yrs, range 1–12 yrs; 20 spayed females, 8 neutered males, 2 intact males). The most common presenting complaints were diarrhea (n=20), vomiting (n=11) and abdominal distention (n=11). Most common physical abnormalities were ascites (n=15) and pleural effusion (n=14). Hypoalbuminemia was often severe and ranged from 0.8–2.2 g/dL, mean 1.35 g/dL. Other abnormal laboratory results included hypoglobulinemia (n=14), hypomagnesemia (n=12), low serum creatinine (n=14), anemia (n=7) and thrombocytosis (n=7). Small intestinal mucosal speckling was present in 2/4 dogs that had abdominal sonography performed. Histopathologic lesions commonly included lymphatic dilation (n=24) and crypt abcessation (n=10) with lamina proprial inflammation ranging from mild to severe (n=30). Clinical course of disease was variable and ranged from resolution of clinical signs with glucocorticoid therapy (n=12) to unresponsive disease (n=9) and peracute death resulting from thromboembolism (n=2).

Yorkshire terrier dogs with PLE can be severely affected with ascites and pleural effusion. Clinical outcomes are variable and prognosis therefore is guarded. Intestinal abnormalities in this disorder include lymphatic dilation, intestinal crypt abscessation, and variable increases in the cellularity of the lamina propria.

ABSTRACT #161
DOPPLER ULTRASOUND ANALYSIS OF GASTROINTESTINAL BLOOD FLOW FOR DIFFERENTIATING FOOD ALLERGIC FROM NON-FOOD ALLERGIC PRURITIC DOGS. J Hobbs, L Gaschen, S Merchant, F Gaschen. Louisiana State University, Baton Rouge, LA.

Doppler ultrasound has been shown to be a non-invasive method of characterizing hemodynamic changes associated with food hypersensitivity in dogs with proven food allergies causing chronic enteropathy. The goal of this study was to identify whether or not dogs with cutaneous signs and documented food hypersensitivity also show changes in gastrointestinal hemodynamic responses in comparison to dogs with similar clinical signs but no food allergy.

Client-owned dogs with pruritus suspected of having a food allergy were included in this study. Transabdominal spectral Doppler analysis of the cranial mesenteric artery (CMA) was performed at time of initial presentation with the dog's regular diet and following a 10 week long hypoallergenic dietary trial with IVD® Rabbit and Potato. The following parameters were recorded at 0, 20, 40, 60, and 90 minutes post-prandially: resistive index (RI), pulsatile index (PI), mean diastolic flow (MDV) and the systolic/diastolic ratio (S/D ratio). Dogs were placed into either a food allergic (FA) or non-food allergic (NFA) group based on the dermatologic work up and response to the hypoallergenic diet. Dogs in the FA group were provoked with their original diet and re-examined at a third time point when clinical signs reoccurred. An ANOVA analysis of repeated measures was used to compare the Doppler parameters within groups between examinations and between groups at each examination.

Thirteen dogs were examined, 5 of which were confirmed to have skin disease due to food allergy. Eight dogs had pruritus due to other causes and did not respond to the dietary trial. At initial examination, there were no significant differences in the post-prandial blood flow parameters between the two groups. After a 10 week dietary trial neither the FA, nor the NFA group showed significant differences in the Doppler parameters compared to the initial examination. However, the FA group showed a significantly elevated ($p<0.05$) S/D ratio at t0 and t20 compared to the NFA group, representing a comparative decrease in diastolic blood flow (lack of vasodilation) at those time points. When provoked with their original diet with recurrence of clinical signs, the FA group showed a significant ($p<0.05$) increase in the MDV at t20 and t40 post-prandially compared to the exam following the dietary trial. The RI Doppler parameter never showed statistical differences in any test.

No significant differences in gastrointestinal blood flow could be identified when switching from a regular to a hypoallergenic diet within either group. This finding may limit the use of this technique as a diagnostic test for food allergies in dogs with cutaneous signs only. However, the FA group did show a statistically significantly more rapid increase in blood flow in the early post-prandial period (t20 and t40) when provoked with their original diet. This response may be explained by local immune reactions to food allergens at the gastrointestinal mucosa.

ABSTRACT #162
EFFECTS OF PREDNISONE ALONE OR PREDNISONE WITH ULTRALOW-DOSE ASPIRIN ON THE GASTRODUODENAL MUCOSA OF HEALTHY DOGS. AH Graham, MS Leib. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

The co-administration of prednisone and ultralow-dose aspirin has been recommended for the management of various diseases, but the safety of this combination in dogs has not been studied. The authors hypothesized that the gastroduodenal lesions associated with prednisone and ultralow-dose aspirin would be similar to those caused by prednisone alone, but both treatments would result in more severe lesions than placebo.

This was a randomized, double-blinded, placebo-controlled study involving eighteen healthy adult purpose-bred dogs divided

into three groups for 27 days of treatments: placebo, prednisone, and prednisone and ultralow-dose aspirin. Gastroduodenoscopy was performed prior to and days 5, 14, and 27 of treatment, and mucosal lesion scores assigned. Mucosal lesion scores were evaluated using a Kruskal-Wallis test. Clinical signs were compared using the Friedman's Chi-Square test (significance at $p<0.05$).

There were no significant differences in gastroduodenal lesion scores between groups at any time during the study, and there were no significant changes in gastroduodenal lesion scores over time within any group. Significantly more frequent diarrhea occurred in the prednisone and aspirin group during treatment, compared to baseline. No significant differences in clinical signs were found between any of the groups. Notably, although statistical significance was not reached, individual dogs in both the PN and PA group developed mucosal lesions consisting of hemorrhages (both), erosions (both), and a single small ulcer (PA), whereas the most severe lesions identified in the NN group were mucosal hemorrhages.

The concurrent use of prednisone and ultralow-dose aspirin did not increase the severity of gastroduodenal lesions compared to prednisone or placebo in healthy dogs. Co-administration of prednisone and ultralow-dose aspirin may increase the incidence of mild, self-limiting diarrhea in individual healthy dogs. The effect of this combination in older dogs with spontaneous disease was not determined from this study.

ABSTRACT #163
SERUM TRIGLYCERIDE CONCENTRATIONS IN MINIATURE SCHNAUZERS WITH AND WITHOUT A HISTORY OF PANCREATITIS

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

Miniature Schnauzers (MS) have been reported to be at increased risk for pancreatitis. One explanation for this might be the documented high prevalence of hypertriglyceridemia in this breed. In humans, severe hypertriglyceridemia has been described as risk factor for pancreatitis. An association between hypertriglyceridemia and pancreatitis has also been suggested for dogs, but a cause and effect relationship is less clear in this species. This is further complicated by the fact that hypertriglyceridemia can be the result rather than the cause of pancreatitis in some cases. The aim of this study was to compare serum triglyceride concentrations between MS with and without a history of pancreatitis.

Twenty-three MS with an initial diagnosis of pancreatitis and 44 age-matched healthy MS with no history of pancreatitis were included in this study. The diagnosis of pancreatitis was based on the presence of clinical signs associated with pancreatitis (e.g., vomiting, anorexia, abdominal pain) and a serum Spec cPL™ concentration $>400\,\mu g/L$ (the current cut-off value for pancreatitis). After recovery from the initial episode of pancreatitis, the owners of the dogs were asked to have a follow-up sample submitted. None of the 23 MS had any clinical signs of pancreatitis at the time the follow-up sample was collected, and none of the MS had any other known diseases that might affect lipid metabolism. Only the follow-up sample, which was obtained after clinical resolution of the initial episode of pancreatitis, was used for statistical analyses. This was done to exclude the possibility that hypertriglyceridemia in the initial sample had developed secondary to pancreatitis. Serum triglyceride concentrations were evaluated in the follow-up sample for all 23 MS and compared with those of the 44 age-matched healthy MS with no history of pancreatitis. Body weights were also compared between MS with and without a history of pancreatitis.

Follow-up samples from the 23 MS were collected within 2 to 41 weeks (median: 8.5 weeks) after the diagnosis of pancreatitis. There was no significant difference of the mean body weight between MS with and without a history of pancreatitis ($p=0.438$). Of the 23 MS with a history of pancreatitis, 18 (78.3%) had serum triglyceride concentrations above the reference range, while only 18 (40.9%) MS with no history of pancreatitis had serum triglyceride concentrations above the reference range (odds ratio: 5.2; 95% confidence interval: 1.7–16.6; $p=0.004$). The median serum triglyceride concentration was significantly higher in MS with a history of pancreatitis (median: 555.0 mg/dL) than in MS with no history of pancreatitis (median: 83.5 mg/dL; $p=0.006$).

In conclusion, MS with a history of pancreatitis were significantly more likely to have hypertriglyceridemia than age-matched MS with no history of pancreatitis. These findings provide further evidence that hypertriglyceridemia may be a risk factor for pancreatitis in MS. Additional studies are needed to further clarify the role of hypertriglyceridemia in the development of pancreatitis in MS as well as other dog breeds.

ABSTRACT #164
SERIAL EVALUATION OF CANINE PANCREATIC LIPASE (SPEC cPL™) IN DOGS WITH CLINICAL SIGNS OF PANCREATITIS

LM Prior[1], MA Forman[1], J Shiroma[1], JE Robertson[2], RA Hostutler[1], CD Brown[1]. [1]MedVet, Medical and Cancer Center for Pets, Worthington, OH. IDEXX Laboratories, West Sacramento, CA.

Determining resolution of pancreatitis in dogs is currently based on a combination of improvement in clinical signs (ex. vomiting, inappetance, diarrhea), examination findings (ex. abdominal pain, fever), bloodwork parameters (ex. neutrophilia, amylase, lipase, hyperbilirubinemia), and abdominal ultrasound. These findings are not specific to the pancreas. The Spec cPL assay is a quantitative measurement of pancreatic lipase and is currently used in the diagnosis of pancreatitis. The aim of this study was to determine if elevated Spec cPL values ($>201\,\mu g/L$) decreased in dogs, as the clinical signs of pancreatitis (ex. vomiting) resolved.

Seventy-one dogs were prospectively evaluated and categorized as Spec cPL 201–399 μg/L (Group 1), n=16 and ≥400 μg/L (Group 2), n=55. Fifty-nine dogs with a Spec cPL ≤200 μg/L (Group 3) were retrospectively evaluated and utilized as a baseline control group. All dogs were treated by board-certified internists and evaluations included CBC, biochemical panel, Spec cPL and an abdominal ultrasound performed by a board-certified radiologist. For all dogs with Spec cPL >201 μg/L, multiple CBC, biochemical panel and Spec cPL tests (median 3, range 2–16) were performed during hospitalization and at recheck evaluations.

The median (range) age in Groups 1, 2 and 3 was 8.9 (4.6–14.1), 9 (2.5–15.1), and 7 (1.4–15.6) years, respectively. The median (range) Spec cPL at presentation in Groups 1, 2 and 3 was 306 (203–374), 661 (413–>1000), and 37 (25–196) μg/L, respectively. Vomiting, reported at presentation, was present in Groups 1, 2, and 3 at 11/16 (69%), 37/55 (67%), and 49/59 (83%), respectively. In Groups 1 and 2, 9/11 (82%) and 30/37 (81%), had resolution of vomiting in a median (range) of 2 (1–83) and 2 (1–127) days, respectively. Median (range) Spec cPL, in Groups 1 and 2, at resolution of vomiting was 28 (<30–528) and 521 (<30–>1000) μg/L, respectively. At last recheck evaluation, median (range) Spec cPL, in Groups 1 and 2, was 86 (<30–86) and 244 (<30–>1000) μg/L, respectively. For dogs without resolution of vomiting, in Groups 1 and 2, median (range) of Spec cPL was 283 (77–489) and 364 (<30–>1000) μg/L, respectively. Considering dogs with ≥3 samples, the Spec cPL did not decrease ≤200 μg/L, despite resolution of vomiting, in Group 1, 2/11 (18%) and in Group 2, 13/37 (35%).

In dogs with clinical sign of pancreatitis and elevated Spec cPL (>201 μg/L), a progressive decrease in Spec cPL was noted in dogs with resolution of vomiting and at final recheck evaluation. However, in a subpopulation of dogs the Spec cPL never returned to ≤ 200 μg/L, the established normal range, despite resolution of certain clinical signs of pancreatitis (vomiting).

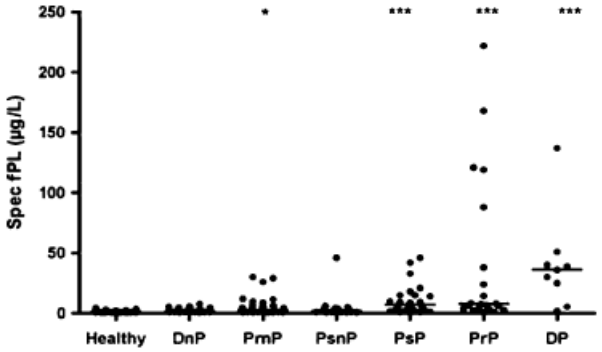
ABSTRACT #165
EVALUATION OF FELINE PANCREAS-SPECIFIC LIPASE (SPEC fPL™) FOR THE DIAGNOSIS OF FELINE PANCREATITIS

MA Forman[1], J Shiroma[1], PJ Armstrong[2], JE Robertson[3], and J Buch[4]. [1]MedVet, Medical and Cancer Center for Pets, Worthington, OH. [2]University of Minnesota, College of Veterinary Medicine, St. Paul, MN. [3]IDEXX Laboratories, West Sacramento, CA. [4]IDEXX Laboratories, Westbrook, ME.

Pancreatitis appears to be a relatively common illness in cats but diagnosis is difficult due to vague clinical signs and clinicopathologic findings. Feline pancreatic lipase immunoreactivity (fPLI) has been shown to be moderately sensitive for the diagnosis of pancreatitis; however test availability has been limited and sensitivity was established using a limited number of cats with pancreatitis. The Spec fPL test is a monoclonal ELISA which has recently become commercially available. The aims of this study were 1) to establish

the reference interval for the test in healthy cats and 2) to determine the sensitivity and specificity of the test in a large group of ill cats with and without pancreatitis.

Forty-one healthy cats and 141 cats with clinical signs consistent with pancreatitis were studied to determine the reference interval and the sensitivity and specificity of the Spec fPL test, respectively. The median ages of healthy and clinical cats were 5 (range 1–14) years and 11 (range 0.6–18) years, respectively. Based on a detailed history and examination, CBC, biochemistry panel, urinalysis, abdominal ultrasonography and clinical outcome, each clinical case was categorized by 2 board-certified internists, blinded to the Spec fPL results, into one of 6 categories (definitely not pancreatitis(DnP), n=26; probably not pancreatitis(PrnP), n=33; possibly not pancreatitis(PsnP), n=19; possibly pancreatitis(PsP), n=29; probably pancreatitis(PrP), n=25; and definitely pancreatitis(DP), n=9). Individual Spec fPL data points (dots) and median values (line) are displayed in the table. Statistical significant difference from healthy cats is indicated (*, ***).



The reference interval, determined from the central 95 percentile of results from healthy cats was 0.7–3.5 µg/L. A concentration ≥5.4 µg/L was determined to be consistent with pancreatitis, using the results to maximize sensitivity and specificity. With 5.4 µg/L as the diagnostic cut off, the sensitivity of the Spec fPL assay for feline pancreatitis (DP and PrP) was 79% and the specificity for cats characterized as PrnP and NP was 82%.

These findings support the use of the Spec fPL as a screening test for feline pancreatitis.

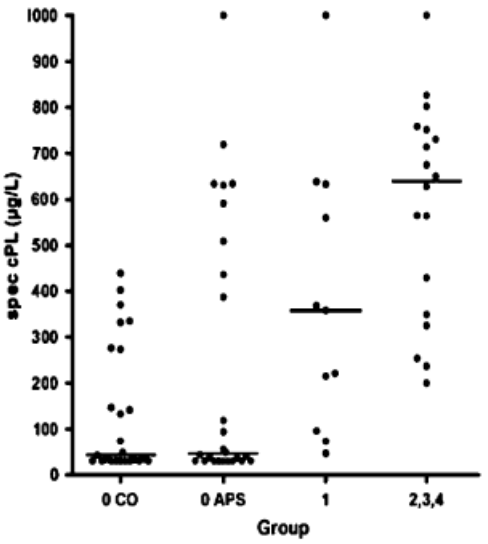
ABSTRACT #166

A MULTI-INSTITUTIONAL STUDY EVALUATING DIAGNOSTIC UTILITY OF SPEC cPL™ IN THE DIAGNOSIS OF ACUTE PANCREATITIS IN DOGS. K McCord[1], J Davis[1], F Leyva[1], PJ Armstrong[2], KW Simpson[3], M. Rishniw[3], MA Forman[4], DS Biller[5], Comparative Gastroenterology Society Members, D Twedt[1]. [1]Colorado State University, Fort Collins, CO. [2]University of Minnesota, Saint Paul, MN. [3]Cornell University, Ithaca, NY. [4]MedVet Center for Pets, Worthington, OH. [5]Kansas State University, Manhattan, KS.

Measurement of pancreas-specific lipase (PL) may aid the diagnosis of acute pancreatitis in dogs. This study evaluated the performance of a commercial immunoassay for canine PL (Spec cPL) in dogs with a clinical diagnosis of acute pancreatitis.

Cases with an initial differential diagnosis that included acute pancreatitis (APS), or did not include acute pancreatitis (CO), were solicited from Comparative Gastroenterology Society (CGS) members. For each dog the history, physical examination, laboratory findings (including total amylase and lipase), abdominal ultrasound and the clinical course were evaluated by a panel of 4 board-certified internists blinded to the results of the Spec cPL. A board-certified radiologist reviewed and graded each ultrasound image. Cases were categorized to one of five pre-defined groups (0–4): 0-not pancreatitis, 1-not primary pancreatitis, 2-possibly pancreatitis, 3-probably pancreatitis or 4-pancreatitis.

The 84 cases enrolled were categorized as follows (group, n): APS (n=57), 0 (26), 1 (11), 2 (9), 3 (8) and 4 (3); CO (n=27), 0 (25) and 1 (2). There was a high level of agreement in the assignment of cases to different groups by the evaluators (kappa 0.87). Spec cPL differed according to group (1-wayANOVA, $P<0.001$) and was higher ($P<0.001$) in dogs with suspected pancreatitis (groups 2,3,4) than those without suspicion of pancreatitis (groups 0APS, and 0CO), but not group 1 ($P>0.05$) (Figure). Amylase and lipase activities did not differ between groups. Spec cPL sensitivity and specificity for cases with clinical score 0 (no pancreatitis), and 2,3,4 (pancreatitis), calculated using current cut off values of <200 µg/L as negative and >400 µg/L positive, yielded a 93% sensitivity and 78% specificity. The likelihood ratio of a negative test (0.029) was better than that for a positive test (1.3).



We conclude that Spec cPL is better able to discriminate dogs with suspected pancreatitis than amylase and lipase, and that dogs with a Spec cPL <200 µg/L are unlikely to have clinical acute pancreatitis.

ABSTRACT #167

CLINICAL SIGNIFICANCE OF INCREASED SERUM FELINE PANCREATIC LIPASE IMMUNOREACTIVITY CONCENTRATIONS IN CATS WITH INFLAMMATORY BOWEL DISEASE. S. Bailey[1], L. Benigni[1], J. Eastwood[1], O.A. Garden[1], L. McMahon[1], K. Smith[1], J.M. Steiner[2], J.S. Suchodolski[2], K. Allenspach[1]. [1]Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, UK, and [2]Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

The aim of this study was to determine the prevalence of increased serum feline pancreatic lipase immunoreactivity concentrations (fPLI) in cats diagnosed with inflammatory bowel disease (IBD); and to investigate the clinical significance and compare the clinical characteristics between cats with IBD with serum fPLI concentrations within the reference range (2.0–6.8 µg/L), within the questionable range (6.9–11.9 µg/L) and above the diagnostic cut off for pancreatitis (≥12 µg/L).

Medical records for 23 cats diagnosed with IBD and with serum fPLI concentrations available were retrospectively studied at the Royal Veterinary College, University of London. The cats were classified into three groups according to their serum fPLI concentration. A Kruskal Wallis test and Fisher's Exact test were used to analyze for any differences between the groups for continuous and categorical data, respectively.

Of the 23 cats, 7 cats had a serum fPLI concentration within the reference range, 9 cats within the questionable range, and 7 cats above the diagnostic cut off for pancreatitis. The clinical outcome of cats with increased serum fPLI concentrations was not significantly different from cats with normal serum fPLI concentrations. No significant differences were identified between the three groups for age, serum ALT and ALP activities, feline IBD clinical disease activity index, pancreatic ultrasound findings, intestinal histopathology scores, treatment, or clinical response. However, cats with serum fPLI concentrations ≥12.0 µg/L had a significantly lower median serum albumin concentration (27.1 g/L) compared to cats with

serum fPLI concentrations within the questionable range (34.2 g/L; $p < 0.05$), and a significantly lower median cobalamin concentration (204 ng/L) compared to cats with fPLI concentrations within the reference range (1142.5 ng/L; $p < 0.05$).

A high number of cats with IBD had increased serum fPLI concentrations. However the outcome for cats with increased serum fPLI concentrations was not different from cats with normal serum fPLI concentrations. It is noteworthy that cats with serum fPLI concentrations $\geq$12.0 µg/L have lower serum albumin and cobalamin concentrations than cats with serum fPLI concentrations in the questionable and reference range, respectively.

ABSTRACT #168

SERUM D-LACTATE CONCENTRATIONS IN CATS WITH GASTROINTESTINAL DISEASE. DP O'Brien[1], RA Packer[2], GE Moore[2], CY Chang[2], GA Zello[3], S Abeysekara[3], JN Naylor[3,4], JM Steiner[5], and JS Suchodolski[5]. [1]University of Missouri, Columbia, MO. [2]Purdue University, West Lafayette, IN. [3]University of Saskatchewan, Saskatchewan SK. [4]Ross School of Veterinary Medicine, St Kitts. [5]Texas A&M University, College Station, TX.

The D-enantiomer of lactic acid is not normally found in any appreciable quantities in serum from mammals. However, recently, we found markedly increased serum D-lactate concentrations in two cats with encephalopathy and pancreatic and gastrointestinal disease, presumably due to an alteration of the gastrointestinal microbiota and bacterial production of D-lactate. The purpose of this study was to determine if serum D-lactate concentration is increased in cats with gastrointestinal disease and if those elevations correlate with specific neurologic or gastrointestinal findings.

Serum D- and L-lactate concentrations were measured by stereospecific high-pressure liquid chromatography using a 3-mm octadecylsilane-packed analytical column coated with N,N-dioctyl-L-alanine as the chiral selector for the separation of lactic acid enantiomers. Serum samples from 30 normal cats were used as controls. Left-over serum samples from cats with abnormal gastrointestinal function test results, that were submitted to the GI Laboratory at Texas A&M University were randomly selected. Questionnaires were sent to veterinarians to survey for gastrointestinal and neurologic signs, and results of other laboratory tests. D-lactate and L-lactate concentrations were compared between normal cats and 100 cats with gastrointestinal disease and a completed questionnaire using a Wilcoxon rank sum test. Association of D-lactate concentration with tests of GI dysfunction and neurologic signs were evaluated with multiple regression and logistic regression analysis, respectively.

All 100 cats had a clinical history of gastrointestinal signs and/or abnormal gastrointestinal function test results. Only 3 cats had obvious neurological signs. D-lactate concentrations of cats with gastrointestinal disease (median and range: 0.41 and 0.01–8.33 mmol/l) were significantly higher than in healthy control cats (median and range: 0.22 and 0.05–0.87 mmol/l; $P = 0.0069$). L-lactate concentrations were not significantly different between the two groups. D-lactate concentrations were not significantly associated with serum concentrations of fPLI, cobalamin, or folate or with the presence of neurologic abnormalities.

In this study, no association between D-lactate concentrations and neurologic signs were observed, although the number of cats with obvious neurologic signs may have been too small to detect such an association. The cat with the highest D-lactate concentrations (8.33 mmol/l) was reported as depressed, listless and weak. Serum D-lactate concentrations were also not associate with other tests of GI function. We conclude that D-lactate concentrations are increased in cats with gastrointestinal disease, possibly due to disarrangements of the intestinal microbiota. These findings warrant further investigations into the role of alterations of the intestinal microbiota in patients with gastrointestinal disease.

ABSTRACT #169

ASSOCIATION OF SERUM COBALAMIN AND METHYLMALONIC ACID CONCENTRATIONS IN DOGS. N Berghoff, KC Stupka, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Cobalamin (Cbl) is a co-factor for several enzymatic reactions in mammals, one of which leads to the conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA. In human and veterinary patients with Cbl deficiency, the metabolite methylmalonic acid (MMA) accumulates and can thus be used as a marker for Cbl deficiency at the cellular level. The aims of this study were to calculate a reference range for MMA in dog serum and to determine the proportion of individuals with increased serum MMA concentrations in dogs with varying serum Cbl concentrations.

For determination of the MMA reference range, serum was collected from 43 healthy dogs. A total of 556 leftover canine serum samples were obtained from submissions to the GI Lab. The samples were divided into 10 groups according to their serum Cbl concentration: <150, 150–250, 250–350, 350–450, 450–550, 550–650, 650–750, 750–850, 850–950, and >950 ng/L ($n \geq 40$ for each group). Serum MMA was measured using an isotope-dilution GC/MS method. The reference range was calculated using the central 95th percentile. Groups were compared using a Kruskal-Wallis test with Dunn's post tests. A χ^2-test for trend was calculated to evaluate the association of serum Cbl and MMA concentrations. Statistical significance was set at $p < 0.05$.

The reference range for serum MMA was 414.7–1,192.5 nmol/L. Median and ranges for serum MMA concentrations for groups 1 through 10 were: 1,328.0 (492.0–24,795.0), 1,034.0 (469.1–3,003.0), 759.5 (378.9–4,064.0), 741.9 (397.2–2,447.0), 710.2 (405.0–1,396.0), 690.5 (398.6–1,733.0), 730.3 (391.7–1,807.0), 820.9 (332.0–1,523.0), 673.6 (343.1–1,765.0), and 733.0 (423.1–1,739.0) nmol/L, respectively. Median MMA concentrations were significantly different among the 10 groups of dogs ($p < 0.0001$). In dogs with Cbl <150 ng/L, median MMA concentration was significantly different from that of all other groups ($p < 0.05$ for all), except the "150–250" group. Median MMA concentration of the "150–250" group was significantly different from that of all other groups ($p < 0.05$ for all), except the "750–850" group. No significant differences were found among the remaining groups. Percentages of dogs with MMA concentrations above the upper limit of the reference range in groups 1 to 10 were 62.5%, 31.7%, 19.2%, 16.0%, 10.0%, 14.3%, 6.0%, 9.6%, 10.2%, and 12.5%, respectively. The χ^2-test for trend was significant ($p < 0.0001$).

These data show that a large percentage of dogs with Cbl concentrations below the lower limit of the reference range (252–908 ng/L) have an increased serum MMA concentration. A significant trend for an increasing serum MMA concentration with a decreasing serum Cbl concentration was observed. Several dogs with Cbl of up to 450 ng/L had increased MMA concentrations, suggesting that a portion of dogs with Cbl concentrations in the low-normal range have evidence of Cbl deficiency. This may indicate a need to supplement these dogs with Cbl, despite a serum Cbl concentration within the reference range. It remains to be determined why some dogs with high-normal Cbl concentrations have an increased serum MMA concentration.

ABSTRACT #170

COMPARISON OF GASTROINTESTINAL MOTILITY IN DOGS TREATED WITH METOCLOPRAMIDE, CISAPRIDE, ERYTHROMYCIN OR MAROPITANT USING THE SMARTPILL™. KW McCord[1], P Boscan[1], K Dowers[1], A Bradley[1], S Cosgrove[2], K Dame[2], J Civil[2], D Holmberg[3], D Sacilowski[3] and DC Twedt[1]. [1]Colorado State University, Fort Collins, CO, [2]Pfizer Animal Health, Kalamazoo, MI. [3]SmartPill Corporation, Buffalo, NY.

The effects of the drugs metoclopramide, cisapride, erythromycin and maropitant on gastrointestinal (GI) motility in the dog are poorly described. The aim of this study was to determine the effect of these drugs on motility in normal dogs using the SmartPill™. The SmartPill™ is a noninvasive wireless sensor capsule that is given orally and transmits data on pressures, transit time, luminal pH and temperature as it passes through the stomach, small and large bowel. This instrumentation has been validated to evaluate GI function in humans. A computer software displays the data and calculates the integral under the pressure curve, which is the area under the curve (AUC) and a motility index (MI=AUC/time).

Ten normal adult dogs were used in a randomized, crossover, blinded design. All dogs received metoclopramide 0.5 mg/kg SQ TID, cisapride 0.5 mg/kg PO TID, erythromycin 1 mg/kg PO TID, maropitant 1 mg/kg SID and a saline placebo 1 ml/dog SQ SID. Drugs were administered daily for three days to obtain a steady

state and on day four the SmartPill™ was administered following a standard meal. All medications were continued until the SmartPill™ left the GI tract. There was at least 7-day washout period between treatments. Data was compared using a linear mixed model with 95% confidence interval. Significance was $p < 0.05$.

The transit times for each treatment had large individual variation within and between dogs. Gastric and large bowel parameters were not significantly different between drugs and placebo. Small bowel mean pressures and MI values were higher for the cisapride treatment group when compared to control ($p < 0.05$). The AUC and MI values for small bowel were higher for maropitant when compared to control ($p < 0.05$).

Using the SmartPill™ technology there were no major differences in gastrointestinal motility measurements between metoclopramide, cisapride, erythromycin, maropitant and placebo. However, both cisapride and maropitant may decrease the contraction pressure pattern in the small bowel without altering the transit time.

ABSTRACT #171

VARIABILITY ASSOCIATED WITH REPEATED MEASUREMENTS OF GASTRIC EMPTYING USING THE SMARTPILL PH.P WIRELESS CAPSULE AND SCINTIGRAPHY IN DOGS. C.S. Boillat, F.P. Gaschen, L. Gaschen, R. Stout, G. Hosgood. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

This study evaluated and compared the repeatability of measurements of gastric emptying (GE) using the SmartPill pH.p® wireless capsule (SP) and scintigraphy in healthy dogs.

Six healthy adult dogs weighing 21.5±1.8 kg were used. A ^{99m}Tc-mebrofenin radiolabeled test meal was offered after *per os* administration of the SP. Serial abdominal images were acquired for 270 minutes. A dedicated remote receiver was used for data collection from the SP until SP was expelled in the feces. Each dog was examined 3 times 1 to 2 weeks apart with the above procedure.

The mean gastric emptying half-times measured by scintigraphy (T 1/2-GES) for each dog ranged from 99.9 to 181.2 minutes. The mean gastric emptying times measured by the SP (GET-SP) for each dog ranged from 385.3 to 669.7 minutes. The mean coefficient of variation (CV) was 11.8% for T 1/2-GES and 7.8% for GET-SP. The intra-class correlation coefficient was 69% for T 1/2-GES and 71% for GET-SP. A nested analysis of covariance showed that changes in scintigraphy do not cause significant variation in GET-SP, and GET-SP and scintigraphy are alike, suggesting that both methods are comparable for GE evaluation.

Intra-individual variability of GE in healthy dogs is similar to values reported in people. These variations must be considered in interpreting individual test results. Repeatability of SP-measurements is at least as good as that of scintigraphy, the current gold standard for evaluation of GE. SP is a valuable, non-radioactive, out-patient alternative to scintigraphy for assessment of GE in dogs.

ABSTRACT #172

THE ESOPHAGEAL TRANSIT TIME OF TABLETS OR CAPSULES IN CATS FOLLOWING ADMINISTRATION WITH FLAVORx® PILL GLIDE OR PILL DELIVERY TREATS. MacPhail CM, Bennett AD, Gibbons DS, Lappin MR. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

Retention of tablets or capsules in the feline esophagus is known to be associated with local esophagitis and subsequent esophageal stricture formation. In previous work, tablets or capsules administered without water were found to be commonly lodged in the esophagus for greater than 5 minutes. Passage into the stomach can be facilitated by following dry-pilling with a 6 ml oral bolus of water or by coating medications with butter. The objective of this study was to evaluate the esophageal passage of tablets and capsules when administered with either a pill delivery treat (Pill Pockets®) or a one-step pill gun with flavored liquid (FlavoRx® Pill Glide).

Eight normal cats were enrolled in the study. Physical examination, serum biochemistry profile, and CBC were performed to ensure good health. Barium sulfate tablets, sized to 4×2 mm, and barium sulfate-filled #4 gel capsules were used in this study. Cats were placed in a specially designed plexiglass box and fluoroscopy was used to evaluate tablet or capsule passage from 0 to 30 seconds, at 60, 90, and 120 seconds, and then at one-minute intervals until the tablet or capsule was in the stomach. Four separate medication administrations were performed in each cat on different days: tablets with pill delivery treats (T-PP), tablets with FlavoRx® pill glide (T-FG), capsules with pill delivery treats (C-PP), and capsules with FlavoRx® pill glide (C-FG).

The percentage of successful passages into the stomach at 30 seconds was 62.5% for T-PP, 62.5% for T-FG, 75% for C-PP, and 100% for C-FG. The percentage of successful passage at 60 seconds was 75% for T-PP, 87.5% for T-FG, 100% for C-PP, and 100% for C-FG. The average transit time was 59.3 sec for T-PP, 36.1 sec for T-FG, 24.5 sec for C-PP, and 15.9 sec for C-FG. The maximal time in the esophagus was 240 sec, 90 sec, 60 sec, and 29 sec for T-PP, T-FG, C-PP, and C-FG, respectively.

The results of this study demonstrate that either pill delivery method is acceptable for successful passage of tablets or capsules into the stomach of cats.

ABSTRACT #173

GASTROINTESTINAL DISEASE IN DOGS WITH EXCESSIVE LICKING OF SURFACES. V. Bécuwe, M.C. Bélanger, D. Frank, J. Parent, P. Hélie. Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.

Excessive licking of surfaces (ELS) is often considered as an obsessive-compulsive disorder and treated with antidepressant drugs. However, dogs with ELS respond poorly to this therapy. We hypothesized that ELS was a manifestation of nausea or discomfort, resulting from an underlying gastrointestinal (GI) disorder. The aims of the study were to 1) describe the ELS behavior, 2) perform a thorough clinical evaluation of the digestive system of dogs presented with ELS and 3) evaluate the outcome of this behavior after appropriate treatment of the underlying GI disorder.

Twenty dogs with ELS (L group) were divided in 2 subgroups of 10 dogs: L0 without and LD with digestive signs such as vomiting and diarrhea. Ten healthy dogs were assigned to a control group (C). Behavioral, physical and neurological exams were performed, prior to a complete evaluation of the GI system (CBC, serum biochemical profile, urinalysis, measurement of total serum bile acids and canine specific pancreatic lipase immunoreactivity, fecal flotation by zinc sulfate, fecal culture, abdominal ultrasonography and upper GI endoscopy with biopsies). Based on results, a board certified internist recommended an appropriate treatment. Dogs were monitored for a subsequent 90 days during which the licking behavior was recorded.

The underlying GI disorders found in the L group included eosinophilic infiltration, lymphoplasmacytic infiltration, delayed gastric emptying, irritable bowel syndrome, chronic pancreatitis, gastric foreign body, giardiasis. Complete resolution of ELS was observed in 9/18 dogs. At day 60, the LD dogs had a better outcome for ELS improvement than the L0 dogs ($P=0.02$). There was no significant difference in behavior of L dogs and C dogs.

GI disorders should therefore be considered in the differential diagnosis of excessive licking of surfaces in dogs.

Previously presented at ECVIM in Belgium, September 2008.

ABSTRACT #174

HIGH RESOLUTION MAGNETIC RESONANCE IMAGING OF THE CANINE BRAIN AT 7 TESLA: A COMPARISON STUDY WITH 0.2 AND 1.5 TESLA. BT Kang[1], KJ Ko[1], DP Jang[2], JY Han[2], CY Lim[1], DI Jung[3], JH Yoo[1], C Park[4], YB Kim[2], EJ Woo[5], ZH Cho[2], HM Park[1]. [1]Konkuk University College of Veterinary Medicine, Seoul, South Korea. [2]Gachon University of Medicine and Science, Neuroscience Research Institute, Incheon, South Korea. [3]Gyeongsang National University College of Veterinary Medicine, Jinju, South Korea. [4]University of California Davis School of Veterinary Medicine, Davis, CA. [5]Kyung Hee University Department of Biomedical Engineering, Yongin, South Korea.

The purpose of this study was to describe the relevant canine brain structures using magnetic resonance imaging (MRI) at 7T. T2-weighted imaging was performed on five healthy laboratory beagle dogs using 0.2, 1.5 and 7T clinical scanners. The anatomic

features were subjectively evaluated by direct comparison of the images obtained from the three different magnetic fields. The signal-to-nose ratio (SNR) and contrast-to-noise ratio (CNR) were calculated and compared between 1.5 and 7T. The T2-weighted images at 7T provided good spatial and contrast resolution for the identification of the clinically relevant brain anatomy; these images provided better delineation and conspicuity of the brain stem structures, which were difficult to unequivocally identify at 0.2 and 1.5T. The SNR and CNR of the images at 7T were significantly increased up to 218% and 615% compared to the 1.5T images. If the image sequences could be optimized, 7T clinical MRI could provide a good experimental and diagnostic tool for the evaluation of canine brain disorders.

ABSTRACT #175
CLINICAL SIGNS, MAGNETIC RESONANCE IMAGING FINDINGS AND SURVIVAL IN DOGS WITH INTRACRANIAL MENINGIOMAS AND GLIAL CELL TUMOURS. A de Stefani[1], A Sparkes[1], L S Garosi[2], L De Risio[1], F J Llabres[2], SR Platt[3]. [1]Animal Health Trust, Newmarket, Suffolk, UK. [2]Davies Veterinary Specialists, Higham Gobion, UK. [3]Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA.

The aims of our study were to retrospectively assess possible associations between clinical signs, magnetic resonance imaging (MRI) findings and survival time in dogs with histopathologically confirmed meningiomas and glial cell tumours.

Our medical database was searched for dogs with intracranial neoplasia (1993–2004). Cases of meningioma (n=31) or glial cell tumour (n=27) with complete medical history, MRI and follow-up until time of death were included. Data were analysed using relevant non-parametric tests (chi-squared, Fishers Exact, Mann-Whitney, Spearman Rank, and Log-Rank Test). For all tests, significance was set at $P \leq 0.05$.

Fifty-eight dogs satisfied the inclusion criteria. Association among clinical signs (presence of seizure activity, altered mentation, blindness and compulsive gait) and tumour site, tumour volume (tumour to total brain volume ratio) and MRI findings (peritumoural oedema, intra-tumoural haemorrhage and parenchymal shift) were assessed. In addition associations between tumour type and, tumour site, volume and MRI findings were evaluated. The influence of tumour site, tumour volume and tumour type on survival time was investigated. The tumour volume was significantly higher (P=0.0006) for gliomas (mean 5.3%) than meningiomas (mean 2.5%). There was no significant difference in the prevalence of oedema or parenchymal shift between the tumour types, but gliomas were significantly (P=0.03) more likely to have intratumoural haemorrhage (30% vs 6%). There was a significant (P=0.002) difference in anatomical distribution between meningiomas (most common in parietal and olfactory lobes, 58%) and gliomas (most common in frontal and temporal lobes, 78%). Tumour site significantly affected the likelihood of seizure activity being observed (P=0.004) or of a compulsive gait being present (P=0.04), but did not significantly affect the likelihood of altered mentation or visual deficits. Survival analysis was performed on 35 dogs that underwent surgery followed by radiation therapy. The median survival time from time of diagnosis of dogs with gliomas (327d) was not significantly different (P=0.26) from that of the dogs with meningiomas (486d) and no association between tumour site and survival (P=0.6) or between tumour volume and survival (P=0.14) was identified.

. . . Association between tumour type, volume, MRI findings and anatomical distribution were identified in this study; however these variables did not influence survival in a subpopulation of dogs.

ABSTRACT #176
ORAL HYDROXYUREA THERAPY FOR DOGS WITH SUSPECTED INTRACRANIAL MENINGIOMA: A RETROSPECTIVE COHORT STUDY (2004–2009). MA Cautela[1], CW Dewey[1], S Cerda-Gonzalez[1], DJ Fletcher[1], G Barone[2]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY; [2]Long Island Veterinary Specialists, Plainview, NY.

Meningioma is the most common canine brain tumor and it can be both difficult and expensive to treat. Supportive therapy typically consists of oral glucocorticoid (GC) therapy±antiseizure drugs. Survival times with supportive therapy are variable, but are typically between 1 and 4 mos. Standard modalities for definitive treatment of canine intracranial meningioma include surgical resection, megavoltage irradiation, or a combination of these options. Despite improved survival times with definitive therapies, owners often decide against these treatments due to both risk factors and cost. Hydroxyurea (HU) is an oral chemotherapeutic drug used with some success in human meningioma treatment. We have used oral HU in dogs (20 mg/kg SID) with suspected and confirmed meningiomas for several yrs. HU is inexpensive and has minimal adverse effects. Our clinical impression is that dogs treated with HU live longer than those treated with GC alone. The purpose of this investigation was to compare survival times between dogs with MRI-diagnosed intracranial meningioma treated with HU and GC and those treated with GC alone via a retrospective cohort study.

Case record inclusion was restricted to dogs from our 2 hospitals that had not received either surgical or radiation therapy. Diagnosis of intracranial meningioma was based on MRI interpretation by a board-certified radiologist. Data derived from each record included signalment, clinical signs, mass location, and survival time from diagnosis. 43 records were identified, 33 HU/GC dogs and 10 GC dogs. Survival times were estimated for each group using the Kaplan-Meier product limit method and compared using the log-rank test ($p < 0.05$). A hazard ratio was also calculated. Survival was significantly longer in the HU/GC group (p=0.025) with these dogs living 2.97 times longer than dogs in the GC group (95% CI=1.15–7.68). At the time of data analysis, the median survival times for HU/GC and GC groups were 28 wks and 14 wks, respectively. 8 dogs (24%) were still alive in the HU/GC group and none in the GC group.

In this study, oral HU use in dogs with suspected intracranial meningioma was associated with longer survival times than those previously reported for dogs treated with GC alone and significantly increased survival likelihood compared with the cohort GC-only group. Our results support the use of oral HU for dogs with intracranial meningioma.

ABSTRACT #177
IMMUNE CELL INFILTRATION INTO CANINE MENINGIOMAS. CL Mariani, T Davis, NJ Olby. North Carolina State University, Raleigh, NC.

Meningiomas are the most common intracranial tumor in dogs. However, very little is known about the immune response to brain tumors in this species. Immune responses against tumor tissue may be beneficial for the host, resulting in delayed tumor growth or regression. Conversely, certain immune cell populations such as regulatory T cells (Tregs), may inhibit anti-tumor immune responses, facilitating tumor growth and progression. The immune system may also influence the expression of certain molecules contributing to tumor cell invasion, such as matrix metalloproteinases. Therefore, analysis of immune cell populations in tumor biopsy samples may provide useful prognostic information. In addition, the current therapy for canine meningiomas is inadequate, and novel treatments such as immunotherapy are needed to improve outcomes. Before exploring immunotherapeutic options, the populations of immune cells present in meningiomas must be characterized, which is the purpose of the current study.

Sections of meningioma tissue were obtained from archival formalin-fixed, paraffin-embedded blocks. Immunohistochemical evaluation of CD3 (T cells), CD79a (B cells), FoxP3 (Tregs), CD18 (microglia, macrophages), CD11d (dendritic cells), toll-like receptor 4 (TLR4) and TLR9 was performed. Consistent populations of CD3+ T cells and CD18+ cells were identified within and surrounding tumor tissue. In contrast, B cells and Tregs were rarely identified, although some samples showed clusters of positive cells. TLR4 and TLR9 positive cells were also noted. Further work to correlate these cell populations with outcome is underway. These observations may form the basis for intervention with immunostimulatory compounds such as TLR agonists.

ABSTRACT #178
FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING FINDINGS OF NON-SUPPURATIVE MENINGOENCEPHALITIS IN SEVEN DOGS. BT Kang[1], DP Jang[2], CY Lim[1], SH Gu[1], DI Jung[3], JH Yoo[1], C Park[4], YB Kim[2], EJ Woo[5], ZH Cho[2], HM Park[1]. [1]Konkuk University College of Veterinary Medicine, Seoul, South Korea. [2]Gachon University of Medicine and Science, Neuroscience Research Institute, Incheon, South Korea. [3]Gyeongsang National University College of Veterinary Medicine, Jinju, South Korea. [4]University of California Davis School of Veterinary Medicine, Davis, CA. [5]Kyung Hee University Department of Biomedical Engineering, Yongin, South Korea.

The purpose of this study was to characterize the [18F]2-deoxy-2-fluoro-D-glucose positron emission tomography (FDG-PET) findings of encephalitis in dogs and to assess the role of the FDG-PET in the diagnosis of meningoencephalitis. The medical records, magnetic resonance (MR) and FDG-PET images of five dogs with necrotizing meningoencephalitis (NME), one dog with granulomatous meningoencephalitis (GME) and one dog with meningoencephalitis of unknown etiology (MUE) were reviewed. The T2-weighted images (WI) and fluid attenuated inversion recovery (FLAIR) images were characterized by hyperintensity, whereas the signal intensity of the lesions on the T1-WI images was variable. The metabolic changes on the brain FDG-PET corresponded well to the hyper- and hypointense lesions seen on the MR imaging. Glucose hypometabolism was identified in the NME, whereas hypermetabolism was noted in the GME. FDG-PET aided in the diagnosis of meningoencephalitis when the metabolic data was combined with clinical and MR findings. FDG-PET cannot be used alone to diagnose meningoencephalitis.

ABSTRACT #179
STEROID RESPONSIVE MENINGITIS-ARTERITIS TREATMENT WITH THREE POTENTIAL DIFFERENT PROTOCOLS: CLINICAL SIGNS, LABORATORY AND LONG TERM FOLLOW UP IN 48 DOGS. A Negrin[1], GB Cherubini[2], HA Volk[3], L Gaitero[1], S Añor[1]. [1]Autonomous University of Barcelona, Barcelona, Spain, [2]Dick White Referrals, Newmarket, UK. [3]The Royal Veterinary College, University of London, UK.

The aims of this study were to describe the clinical signs, laboratory and follow up results, in particular the presence of paresis/ataxia and the CSF characteristics in 48 dogs affected by steroid-responsive meningitis-arteritis (SRMA) and treated with three different therapeutic protocols (Prednisolone, Prednisolone and Cytosine Arabinoside, Ciclosporine and Cytosine Arabinoside). In addition, the association between number of relapses and drug protocol (dose and duration), number of relapses and outcome, and side effects and survival were investigated. A retrospective search was performed for dogs fulfilling the following criteria: presence of cervical/diffuse spinal pain, diagnosis of aseptic suppurative meningitis based on neurological examination, CSF analysis, serum and CSF negative PCR results for *Toxoplasma gondii*, *Neospora caninum*, *Ehrlichia canis* and Canine Distemper virus, peripheral blood leukocytosis, normal spinal radiographs and complete/partial resolution of clinical signs with immunosupressive therapy. The same type and dose of each drug were used in all protocols. Long term follow up was obtained up to December 2008. A total of 48 dogs were included in the study. Boxers were the most prevalent breed (12), other breeds were not overrepresented. Mean age of onset of neurological signs was 14 months (4–84 months) and mean duration was 13 days (2–27 days). Clinical signs at presentation were: cervical pain (85%), pyrexia (73%), depression (56%), stiff gait and thoracolumbar pain (31%), ataxia (19%) and paresis (11%). On CSF analysis, median WBC count was 355/μL (31–8200/μL) with a median neutrophil percentage of 78% (49–97%). Median total protein concentration was 0.39 g/L (0.1–7.3 g/L). Dogs with multifocal spinal pain had a significant higher median WBC count than dogs with cervical pain only, while presence of paresis/ataxia was not statistically correlated with WBC count in CSF. Treatment protocols included: Prednisolone (Pred) (38 dogs), Pred and Cytosine Arabinoside (CA) (5 dogs) and Cyclosporine (Cyclo) and CA (5 dogs)..Remission of clinical signs was obtained in all cases at 1.5 months after treatment initiation, with a mean duration of 7.8 months. Eighty one percent (39/48) of cases were still alive and median life span since diagnosis was 16.7 months (8–83). Relapses of neurological signs were observed in 16/36 (44%) dogs treated with Pred, most of them (63%) at 5 months after treatment initiation. All 5 dogs treated with Cyclo-CA relapsed 1 month after treatment initiation, while none of the 5 dogs receiving Pred and CA had relapses during treatment. This study suggests that slower tapering of Pred alone may be more effective in avoiding relapses; moreover, the combination of Pred+CA could be a promising alternative as it seems to be effective to prevent from relapses and to decrease side effects, as well as to ensure good survival rates.

ABSTRACT #180
THREE-DIMENSIONAL TIME-OF-FLIGHT MAGNETIC RESONANCE ANGIOGRAPHY OF INTRACRANIAL VESSELS IN A CANINE MODEL OF ISCHEMIC STROKE WITH PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION. BT Kang[1], DP Jang[2], SH Gu[1], CY Lim[1], DI Jung[3], JH Yoo[1], C Park[4], YB Kim[2], EJ Woo[5], ZH Cho[2], HM Park[1]. [1]Konkuk University College of Veterinary Medicine, Seoul, South Korea. [2]Gachon University of Medicine and Science, Neuroscience Research Institute, Incheon, South Korea. [3]Gyeongsang National University College of Veterinary Medicine, Jinju, South Korea. [4]University of California Davis School of Veterinary Medicine, Davis, CA. [5]Kyung Hee University Department of Biomedical Engineering, Yongin, South Korea.

The purpose of this study was to evaluate the potential efficacy of 3-dimensional time-of-flight magnetic resonance angiography (TOF-MRA) to validate canine ischemic stroke model. Ischemic stroke was induced by permanent middle cerebral artery occlusion (MCAO) in five healthy beagle dogs. T2-turbo spin echo images and TOF-MRA were serially obtained three times with a 1.5-tesla magnetic resonance system: before, 3 and 10 days after the MCAO. TOF-MRA of the dogs showed the main cerebral arteries and their branches. In three dogs, angiograms of the brain obtained at 3 days after the MCAO showed complete occlusion of the MCA; in addition, T2 hyperintensity was found unilaterally in the striatocapsular and cerebral cortex lesions. However, partial occlusion of the proximal part of the MCA was identified in the other two dogs, with T2 hyperintensity found only in the striatocapsular lesions. Because improvement of the ischemic lesions was observed in all dogs, without reperfusion of the occluded vessels, recruitment of blood flow through collateral vessels was suggested. The occluded sites were confirmed at necropsy. The results of this study demonstrate the potential of TOF-MRA to provide a detailed description of intracranial arteries and aid in the diagnosis of flow impairment in canine ischemic stroke.

ABSTRACT #181
PREVALENCE OF CEREBRAL MICROBLEEDS IN AGED DOGS. D Insua[1], ML Suarez[2], G Santamarina[2], A González[2], M Sarasa[1], P Pesini[1]. [1]Araclon Biotech S.L. Zaragoza, Spain. [2]Department of Veterinary Clinical Sciences, University of Santiago, Lugo, Spain.

Cerebral microbleeds are focal lesions that can be visualized on magnetic resonance imaging (MRI). Hystopathological analysis shows that these are hemosiderin deposits from red blood cells that presumably have leaked out of small brain vessels. Little is known on microbleed prevalence, risk factors, and clinical correlates in the general population. Recent studies with gradient-echo T(2)* MRI technique to detect hemosiderin components in the brain have reported that latent microbleeds are more frequent in Alzheimer Disease (AD) patients than in normal controls. Furthermore, hystopathological studies have found that senile plaques are sites of microhaemorrhages. These co-localization raises the questions of whether microhaemorrhages are early events in plaque formation and whether therapies which stabilize cerebral microvessels can prevent the onset or slow the progress of dementias associated with plaque formation.

We have used the iron Perls staining to study the appearance and extension of microhaemorrhages in the brain of young (n=6), aged dogs without cortical amyloid deposits (n=7) and aged dogs with

cortical amyloid deposits (n=7). Five animals in this last group were diagnosed with canine counterpart of AD characterized by cognitive impairment, cortical amyloid deposits and neuronal loss. All the brains were obtained by necropsies carried out in our VTH with the explicit consent of the owners.

Extensive hemosiderin deposits were found in all the aged animals irrespective whether they did present or not cortical amyloid pathology. These deposits were intensely stained and particularly dense around the fiber tract of the internal capsule, the globus pallidum and the olfactory tubercle. The caudate nucleus was also considerably affected whereas the putamen used to be spared. Less dense deposits spread along the diagonal band, the septum, anterior commissure, and throughout the subcortical white matter. No appreciable hemosiderin deposits were seen in the cortical grey matter. A huge number of hemosiderin containing phagocytic microglia was seen within the stained areas. Furthermore hemosiderins deposits, although lighter and smaller, were found in the same brain regions of two young dogs.

These results showed that microbleeds have a great prevalence in the dog. In addition they do not give support to the existence of a mechanistic relation between this lesion and the presence of cortical amyloid-β pathology.

ABSTRACT #182

PRELIMINARY OBSERVATIONS BETWEEN COX-2 INHIBITOR ADMINSTRATION AND ACUTE CEREBROVASCULAR DISEASE IN DOGS

FA Wininger, RS Bagley, AV Chen. Veterinary Clinical Sciences-Washington State University, Pullman, WA.

Reasons for the increased recognition of acute cerebrovascular disease (ACVD) in dogs are currently being evaluated. ACVD in humans have been associated with the theoretical hypertensive, prothrombotic effect of new generation NSAIDs. This purpose of this study was test the hypothesis that the increased use of cyclooxygenase-2 inhibitor drugs in dogs may correlate with ACVDs.

A retrospective study of dogs with ACVD was performed to determine if a relationship with NSAID administration existed. 67 dogs met the inclusion criteria of a presumptive ACVD diagnosis based on clinical findings, cerebrospinal fluid cytology and MRI findings. All dogs had a minimum of one blood pressure measurement and a urinalysis. 33 of the 67 dogs had fibrin split products and D-dimers measured in serum. An age and breed matched cohort of an additional 67 dogs presenting for intracranial disease was used as a control population. To be included in the NSAID group, drug administration was within a year of presentation. Data was analyzed with chi-squared analysis. 4 of 67 dogs with ACVD had a NSAID administered within 5 days of presentation compared to 7 of 67 in the non-ACVD group (chi-square=0.973, p-value=0.324). The type, frequency and duration of NSAID administration were highly variable. From the data evaluated no other risk factors have currently been identified. Based on this preliminary information, it is difficult at this time to determine a significant association between ACVD and the administration of COX-2 specific NSAIDs in dogs.

ABSTRACT #183

MULTIPLEX ANALYSIS OF CYTOKINES IN THE CEREBROSPINAL FLUID OF DOGS AFTER STROKE

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Inflammation contributes to brain injury following human and animal strokes, and elevated inflammatory cytokines in CSF have been associated with clinical deterioration. As an initial characterization, pro- and anti-inflammatory CSF cytokines/chemokines were evaluated in dogs with stroke.

Cerebrospinal fluid was collected from 10 healthy beagles with normal brain MRI and 18 various dog breeds with clinical signs and MRI consistent with cerebrovascular infarction. Cerebrospinal fluid was collected in glass or polypropylene tubes, immediately frozen and stored at −80 °C until use. Cerebrospinal fluid was evaluated with a canine-specific multiplex immunoassay for GM-CSF, IFN-γ, IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, IFN-inducible protein (IP)-10, keratinocyte chemoattractant (KC), monocyte chemoattractant protein (MCP)-1 and TNF-α (Millipore) using a Luminex 200 instrument.

GM-CSF, IFN-γ, IL-6, IL-8, IL-10, IL-15, IL-18, IP-10 and TNF-α were not detected in the majority of dogs, however KC and MCP-1 concentrations were elevated significantly ($p<0.01$ and $p<0.0001$, respectively) in stroke dogs compared to normal dogs. Among stroke dogs, cytokine levels were not significantly changed by administration of anti-inflammatory medications (within two weeks of CSF collection) or by time of CSF collection after infarction. KC elevations occurred in stroke dogs compared to normal dogs when CSF was collected within 48 hours of stroke ($p<0.05$) or greater than five days after stroke ($p<0.01$). IL-2 and IL-4 levels were higher in CSF collected in polypropylene versus glass tubes in stroke dogs ($p<0.005$ and $p<0.05$, respectively).

While additional inflammatory mediators in canine stroke require evaluation, this preliminary analysis identified elevation of the chemokines KC and MCP-1 in stroke dogs consistent with post-infarct inflammation.

ABSTRACT #184

VALIDATION OF R&D SYSTEMS KITS TO DETECT CYTOKINE LEVELS IN CANINE CEREBRAL SPINAL FLUID

AD Dierenfeld[1], PM Boggiatto[2], J Parkes[3], M Passage[4], JK Jens[2], EM Snella[2], KL Kline[3], CA Petersen[2], PI Dickson[4], NM Ellinwood[1]. [1]Departments of Animal Science, [2]Veterinary Pathology, [3]Veterinary Clinical Sciences, Iowa State University, Ames, IA; [4]Department of Pediatrics, LA Biomed at Harbor-UCLA, Torrance, CA.

Mucopolysaccharidosis (MPS) type I and type IIIB are lysosomal storage diseases caused by deficiency of lysosomal enzymes and subsequent lysosomal accumulation of dermatan and heparan sulfates (MPS I) and heparan sulfate (MPS IIIB). The phenotypes vary, although both forms are neuropathic in humans. Spontaneous canine models exist and have been critical in developing therapies for the corresponding human disease. There is interest in minimally invasive methods and techniques to monitor response to therapy that relies on authentic biomarkers and which can help to document any response to protein or gene therapy based treatments. Non-specific immune activation in the CNS is also hypothesized to play role in the pathology of these conditions. To investigate if CSF could be used as a source of biomarkers, we evaluated normal and MPS I and IIIB affected dog samples. Additionally, to confirm lack of immune response, we also assessed samples of animals treated as part of a previously reported intravenous and intrathecal based recombinant enzyme threatment for MPS I, in which animals showed tolerance as a result of a enzyme replacement protocol begun during the neonatal period.

ELISA Quantikine® IFNγ, IL-10, and DuoSet® IL-12/IL-23 p40 kits (R&D Systems) were validated for CSF by spiking CSF samples with serial dilutions of the standard, resulting in 90-95% of the spiked samples consisting of CSF. All spiked CSF samples showed expected values when assayed. CSF samples of normal, MPS IIIB affected, MPS I affected, and MPS I treated dogs were then evaluated using these validated kits. These samples were also assayed with TNF-α, a kit previously validated for canine CSF. Cytokine levels for all samples were below the level of detection for TNF-α, IFNγ, and IL-10. Efforts were made to extend the standard curve to include lower values. However this was unsuccessful. Slightly elevated levels were seen in 2 of 23 animals tested using the IL-12/IL-23 p40; values ranged from 45 to 85 pg/ml, slightly above the minimum detectible level of 31.25 pg/ml. The IL-12/IL-23 p40 positive CSF samples derived from a normal untreated dog and an MPS I enzyme treated dog, so no trend as to IL-12 levels between the various groups could be documented. The treated dog received quarterly intrathecal injections of rhIDU, and showed elevated levels of 85, 81, and 45 pg/ml over the first fifteen months of treatment, though the levels decreased below the limit of detection by the 18th month.

Validation of these kits for canine CSF will aid future research to determine immune response in the nervous system. The kits are limited to detecting elevated levels of cytokines in CSF. Normal dog CSF cytokine levels are absent or below the limit of detection with these assays. Although the levels for this particular study were not

significantly elevated, these findings support our initial immune tolerance findings in that they do not document elevated CSF cytokine levels.

ABSTRACT #185
MAGNETIC RESONANCE IMAGING FINDINGS IN 60 DOGS WITH CERVICAL SPONDYLOMYELOPATHY. Ronaldo C. da Costa[1], Joane Parent[2]. [1]College of Veterinary Medicine, The Ohio State University, Columbus, OH, [2]Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, QC.

Medical and radiological records of dogs having cervical spondylomyelopathy (CSM) were searched between 2003 and 2006. Inclusion criteria was a definitive diagnosis of CSM achieved by magnetic resonance imaging (MRI) in large or giant breed dogs with clinical signs of cervical spinal disease. Our goal was to report the frequency of spinal changes seen on MRI of CSM cases.

Sixty dogs met the inclusion criteria. Forty three (71.7%) were large breed dogs and 17 (28.3%) were giant breed dogs. Thirty one of the 43 large dogs were Doberman pinschers. Their median age was 6.5 years (mean 6.8) with 18 males and 13 females. The 12 other large dogs were 3 Weimaraners, 2 Dalmatians, 3 mixed breed, 1 Labrador, 1 Boxer, 1 German shepherd and 1 Pit Bull. Their median age was 9 years (mean 8.7) with 8 males and 4 females. Among the giant dogs, 9 were Great Danes, 3 Bernese mountain dogs, 2 Rottweilers, 1 Irish Wolfhound, 1 Mastiff and 1 Swiss Mountain dog. Their median age was 2.8 years (mean 3.6) with 14 males and 3 females.

MRI findings in Dobermans revealed that 15 dogs had a single site of spinal cord compression while 15 dogs had 2 or more compressive sites (1 dog had only intervertebral foraminal stenosis). The main compression was located at C6-7 in 15 dogs, C5-6 in 14 dogs, and C4-5 in 1 dog. The compression was caused solely by intervertebral disk protrusion in 20 dogs, disk protrusion and ligament hypertrophy in 5 dogs, disk protrusion and osseous compressions (facet arthritis or malformation) in 3 dogs, and purely osseous in 2 dogs. Eighteen of the 30 dogs with cord compression (60%) had spinal cord increased signal intensity (ISI) on T2-weighted images.

MRI findings in other large breed dogs revealed that 5 dogs had a single compression, while 7 dogs had 2 or more compressions. The main compression was located at C6-7 in 5 dogs, C5-6 in 5 dogs and C4-5 in 2 dogs. The compression was caused by disk protrusion in 6 dogs, disk protrusion and osseous compressions in 4 dogs, purely osseous in 1 dog, and a combination of osseous and ligament hypertrophy in 1 dog. Six out of 12 dogs (50%) had spinal cord ISI.

MRI findings in giant breed dogs showed that 6 dogs had a single compression, while 11 dogs had two or more compressive sites. The main compression was at C6-7 in 7 dogs, C5-6 in 6 dogs, and C4-5 in 4 dogs. The compression was caused by osseous malformation and/or facet arthritis in 15 dogs, intervertebral disk protrusion in 1 dog, and a combination of disk protrusion and ligament flavum hypertrophy in 1 dog. Eight out of 17 dogs (47%) had ISI within the spinal cord.

The results show that large breed dogs (Dobermans and non-Dobermans) commonly have intervertebral disk-associated spinal cord compression (38 out of 42 dogs, 90%), with the compression most often located at C5-6 or C6-7 (92.8% of dogs). In contrast, most giant breed dogs (88%) had osseous compressions caused by lamina or pedicle malformation or facet arthritis. Increased signal intensity within the spinal cord was seen in approximately 50% of all dogs.

ABSTRACT #186
PHOSPHORYLATED NEUROFILAMENT NF-H AS A BIOMARKER OF SPINAL CORD INJURY IN DOGS. Hiroaki Kamishina[1], Yui Kobatake[1], Masaaki Katayama[1], Yutaka Momota[2], Jun Yasuda[1], Reeko Sato[1], Takashi Uemura[3], Gerry Shaw[3]. [1]Iwate University, Morioka, Iwate, Japan. [2]Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan. [3]University of Florida, Gainesville, FL.

Phosphorylated neurofilament NF-H is the major structural protein abundantly present in large-diameter axons of the spinal cord. Following injury, significant amounts of NF-H are expected to be released into the cerebrospinal fluid (CSF) and blood. The objectives of this study were to establish an ELISA assay that reliably quantifies canine NF-H in CSF and blood, and to evaluate the significance of NF-H as a biomarker of spinal cord injury (SCI) in dogs.

The specificity of the ELISA antibodies to canine NF-H was tested by immunohistochemistry and western blotting. Canine spinal cord NF-H was isolated and serially diluted samples were used to quantify the NF-H levels. CSF and plasma collected from 5 normal dogs and 20 dogs with SCI (acute thoracolumbar intervertebral disc disease) were used for NF-H measurements. In SCI dogs, plasma NF-H levels and hindlimb locomotor function were evaluated up to 3 months post-injury.

The assay was confirmed to specifically and quantitatively measure canine NF-H. NF-H was readily detectable both in CSF and plasma of dogs with SCI, but undetectable in normal dogs. In most cases, NF-H levels correlated with hindlimb functional scores; dogs with higher NF-H levels had lower functional scores (severe signs), however, some exceptions existed. The plasma NF-H level decreased by 4 weeks post-injury and returned to baseline by 3 months post-injury when maximum functional recovery was achieved. These findings suggest that measurement of NF-H arrows assessment of lesion severity and may aid in judging prognosis.

ABSTRACT #187
EPIDURAL AND INTRADURAL SCARRING POST LAMINECTOMY AND DUROTOMY: A COMPARISON OF GELFOAM AND SENTRX FILM. NJ Olby, J-H Lim. North Carolina State University, Raleigh, NC.

Many neurosurgical procedures involve performing a laminectomy or craniotomy and durotomy, with the potential for post surgical scars to form both epidurally and intradurally. This scarring can cause disturbance of CSF flow and compression of the brain or spinal cord parenchyma. Many different materials have been developed to limit formation of scar tissue in the central nervous system but some of these materials are very expensive. Products based on cross-linked hyaluronic acid have been developed by SentrX Animal Care to promote healing while limiting scar formation. The aims of this study were to use magnetic resonance imaging (MRI) to compare epidural and intradural scar formation after hemilaminectomy and durotomy in dogs following application of either gelfoam or SentrX film.

Hemilaminectomies and durotomies were performed at L1/2 and T12/13 in six dogs. A piece of gelfoam or SentrX film was placed over each site before closure. MRI of the surgical sites was performed at 24 hours, 2, 6 and 12 weeks postoperatively. Axial and sagittal T2 weighted, T2 haste, T1 pre and post contrast and STIR images were obtained. Images of both hemilaminectomy sites were evaluated for the number of slices in which there was soft tissue within the epidural space, interruption of the subarachnoid space and distortion of the outline of the spinal cord. The identity of the film used at each site was revealed and the parameters measured compared between SentrX film and gelfoam using the students' t test with p values <0.05 taken as significant.

All dogs recovered from surgery well with mild or no deficits in proprioceptive placing after two weeks. The combination of imaging sequences allowed evaluation of soft tissue within the epidural space (T2 and T1 weighted images), the integrity of the subarachnoid space (T2 haste images) and the outline of the spinal cord (T2 weighted images). At two weeks after surgery there was contrast enhancing soft tissue within the epidural space in all hemilaminectomy sites regardless of treatment. By six weeks, the contrast enhancement had disappeared but distortion of the spinal cord was evident with the cord pulled towards the dura mater at the durotomy site by intradural soft tissue. There was consistently less soft tissue within the epidural space and less interruption of the subarachnoid space and spinal cord distortion in the sites treated with SentrX film. This difference was statistically significant when the distance over which soft tissue was present within the epidural space was compared between treatments, but significance was not achieved for the number of slices in which there was distortion of the spinal cord or interruption of CSF flow. In conclusion, SentrX film is a safe and effective alternative to gelfoam for placement over a durotomy site and within a hemilaminectomy defect to limit postoperative scarring.

ABSTRACT #188

BREED SPECIFIC POLYMYOSITIS IN THE HUNGARIAN VIZSLA DOG. AC Haley[1], SR Platt, M Kent, SJ Schatzberg, A Durham, S Cochrane, GD Shelton. [1]University of Georgia, College of Veterinary Medicine, Athens, GA. [2]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA. [3]Veterinary Emergency Clinic and Referral Centre, Toronto, Ontario, Canada, [4]University of California, San Diego, Department of Pathology, School of Medicine, La Jolla, CA.

Inflammatory myopathies are relatively common in dogs and may have a focal (masticatory muscle myositis) or generalized (polymyositis) distribution. Recently, a breed specific myositis presenting as pharyngeal dysphagia and masticatory muscle atrophy has been described in 14 Hungarian Vizsla dogs in the United Kingdom (Foale et al, BSAVA 2008). Here we report a similar syndrome in 5 Vizsla dogs from the North America. This retrospective study provides a preliminary clinicopathologic description and outcome in these 5 dogs.

Five Viszla dogs presented to 5 different veterinary hospitals (1 general practice, 2 referral practices, 2 university practices) with clinical signs of dysphagia (3/5), regurgitation (3/5), excessive salvation (3/5), masticatory muscle atrophy (4/5) and pain on opening the jaw (2/5). All dogs were male (4/5 castrated, 1/5 intact) and ranged in age from 1 to 9 years (mean 5.2 years). Creatine kinase activity was measured in 3 cases and was elevated (range 1061–9758 U/L; mean 5443). Antibodies against type 2M fibers and acetylcholine receptors were negative in those dogs tested (3/3 and 2/2 respectively). Three dogs had radiographically evident megaesophagus (ME). Two dogs had ME at the time of initial presentation while one dog developed ME 19 months after onset of dysphagia. Serum antibody titers against *Toxoplasma gondii* and *Neospora canis*, *Borrelia burgdorferi, Ehrlichia canis* and *Ehrlichia equi* were negative when tested. Electromyography was performed in 2 dogs with no abnormalities. Histopathologic examination of temporalis muscle biopsies was performed in 3 cases with multifocal areas of lymphocytic infiltration in 2 cases. Although cellular infiltrates were not evident in one case, bilateral multifocal hyperintensities in the temporalis muscle were observed on T2-weighted magnetic resonance images (MRI). A complete necropsy was performed on the fifth case and chronic lymphohistiocytic and plasmacytic myositis and fibrosis was evident in the esophagus, myocardium and skeletal muscle. Immunosuppression with prednisone and azathioprine has not resulted in clinical improvement at 2 and 3 months follow up in 2 dogs. Two dogs were lost to follow up. In conclusion this study, in combination with the previous report from the UK, should alert clinicians to the occurrence of a new breed associated polymyositis in the Vizsla dog which warrants further investigation to elucidate pathogenesis, genetic associations, response to treatment and prognosis.

ABSTRACT #189

MUTATIONAL ANALYSIS OF DYSTROPHIN-DEFICIENT MUSCULAR DYSTROPHY IN CAVALIER KING CHARLES SPANIELS. G. L. Walmsley[1], V. Arechavala-Gomeza[2], M. Fernandez-Fuente[1,2], N. Nagel[3], R. Stanley[1], K. Chandler[1], F. Muntoni[2], G. D. Shelton[4] and R.J. Piercy[1,2]. [1]Department of Veterinary Clinical Sciences, Royal Veterinary College, London, UK; [2]Dubowitz Neuromuscular Centre, UCL Institute of Child Health London, UK; [3]Northdale Veterinary Practice, West Sussex, UK; [4]Department of Pathology, School of Medicine, University of California San Diego, La Jolla, CA.

Canine dystrophin-deficient muscular dystrophy, analogous to Duchenne muscular dystrophy of humans, is a severe inherited degenerative disorder of striated muscle. This debilitating and ultimately fatal condition results in a progressive destruction of skeletal and cardiac muscle due to mutations in the gene encoding dystrophin, a structural protein that links the contractile apparatus to the sarcolemma. The disorder is seen in several canine breeds but the genetic cause has only been reported in the Golden Retriever, German Short-haired Pointer and Rottweiler. Here we present the findings of clinical, histopathological and molecular characterisation of this condition in Cavalier King Charles Spaniels.

A 10 month old male neutered client-owned Cavalier King Charles Spaniel from the United Kingdom was presented with a chronic progressive history of lethargy, exercise intolerance and dysphagia. The dog was tetraparetic with poor skeletal muscle mass (body condition score=2/9), reduced spinal reflexes, macroglossia and restricted jaw movement. Investigations documented a marked elevation in creatine kinase activity (33,695 U/l; 61–394 U/l) and electromyography revealed spontaneous activity indicative of a primary muscle disorder (complex repetitive discharges and pseudomyotonia). Dystrophin-deficient muscular dystrophy was diagnosed on the basis of skeletal muscle histopathology, immunohistochemistry and immunoblotting using monoclonal antibodies to the dystrophin rod and carboxy termini. Oligonucleotide primer pairs designed for RT-PCR to amplify overlapping regions of dystrophin cDNA identified, following sequencing, an exon deletion and a frame-shift not present in control cDNA, that is predicted to result in premature termination of the protein product. Sequencing the associated genomic DNA confirmed the causative (and novel) mutation. The ability of antisense oligonucleotide induced exon skipping to restore the reading frame was demonstrated *in vitro* in cultured myoblasts from the affected dog. Sequencing of amplified DNA from an additional Cavalier King Charles Spaniel with dystrophin-deficient muscular dystrophy from North America identified the same mutation.

In conclusion, dystrophin deficient muscular dystrophy in the Cavalier King Charles Spaniel may provide an excellent model for Duchenne muscular dystrophy due to the potential application for trials of antisense oligonucleotide-mediated exon skipping – one of the more promising research directions for genetic therapy in this fatal disorder.

ABSTRACT #190

FREQUENCY OF THE CANINE EXERCISE INDUCED COLLAPSE GENE IN DIVERSE BREEDS. KM Minor[1], EE Patterson[1], SD Gross[1], MK Keating[1], SM Taylor[2], GS Johnson[3], K Todd-Thompson[4], KJ Ekenstedt[1], JM Mickelson[1]. [1]University of Minnesota College of Veterinary Medicine, St. Paul, MN. [2]Western College of Veterinary Medicine, Saskatoon, Saskatchewan. [3]University of Missouri-Columbia College of Veterinary Medicine, Columbia, MO. [4]University of Texas San Antonio School of Medicine, San Antonio, TX.

A (G767T) dynamin 1 (*DNM1*) mutation in Labrador retrievers is highly associated with the syndrome of exercise-induced collapse (EIC). *DNM1* is essential for synaptic vesicle endocytosis. We previously reported a 37% carrier frequency with 3% homozygous affected dogs from a field-trial population of 400 dogs. The aim of this present study was to determine the frequency of the mutation in other Labrador sub-populations, as well as closely and distantly related breeds,

Breeds were chosen based on genetic marker clustering data, and/or breeds with readily available DNA samples with the goal of testing 200 or more dogs per breed. When possible, individuals selected were unrelated to the grandparent generation.

In all Labrador sub-populations (conformation, pet, and service), homozygous affected dogs and 30–40% carriers of the mutation were found. The mutation was also detected in Chesapeake Bay and Curly-coated retrievers (carrier frequency >10%) with clinically affected homozygous individuals in both breeds. A few carrier Welsh Pembroke Corgis (n=10) were also identified but we have not found any affected Corgis to date.

The mutation was not observed in Golden, Flat-coat or Duck tolling retrievers, nor in a number of other less related breeds (Newfoundlands, American water spaniels, Portugese water dogs, Jack russell terriers, Wheaton terriers, or Border collies). Ten Border collies (all mutation negative) had recurrent, unexplained collapse.

The *DNM1* mutation is prevalent in all Labrador sub-populations. A comprehensive program to carefully eliminate the mutant allele while still preserving genetic diversity and also accounting for reduction of other inherited diseases, could be implemented.

ABSTRACT #191

BRDU LABELING PATTERN OF THE ROSTRAL MIGRATORY STREAM IN NORMAL CANINE AND FELINE BRAINS. M Lewis[1], S Malik[2], T van Winkle[1], DJ Watson[2], CH Vite[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA. [2]Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA.

In the human and rodent brain, a track of proliferative cells known as the rostral migratory stream extends from the subventric-

ular zone (SVZ) of the lateral ventricle towards the olfactory bulb. This is the migratory pathway for SVZ neural progenitor cells which eventually differentiate into olfactory interneurons. We have characterized the pattern of proliferative cells in the normal canine and feline brain using 5-bromo-2-deoxyuridine (BrdU) labeling. All procedures were approved by the IACUC. BrdU was administered intravenously to dogs (n=2, 75 mg/kg) or cats (n=5, 25 mg/kg) every 24 hr for 5 days. The dogs were perfused with saline followed by formalin and the brains were removed 1 or 5 days after the final BrdU infusion. Cats were perfused at 1 or 10 days followed the final BrdU infusion. Two additional dogs and one cat received a single dose of 75 mg/kg and were perfused 6 hr later. Animals that did not receive BrdU were used as controls. Frozen sagittal sections were prepared on a cryostat and stained with Nissl, hematoxylin and eosin, Luxol fast blue, anti-BrdU antibody, and anti Ki67 antibody. In canine and feline brains, a prominent, dense, continuous track of Nissl-stained cells was found, beginning in the subependymal layer at the base of the lateral ventricle, curving around the caudate nucleus and continuing ventrally to the olfactory peduncle. In brains from dogs and cats that received multiple doses of BrdU, we found BrdU-immunoreactive cells in the same track, continuously present from the ventral wall of the lateral ventricle to the rostral olfactory peduncle. The BrdU-labeled nuclei were single or in closely apposed small groups. These cells were not stained in control sections on which the primary (anti-BrdU) antibody was omitted. A similar pattern of staining was found in animals receiving one dose of 75 mg/kg, indicating cell division continuously along the track. Ki67 staining was identical to that seen in the BrdU stained sections. The distribution of dividing cells in the canine and feline brain is similar to the pattern of migrating subventricular zone neural progenitors in the rodent and human brains. The substantial size of this track in the dog and cats brain likely reflects the importance of the olfactory system in these animals. We will present our ongoing immunohistochemical studies in these and additional animals to describe the phenotype of these cells and the anatomical route and dimensions of the track.

ABSTRACT #192

INTRATHECAL ENZYME THERAPY IN MUCOPOLYSACCHARIDOSIS I CATS REDUCES STORAGE THROUGHOUT THE BRAIN. M Haskins[1], P Wang[1], S Walkley[2], J Rhodes[1], P O'Donnell[1], C Bryan[1], NM Ellinwood[3], R Cahayag[4], A Cheng[4], C Henschel[4], CA O'Neill[4], J White[4], CH Vite[1]. [1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA; [2]Albert Einstein College of Medicine, Bronx, NY; [3]Iowa State University, Ames, IA; [4]BioMarin Pharmaceutical Inc., Novato, CA.

Mucopolysaccharidosis (MPS) I is a lysosomal storage disease caused by deficient activity of the lysosomal hydrolase a-L-iduronidase (IDUA). The most common subtype in human patients has multisystemic disease including severe mental retardation associated with storage of glycosaminoglycans (GAGs) in the central nervous system (CNS). An orthologous cat model of MPS I has widespread storage of GAGs in the CNS. Approved clinical therapy for MPS I is weekly intravenous enzyme replacement with recombinant human IDUA (Aldurazyme; ALD) which is expensive and does not result in cure of the neurological disease. This study was designed to determine if intrathecal administration of ALD could alter the CNS lesions and how long the effect would persist.

Twelve MPS I cats between 18 and 27 months of age were divided into 4 groups. The cats were anesthetized with IV propofol, intubated, placed in lateral recumbancy, and ~2 cc of CSF collected. Two of the three cats in each group received intrathecal injections of 0.1 mg/kg of recombinant human α-L-iduronidase (Aldurazyme;ALD) in Elliots B solution on a Monday, Friday, and Tuesday. The remaining cat was treated with an equivalent volume/kg of Elliots B vehicle. Group 1 was sacrificed 2 days following the last injection; groups 2, 3, and 4 at 1 month, 2 months, and 4 months, respectively. The 4-month enzyme and GAG data are being analyzed together with 2- and 4-month immunocytochemistry and all the quantitative ganglioside data. Three cats given ALD and one given vehicle alone developed abnormal posture with lowered forelimbs, almost resting on their elbows. IDUA was not detected in CSF or blood from 2–28 days post-treatment. Brain IDUA activities were 4.2- and 3.8-fold normal at 2 days, dropping to 17% and 29% of normal at 1 month, and to affected levels by 2 months. Brain GAG levels were dramatically reduced at 2 days and 1 month, and were 30% and 48% below affected levels at 2 months. Blinded interpretation of brain ganglioside (GM2, GM3) immunohistochemistry and unesterified cholesterol showed a qualitative reduction at 2 days. These preliminary data indicate a need to repeat intrathecal injections at 2–3 month intervals to maintain suppression of GAG storage.

ABSTRACT #193

NEURAL STEM CELL SOURCES IN ADULT DOGS. JH Lim, NJ Olby, CL Mariani. North Carolina State University, Raleigh, NC.

Understanding adult neurogenesis is important to fully understand the potential for regeneration within the central nervous system (CNS), and to identify neural progenitors that could be used clinically for transplantation. We investigated whether neurospheres could be isolated from the adult canine CNS and whether adipose tissue-derived stromal cells (ADSCs) could be induced to differentiate into neural lineages.

Brain tissue was obtained from the dentate gyrus and the subventricular zone, and subcutaneous adipose tissue was collected from cadavers of adult dogs aged from 1 to 6 years. Following enzymatic dissociation, cells were cultured in appropriate media to produce neurospheres (brain tissue) or ADSCs (adipose tissue). Third passage ADSCs were induced to adipogenesis, osteogenesis and neurogenesis by addition of appropriate inductive agents. RT-PCR analyses were performed to identify neural progenitor cell surface markers in the induced ADSCs and neurospheres. Expression of neuronal lineage markers was evaluated immunohistochemically.

Neurospheres and ADSCs grew rapidly from the brain and adipose tissue respectively. Morphologically the ADSCs had fibroblast-like characteristics and could be induced to produce adipogenic and osteogenic lineages. Under neurogenesis culture conditions, ADSCs formed spherical cellular aggregates that resembled neurospheres. RT-PCR analyses revealed that these aggregates showed a similar phenotype to brain-derived neurospheres with expression of Sox2 and CD90. Neurospheres from both sources expressed Nestin, GFAP and Tuj1. In conclusion, we cultured neurospheres from adult canine brain tissue and generated neural progenitor cells from canine adipose tissue. The ADSCs have the potential for future autologous cell transplantation therapy for neurological disorders.

ABSTRACT #194

DOGS WITH CORTICAL BETA-AMYLOID PATHOLOGY HAVE LESS SEROTONERGIC NEURONS IN THE ROSTRAL RAPHE NUCLEI THAN AGED MATCHED HEALTHY CONTROLS. V Bernedo[1], D Insua[2], G Santamarina[1], ML Suárez[1], M Sarasa[2], P Pesini[2]. [1]Department of Veterinary Clinical Sciences, University of Santiago, Lugo, Spain. [2]Araclon Biotech Ltd. Zaragoza, Spain.

Dogs may naturally suffer an age-related cognitive dysfunction, which is sometimes described as the canine counterpart of Alzheimer disease (AD). This disorder reproduces key neuropsychological and hystopathological features of AD. Recently, we have found that dogs with the canine counterpart of AD lose noradrenergic neurons in the A6–A7 brainstem groups. However, no studies have previously investigated whether the serotonergic system is affected in this canine condition.

In the present study, we have used unbiased stereological procedures to estimate the number of the dorsal and median raphe nuclei (DRN and MRN, respectively) serotonergic neurons immunolabeled with an anti-tryptophan hydroxylase (TrH) monoclonal antibody in young and aged dogs without beta-amyloid (Aβ) cortical deposits and in aged dogs with Aβ cortical deposits.

No significant variations were found between young and aged dogs without Aβ cortical deposits. In contrast, aged dogs with Aβ cortical pathology had 33% fewer serotonergic neurons in the DRN and MRN than aged dogs without Aβ cortical deposits ($p=0.01$). However, in spite that the effect size of cortical beta-amyloid pathology on the number of serotonergic neurons could be considered

large in statistical terms, these two variables did not correlated significantly.

These results suggest that degeneration of the serotonergic neurons could be involved in the cognitive damage that accompanies Aβ cortical pathology in the dog but it cannot be satisfactorily explained as secondary to cortical pathology in the way that the degeneration of the cholinergic and noradrenergic neurons in AD is generally explained. It should be considered that in the loss of serotonergic neurons other mechanisms might be implicated.

ABSTRACT #195
EVALUATION OF THE CARCINOEMBRIONIC (CEA) AND CARBOHYDRATE 15-3 (CA 15-3) ANTIGEN IN MALIGNANT MAMMARY TUMORS IN BITCHES. CM Oliveira, FHM Jardini. School of Veterinary Medicine – University of São Paulo, Brazil.

In last years, through the use of biomarkers produced in cancerous tissue, diagnostics and therapeutics of human breast cancer changed significantly. These markers used in diagnosis, prognosis and monitoring of the tumor are able to signal biological behavior of cancers. Several substances are related to human breast cancer being CEA and CA 15-3 currently used. However in veterinary medicine these substances are not used regularly and there is a curiosity to investigate whether those biomarkers may be recommended in animals in the same manner as in humans.

Serum samples of 39 bitches, aging 5 to 13 year-old, with mammary tumors were collected immediately before and 2 weeks, 1, 3, 6 and 12 months after surgery, and frozen (−20 °C). Fragments of the tumors were collected during surgery and fixated in 10% formalin and included in paraffin up to 48 hours after excision. The animals had radiographs and ultrasound scans done 3, 6 and 12 months after surgery in order to look for metastasis. The serum quantification of CEA and CA 15-3 was done by sequential immunometric chemoluminescence. For the detection of CEA in mammary- and lymph node tissue a commercial test kit (Dako®, LSAB2) was used; fragment of human breast cancer was used as control. Microscopic evaluation was based on coloration grades of Hercep test.

In none of the serum samples of the 39 bitches, with a primary complex and metastasis, CEA and CA 15-3 were found. Those findings may suggest that (1) none of the tumors produced the markers, (2) the markers were produced but not released, (3) biomarkers were in the blood but structural differences did not allow interaction with antibodies, or (4) other factors could interfere with biomarkers concentrations in the blood. There was no expression of CA in 1:100 and 1:200 dilutions on the tumoral fragments, independent of their histological type. The negative result may be explained by (1) no expression of CA, (2) no production of that protein, (3) production of the protein, but not detected by the antibodies, (4) the methodology did not allow an adequate interaction, or (5) the protein concentrations were inadequate to detect CA. Thus, findings observed in the present study showed that evaluation of CEA and CA 15-3, in serum and tumor tissue, were not adequate for dogs.

ABSTRACT #196
EXPRESSION OF HER-2/NEU PROTEIN IN MAMMARY TUMORS OF BITCHES. CM Oliveira. School of Veterinary Medicine – University of São Paulo, Brazil.

Mammary neoplasias in bitches make out an important portion of canine tumors. But different than in humans, early diagnosis has not been done and treatment usually has been limited to its excision. In humans knowledge of tumor markers, it is important to recognize markers for helping in diagnosis, in staging and as well as to evaluate prognosis, therapy and remission. Her-2 (human epidermal growth factor receptor-type 2) is one of these markers; it codifies synthesis of $p185^{erb}$ (Her-2/neu), a protein expressed in 30% to 40% of human breast cancers and is associated with a bad prognosis. Her-2 has raised some interest as an indicator to evaluate the sensibility to chemotherapy and it is associated to histology grade and survival rates. It is known that treatment with Herceptin ^{R} is only effective on tumors overexpressing Her-2 at their surface.

The objective of this study was to verify the expression of Her-2 in malignant and benign mammary tumors of bitches.

Fragments of tumors from 51 bitches where fixated in 10% formalin and included in paraffin, prepared and stained with Hematoxilin-eosin. Following tumors were identified: benign (n=10) and malignant (n=41). Detection of Her-2 was performed by immunohistochemistry (ABC) with streptavidine peroxidases. Results were classified in accordance to intensity of color and were denominated as negative (scores=0 or 1+) and positive (scores=2+ [weak] or 3+ [strong]). From those 51 tumors, Her-2/neu was present in 76.5% of the cases (n=39) and absent in 23.5% (n=12). The positive cases that expressed that protein, 79.5% were from malignant tumors and 20.5% were benign. Of the 51 analyzed tumors, the expression of Her-2/neu was positive (scores=2+ or 3+) in 58.8% and negative (scores=0 or 1+) in 41.2%. In conclusion benign and malignant tumors of bitches expressed Her-2/neu protein; however only malignant tumors presented strong positive expression of Her-2/neu.

ABSTRACT #197
CERULOPLASMIN CONCENTRATIONS IN DOGS WITH MULTICENTRIC LYMPHOMA UNDERGOING CHEMOTHERAPY. SRR Lucas, A. Merlo, RMS Mirandola, TP Gasparin. School of Veterinary Medicine and Animal Science – University of São Paulo, SP, Brazil.

Acute phase proteins (APPs) are synthesized under stimulation of cytokines such as IL-1, IL-6 and TNF-α. and represent a very fast and nonspecific response that occurs following inflammatory, infectious and neoplastic conditions in dogs. Ceruloplasmin (Cp) is a positive APP, responsible for the transport of copper and protection of cells and tissue against oxidant compounds. The aim of this study was to evaluate Cp concentrations at diagnosis and during chemotherapy in dogs with multicentric lymphoma (ML). Cp was measured using orto dianisine technique, in two groups of dogs: 10 healthy dogs (control) and 13 dogs with ML. All dogs were submitted to chemotherapy. (approved by the Bioethics Committee). Dogs with signs of concurrent disease or that were previously treated with prednisone were excluded from the study. Cp measurement was done before treatment and once a week, during the first month of chemotherapy, and each 3-week intervals until the relapse for dogs with ML, and until the 16^{th} week in control dogs. ANOVA test followed by multiple Tukeýs tests were used to compare the groups. There was no difference between the mean of Cp concentration in dogs with ML at the diagnosis when compared to healthy dogs ($p > .05$). Levels of Cp decreased significantly at 4^{th} week when compared to the first week, but Cp increase was not observed at the relapse. At all other times during the treatment, Cp concentrations for dogs with lymphoma were not significantly different from controls submitted to chemotherapy. As conclusion, Cp levels in ML at diagnosis were similar to healthy dogs, decreased when lymphoma remission was achieved and there was no change at the relapse.

ABSTRACT #198
FLOW CYTOMETRIC PROGNOSTIC FACTORS IN CANINE B CELL LYMPHOMA. EC Marcus, SE Lana, AC Avery. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

Recent studies suggest that length of survival can be predicted based on the type of non-neoplastic infiltrating cell populations in human B cell lymphoma. However, these trends have not yet been investigated in canine diffuse large B cell lymphoma. In addition, prognosis in canine B cell leukemia can be predicted by cell size, but similar information is not available for canine B cell lymphoma. The purpose of this study was to evaluate lymph node aspirates from confirmed cases of canine B cell lymphoma via flow cytometry to determine if correlation exists among the number macrophages and T cells present, B cells size, survival and disease free interval.

The study population included all dogs admitted to the Veterinary Teaching Hospital with B cell lymphoma within a two year period that had lymph node aspirates assessed via flow cytometry. Follow up data including disease free interval and survival duration from 43 cases of B cell lymphoma was collected and compared to immunophenotype data collected via flow cytometry. Relative size of B cells was determined by measuring geometric mean linear forward scatter.

Preliminary data analysis suggests a negative correlation between B cell size and disease free interval. B cell size may provide valuable prognostic information to clinicians and clients as they assess treatment plans for canine B cell lymphoma patients.

ABSTRACT #199
DENAMARIN® FOR THE PREVENTION OF LOMUSTINE (CCNU) INDUCED HEPATOTOXICITY IN TUMOR BEARING DOGS: INTERIM ANALYSIS. GM Hammond, MS Kent, AM Irish, TA Guerrero, CO Rodriguez, KA Skorupski. Veterinary Medical Teaching Hospital, University of California, Davis, Davis, CA.

Liver enzyme elevations occur in up to 86% of dogs treated with CCNU. Therapy may be prematurely discontinued, delayed, or the drug dose decreased due to liver enzyme changes or concerns about developing hepatotoxicity.

S-Adenosylmethionine (SAMe) and silybin are commonly used hepatoprotectants. Denamarin® incorporates both SAMe and silybin in a patented veterinary formulated product.

The goal of this clinical trial was to evaluate Denamarin as a hepatoprotectant in tumor bearing dogs undergoing CCNU therapy. Reported are the results of a planned interim analysis after the enrollment of 30 dogs.

A prospective non-blinded, randomized clinical trial was performed at the UC Davis VMTH. Dogs prescribed CCNU chemotherapy and that had a normal pre-treatment ALT were eligible. Dogs were randomly assigned to either Group 1, receiving Denamarin starting at the time CCNU therapy was initiated, or Group 2, receiving Denamarin only if they experienced a grade 4 hepatoxicity.

The two groups had no differences in pre-therapy ALT, bilirubin or tumor type. The mean post-therapy ALT elevation for Group 1 and Group 2 dogs was 119 IU/L ($p=0.10$) and 815 IU/L ($p=0.046$), respectively. Mean post therapy bilirubin elevation for Group 1 and Group 2 dogs were 0.4 mg/dl ($p=0.16$) and 0.15 mg/dl ($p=0.07$). Therapy alterations occurred in 3 dogs in Group 2, whereas one dog in Group 1 had chemotherapy altered due to liver enzyme elevations.

This data indicates that dogs receiving Denamarin may have less severe liver enzyme elevations after CCNU therapy and supports continued enrollment in this prospective clinical trial.

ABSTRACT #200
EFFECTS OF MUTANT BETA-CATENIN ON HEPATOCYTE GROWTH HOMEOSTASIS. Timothy J. Stein, DVM, PhD and Eric P. Sandgren, VMD, PhD. Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI.

Beta-catenin, a dual-function protein integral to cell-cell adhesion and intracellular transmission of Wnt signals, is altered in numerous human cancers including hepatocellular carcinoma. The comparative hepatocyte growth assay is a transplantation-based growth assay developed to quantify the effects of genetic alterations on hepatocyte growth homeostasis. For this study the comparative hepatocyte growth assay was used to assess the effects of a mutant beta-catenin on hepatocyte growth homeostasis.

The comparative hepatocyte growth assay was used to quantify the effects of mutant β-catenin on the growth kinetics of transplanted hepatocytes in growth permissive and restrictive environments. Two populations of transgenic mice were generated for donor hepatocytes; one harboring a liver-specific inducible mutant beta-catenin+marker transgene (hPAP), and the other carrying only a marker transgene (LacZ). The growth of foci derived from hepatocytes expressing the mutant beta-catenin were compared to that of hepatocytes expressing only a marker transgene. The ability of mutant beta-catenin to give rise to clonal populations with extreme growth potential compared to normal hepatocytes was assessed through the determination of extreme outliers.

The expression of mutant β-catenin was unable to increase the growth of foci in growth permissive or restrictive environments. The production of foci with extreme growth potential was not different from control hepatocytes.

β-catenin does not affect measures of neoplasia assessed by the comparative hepatocyte growth assay. The contribution of beta-catenin to HCC is unique from that of other frequently identified genetic mutations in HCC, which have been shown to quantifiably effect hepatocyte growth homeostasis in this assay.

Previously presented at the Veterinary Cancer Society Conference, 2008.

ABSTRACT #201
COMPARISON OF COMPLETE BLOOD COUNT VALUES FOR SAMPLES COLLECTED USING TRADITIONAL AND MICRO-SAMPLING TUBES. JC Whittemore[1], B Flatland.[2] [1]From the Departments of Small Animal Clinical Sciences and [2]Pathobiology, University of Tennessee, College of Veterinary Medicine, Knoxville TN.

Anemia caused by diagnostic sampling is the main indication for blood transfusion in human neonates. Diagnostic sampling may pose the same risk to dogs and cats given their size. Microsampling tubes may be used to decrease sampling volumes; their accuracy under clinical conditions has not been reported.

Samples were collected from clinically healthy dogs (14) and cats (15). Three mL of blood were collected from each animal and transferred into EDTA Vacutainer™ (2.5 mL) and Microtainer™ tubes (0.5 mL) by a licensed veterinary technician blinded to the study purpose. Complete blood counts were performed within 12 hours on all samples. Parameters evaluated were RBC, HgB, Hct, MCV, MCH, MCHC, MPV, WBC, and plasma protein as well as neutrophil, lymphocyte, monocyte, eosinophil, basophil, and platelet counts. Results were compared by Pearson's correlation coefficients, Passing-Bablok regression analysis, Bland-Altman plots, and either paired t-tests or Wilcoxon Rank Sum (MCV, MCH) using MedCalc 10.0.1.

All data were normally distributed except MCV and MCH. Pearson's correlation coefficients were >0.9 for all analytes except monocytes (0.89), basophils (0.73) and eosinophils (0.77). There was no evidence of constant or proportional bias on Passing-Bablok regression analysis or Bland-Altman plots for any analyte. There were no significant differences on paired t-tests. Wilcoxon rank sum testing was significantly different ($p=0.002$) for MCV; however, this difference was not clinically significant.

These results suggest that Microtainer™ sampling tubes provide clinically equivalent complete blood count results to Vacutainers™ while minimizing total diagnostic sampling volumes.

ABSTRACT #202
AGING ASSOCIATED CHANGES IN BLOOD CELLS OF CATS. S Yu, MF Locniskar. Hill's Pet Nutrition, Inc., Topeka, KS.

The purpose of the present study was to investigate the aging effect on blood cells in healthy cats.

Complete blood cell count was measured with a CELL-DYN® 3700 (Abbott Dignostics) in 108 healthy American Domestic Shorthair cats. The measurements included RBC, Hb, PCV, MCV, MCH, MCHC, platelet, WBC, and WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). Cat age ranged from 1.5 to 16 years at the beginning of the study. Of these cats, 42 were males (6 intact and 36 castrated) and 66 were females (28 intact and 38 spayed). Cats were fed a dry cat food for four weeks before the blood cell measurements. The dry cat food met the AAFCO Cat Food Nutrient Profiles for adult maintenance.

RBC ($r=-0.62$, $p<0.01$), Hb ($r=-0.57$, $p<0.01$), PCV ($r=-0.61$, $p<0.01$), platelets ($r=-0.40$, $p<0.01$), lymphocytes ($r=-0.25$, $p=0.010$), and eosinophils ($r=-0.36$, $p<0.01$) were negatively while MCV ($r=0.30$, $p=0.002$), MCH ($r=0.35$, $p<0.01$), and neutrophils ($r=0.28$, $p=0.030$) were positively correlated with age in cats. Age had no effect ($p>0.05$) on MCHC, WBC, monocytes, and basophils.

These data suggest that cats have a tendency of macrocytotic anemia when they get old. Although aging did not affect WBC, increased neutrophils and decreased lymphocytes and eosinophils may suggest altered immunity with aging of cats. The clinical implications of aging associated changes in blood cells need further investigation.

ABSTRACT #203
CLOPIDOGREL THERAPY IN DOGS WITH IMMUNE-MEDIATED HEMOLYTIC ANEMIA. RL Haviland, N Pacifico, D Bianco. Red Bank Veterinary Hospital, Tinton Falls, NJ.

Thromboembolic events (TE) are a common complication of canine immune-mediated hemolytic anemia (IMHA). Canine IMHA has a high mortality rate, especially during the first two weeks of therapy. Clopidogrel (Plavix®) is a second generation antiplatelet drug that may have a synergistic effect with aspirin. This study reports the use of clopidogrel with or without ultra-low dose aspirin therapy in client-owned dogs with primary IMHA.

Dogs were diagnosed with IMHA based on the presence of at least 4 of 5 criteria: spherocytosis, positive saline autoagglutination test, positive direct Coombs' test, reticulocytosis, and hyperbilirubinemia. Secondary causes of IMHA were excluded based on history, physical examination, complete blood count, coagulation studies, serum chemistry, urinalysis, thoracic radiographs, abdominal ultrasound and vector-borne disease testing. Dogs with severe thrombocytopenia or treated with heparin or unable to tolerate oral medications were excluded.

After informed owner consent was obtained, twelve dogs with primary IMHA were treated with clopidogrel using a single loading dose of 10 mg/kg PO on day 1, followed by a maintenance dose of 2 mg/kg PO q24 hrs for 4 weeks. Five dogs received concurrently ultra-low dose aspirin therapy (0.5 mg/kg PO q24 hrs for 4 weeks). All dogs received the same initial treatment, including corticosteroids, doxycycline, and gastroprotectant agents. Additional immunosuppressive therapy, such as azathioprine and cyclosporine, were used in few dogs after 1 week of therapy. All dogs were closely monitored for any evidence of TE, change in appetite, hemorrhagic, gastrointestinal or dermatologic adverse effects over a 6-month period. Physical examination, complete blood count and serum chemistry were performed at 1, 3, and 6 months.

All dogs survived to discharge. Two dogs relapsed within 6 months of therapy, and one of them was euthanized. No evidence of thrombosis or hemorrhage was identified at necropsy. The other eleven dogs were still alive at the conclusion of the study, and no evidence of TE was identified over a 6-month period. No hemorrhagic, gastrointestinal, or dermatologic adverse effects attributable to clopidogrel were noted in any dog. Clinicopathologic abnormalities were considered secondary to immunosuppressive drugs. Clopidogrel, either alone or in combination with ultra-low dose aspirin therapy, was well tolerated in this group of dogs with primary IMHA. Larger prospective controlled studies should be performed to evaluate the efficacy and safety of clopidogrel therapy for prevention of TE in dogs with primary IMHA.

ABSTRACT #204
PLATELET P-SELECTIN (CD62P) EXPRESSION AND PLATELET-LEUKOCYTE INTERACTIONS IN CLINICALLY HEALTHY DOGS WITH AND WITHOUT ULTRALOW-DOSE ASPIRIN. SA Center, JF Randolph, MB Brooks, KS Sharpe, T Stokol, KL Warner. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Improved survival in canine IMHA has been achieved when ultralow-dose aspirin (ULDAsp, 0.5 mg/kg/day PO) is combined with glucocorticoids and azathioprine. However, it remains unclear whether this benefit reflects inhibition of thromboxane-induced platelet activation, modulatory effects on vascular endothelium and systemic inflammation, or altered drug-protein binding.

This study evaluated whether ULDAsp alters 2 flow cytometric parameters of platelet activation: P-selectin (CD62P) expression as a marker of alpha granule release and platelet-leukocyte aggregate (PLA) formation. We compared pre- and post-treatment basal platelet reactivity, and ex vivo platelet response to thrombin stimulation.

Whole EDTA-anticoagulated blood was collected from 10 clinically healthy dogs before and 24-hrs after 2 days of ULDAsp (0.5 mg/kg once daily). Ex vivo thrombin stimulation (1U/mL, 10 min, 37C) or vehicle-control treatment was followed by paraformaldehyde fixation. Platelets were identified by labeling with an antibody to the constitutive membrane antigen GPIIIa (CD61-FITC). Platelet P-selectin expression was detected by dual labeling with CD61-FITC and CD62P-PE; PLA formation was measured in RBC-lysed samples by dual labeling with CD61-FITC and a pan-leukocyte antibody (CD18-PE). Differences were identified using the Wilcoxon Signed Rank test with a two-sided $P=0.05$.

We found no difference in basal CD62P expression or PLA formation between pre- and post- ULDAsp treatment samples. Although ex vivo thrombin stimulation significantly increased CD62P expression and PLA from basal levels, ULDAsp did not significantly influence either response. Seemingly, monitoring CD62P or PLA would have low utility in assessing a pathophysiologic benefit from ULDAsp in dogs with IMHA.

ABSTRACT #205
EFFECTS OF ASPIRIN AND CLOPIDOGREL ON PLATELET FUNCTION IN HEALTHY DOGS. L Shearer, SA Kruth, D Wood. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Aspirin has been widely used in both human and veterinary patients for its anti-thrombotic properties. Several studies have evaluated the effect of aspirin on platelet function in dogs, with conflicting results. Clopidogrel is an effective anti-thrombotic therapy in humans, but has been minimally evaluated in dogs. The objective of this study was to assess the effect of various doses of aspirin and clopidogrel on platelet function in healthy dogs. Results were correlated with plasma drug metabolite concentrations.

Six healthy dogs were randomized to six treatment groups (aspirin: 0.5, 1.0, 2.0 and 10.0 mg/kg/d; clopidogrel: 2.0 and 4.0 mg/kg/d). Blood samples were collected on days 0, 3, and 7 and evaluated using optical aggregometry (ADP and PAF as agonists) and the PFA-100 analyzer (collagen-ADP and collagen-EPI cartridges). Day 7 plasma drug metabolite levels were measured using high performance liquid chromatography.

No statistically significant effect on platelet function was identified for the currently recommended anti-thrombotic aspirin dose (0.5 mg/kg/d) with either aggregometry or the PFA-100. ADP-induced aggregometry detected inhibition of platelet function by day 7 for the 1.0 and 2.0 mg/kg/d aspirin doses. No effect on platelet aggregation was detected for the 10 mg/kg/d aspirin dose. The PFA-100 detected significant inhibition of platelet function for all aspirin doses, except the 0.5 mg/kg/d dose, on both day 3 and 7 using the collagen-EPI cartridge. Inhibition of platelet function was also detected with the PFA-100 for the 2 and 10 mg/kg/d aspirin doses using the collagen-ADP cartridge. Both doses of clopidogrel yielded significant inhibition of platelet function with both methodologies (ADP agonist) on day 3 and 7.

Plasma drug metabolite levels for aspirin yielded variable results (0.5 mg/kg/d: 0.17–0.68 ug/mL; 1.0 mg/kg/d: 0.74–2.95 ug/mL; 2.0 mg/kg/d: 1.52–5.37 ug/mL; 10 mg/kg/d: 13.50–30.74). Some dogs had consistently low or high plasma metabolite levels regardless of the dose administered, suggesting variability in individual absorption. Measurement of drug metabolite levels for clopidogrel were less variable (2.0 mg/kg/d: 0.043–0.519 ug/mL; 4.0 mg/kg/d: 0.303–0.489 ug/mL), but displayed considerable overlap in the range of values between the two dose groups.

Currently recommended anti-thrombotic dosing of aspirin (0.5 mg/kg/d) is ineffective as measured by optical aggregometry and the PFA-100 analyzer in healthy dogs. Treatment with 1 mg/kg/d of aspirin was the lowest dosage to inhibit platelet function by both methodologies. Clopidogrel may be a reasonable anti-platelet therapy alternative. The anti-platelet effect of aspirin and clopidogrel was readily documented using the PFA-100. Ineffective anti-platelet therapy may be correlated with inadequate plasma drug metabolite concentrations. Plasma metabolite concentrations may subsequently be used to tailor anti-platelet therapy.

ABSTRACT #206
INFLUENCE OF RHFVIIa ON HAEMOSTATIC VARIABLES IN NORMAL AND FACTOR VII DEFICIENT DOGS. R Mischke[1], B Dörsch[1], M Diedrich[1], H-J Schuberth[2], M. Kietzmann[3], M von Depka[4]. [1]Small Animal Clinic, Hannover School of Veterinary Medicine, Hannover, Germany. [2]Institute of Immunology, Hannover School of Veterinary Medicine, Hannover, Germany. [3]Institute of Pharmacology, Toxicology and Pharmacy, Hannover School of Veterinary Medicine Hannover, Germany. [4]Werlhof Institute for Haemostaseology, Hannover, Germany.

Recombinant human factor VIIa (rhFVIIa) is widely used as a haemostatic agent in human medicine. The objective of this study

was to investigate the influence of rhFVIIa on different haemostastic parameters in normal and factor VII deficient dogs.

RhFVIIa was injected in 2 different dosages (100 and 500 µg/kg body mass [BM]) to either normal or factor VII deficient Beagle dogs (n=4–6). Factor VII:C activity, prothrombin time (standard test [PT_{ST}] and modified test [PT_{MT}]), activated partial thromboplastin time (APTT), platelet count and haematocrit as well as antibodies against rhFVIIa were measured at different time points.

Injections of rhFVIIa were well tolerated. The maximum values of factor VII:C activity which were measured 2 or 5 minutes after the rhFVIIa injection of 100 or 500 µg/kg were 4.5±0.25 I.U./mL (1 I.U./mL is equivalent to 100% activity) and 22.0±2.0 I.U./mL in healthy dogs and 4.7±0.28 I.U./mL and 19.1±1.3 I.U./mL in factor-VII-deficient dogs. Terminal half-life values lay between 1 and 1.5 hours. The coagulation activity measured by PT_{MT}, which allowed a more differentiated monitoring of rhFVIIa treatment than PT_{ST}, increased to values of approximately 1.5times (after 100 µg/kg) or 3times (after 500 µg/kg) the initial values ($p<0.001$). A significant shortening of APTT from 14.7±0.27 s to 13.0±0.35 s was restricted to the group of healthy dogs receiving the high factor VIIa dosage and lasted for 1.5 hours. This group also showed the most remarkable effects on platetet count and haematocrit. Antibodies against rhFVIIa developed in nearly all the dogs.

In conclusion, PT_{MT} is a useful screening test to monitor rhFVIIa treatment in dogs. Factor VII:C activity values and PT_{MT} results measured after injection of 100 µg/kg rhFVIIa indicate that lower dosages should be effective for treatment of bleeding episodes in factor VII deficient dogs. High rhFVIIa concentrations have to be used to affect the APTT in dogs indicating a possible effect in haemophilic dogs.

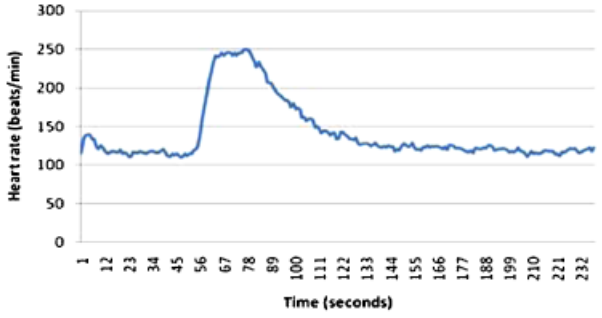
ABSTRACT #207

EXERCISE-ASSOCIATED HEART RATE RECOVERY TIME IN NORMAL DOGS. R Manley, E Côté, LA Pack. Atlantic Veterinary College, Charlottetown, PE, Canada.

Heart rate (HR) recovery time (HRRT) is the interval from peak HR during physical exertion to spontaneous return to resting HR. It indicates cardiovascular health and mortality risk in humans. We sought preliminary data that define normal HRRT in dogs.

Sixteen healthy dogs of various somatotypes, ages, and both sexes were outfitted with an electrocardiographic event recorder (King of Hearts Express, Alaris Medical). Each dog engaged in an exercise and cool-down protocol consisting of: 1 minute acclimation at the foot of a stairwell; climbing and immediately descending 6 flights of stairs (40 vertical feet; 12.25 m) at a brisk but comfortable pace, accompanied by a handler; and cool-down at the starting point. The procedures were repeated in triplicate with a minimum 30-minute rest period between each.

The procedure was tolerated by all dogs. The median resting HR prior to exercise was 110 beats/min. The median peak HR during exercise was 250 beats/min. Median duration of exercise was 52.5 seconds. The median HR decreased to 180 beats/min 40 seconds after onset of exercise; 145 beats/min 54 seconds after onset of exercise; and 135 beats/min 61 seconds after onset of exercise.



These results provide preliminary data that may be useful for assessing the cardiovascular function of dogs.

ABSTRACT #208

INFLUENCE OF AGE ON PULMONARY ARTERIAL PRESSURE IN HEALTHY BEAGLE DOGS: ANALYSIS BY RIGHT VENTRICULAR CATHETERIZATION, PULSED DOPPLER ECHOCARDIOGRAPHY AND PULSED DOPPLER TISSUE IMAGING. E. Mercier[1], M. Mathieu[2], C. Clercx[1], K. Mc Entee[1,2]. [1]Department for Clinical Sciences B44, Faculty of Veterinary Medicine, University of Liège, Belgium; [2]Laboratory of Physiology, Faculty of Medicine, Free University of Brussels, Belgium.

Pulmonary arterial pressure (PAP) increases with age in healthy human subjects and this has been attributed to vascular resistance; but no data exists in dogs. Pulmonary hemodynamics can be assessed directly by right heart catheterization, or indirectly by conventional Doppler-echocardiography and Doppler tissue imaging (TDI). The aims of this study were to assess the influence of aging on pulmonary hemodynamics and hemorheological properties in healthy dogs and to compare measurements obtained by cardiac catheterization, with systolic time intervals of pulmonary flow and myocardial velocities of the tricuspid lateral annulus.

Fourteen healthy experimental beagle dogs from two age groups were used in the study, 8 young dogs aged 10 months to 5 years (mean: 2.7 years) and 6 old dogs aged 8 to 15 years (mean: 12.1 years). Hematology, total protein and fibrinogen concentrations and whole blood viscosities were measured. Pulmonary flow, its maximal velocity, acceleration and ejection times were recorded by pulsed Doppler-echocardiography. Early (E′) and late (A′) diastolic myocardial velocities, isovolumic contraction velocity and systolic myocardial velocity of the free tricuspid annulus were measured by pulsed-wave TDI. Under general anesthesia, systolic (s), mean (m), diastolic (d) and occluded PAP, right atrial pressure and cardiac output were measured using a pediatric thermodilution Swan-Ganz catheter, while stroke volume, pulmonary vascular resistance (PVR), pulse pressure and compliance (C) were calculated. Values are given as mean±SE.

Hemorheological measurements were not different between the 2 groups. Systolic PAP (30,6±2,1 mmHg), mPAP (19.3±1.8 mmHg) and dPAP (13.4±1.4 mmHg) were higher in old dogs compared with young dogs (respectively 21.4±1,5 mmHg, 13.7±1,2 mmHg and 9.5±1.1 mmHg). This increase was attributed to a higher PVR and lower C. Acceleration and ejection times of pulmonary flow were not modified. The A′ wave was increased in old compared to young dogs (0.147±0.003 m/s vs 0.109±0.009 m/s, $p=0.02$) and the E′/A′ ratio was decreased (0.90±0.05 vs 1.13±0.06, $p=0.01$). Systolic, m and dPAP were correlated with age; PVR and C were inversely related. The A′ wave was correlated with age ($r=0.72$, $p<0.005$) and with sPAP.

Results of the present study show that (1) in healthy Beagle dogs, PAP increases with aging, (2) factors implicated in this increase are PVR and C, (3) systolic time intervals of the pulmonary flow are not sensitive to detect this increase, (4) the tricuspid free annulus late diastolic velocity increases with aging and is correlated with sPAP.

ABSTRACT #209

EVALUATION OF HDO® (HIGH DEFINITION OSCILLOMETRY) – A NEW NIBP TECHNIQUE – IN COMPARISON TO INVASIVE MEASUREMENT (HSE -HYDRO SACHS ELECTRONICS) IN ANESTHETISED DOGS. Ch Baumgartner[1], W Erhardt[1], Ch Faltermeier[1], J Reinert[1], J Henke[1], B Egner[2], I Haas[3]. [1]ZPF (Center of Preclinical Research), Technical University of Munich, Germany, [2]Medical Center for Small Animals, Hoerstein, Germany, [3]FZMB Bad Langensalza, Germany.

Precise measurement of high and low blood pressure situations is mandatory also in veterinary medicine. Even more if cardiac output situation is affected, like in arrhythmia or shock, accurate blood pressure information can be a vital parameter for treatment decision and survival. So far available NIBP technologies had clear limitations. Invasive techniques are regarded as gold standard however, these are not applicable for day-by-day routines.High Definition Oscillometry (HDO) was evaluated against direct line as a potential non-invasive approach. To investigate accuracy in the above situations, 7 beagle dogs where put under anesthesia (propofol) and blood pressure was affected by using either phenylephrin, dopamin or isufluran. Systolic, mean, diastolic arterial blood pressure (SAP/

MAP/DAP) and pulse/min (bpm) were determined 75 times within 120 Minutes per individual.

Mean, standard deviation (SD) and standard error of the mean (SEM) were calculated. The agreement of non invasive SAP and DAP with the corresponding simultaneous invasive values was analyzed according to the Bland-Altman method. Mean difference (bias) and SD of the difference (precision) were calculated. A significant correlation for SAP was observed ($r=0.895$; $p<0.001$). Mean difference between the two methods was 0.18 ± 9.15 mmHg. Also for DAP a significant correlation between the two methods was investigated ($r=0.875$; $p<0.001$), with a mean difference of 5.6 ± 7.3 mmHg. As these results reflect all situations including severe arrhythmia, high and low heart rate (49–210 bpm) and blood pressure within 67-310 SAP, HDO may be regarded as a clear alternative for invasive methods.

ABSTRACT #210
ACTION DURATION-RELATED DIURETIC EFFECTS AND RENNIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN HEALTHY DOGS WITH A FUROSEMIDE. Y Hori, N Ohshima, K Kanai, F Hoshi, N Itoh, S Higuchi. Kitasato University, Aomori, Japan.

This study investigated the difference in action duration-related diuretic effects and activity of rennin-angiotensin-aldosterone (RAA) system to a furosemide as a model of short-acting and long-acting loop diuretics.

Six dogs were anesthetized and were randomized to placebo, intravenous bolus administration (IB), and chronic rate infusion (CRI) groups. This study was conducted with crossover study. Furosemide, which was dissolved in sterile saline, was infused for 8 hours in CRI group, or was injected at 0- and 4-h in IB group. Total dosage of furosemide was 4 mg/kg and total amount of sterile saline was equal to each group at 18 ml/head. Blood and urine samples were collected at baseline, 1, 2, 4, 5, 6, and 8-h.

Compared with baseline, the IB group increased significantly the urine output at 1- and 5-h. In contrast, the CRI group increased significantly the urine output compared to baseline, and the increase persisted for 4 hours. Compared to placebo, the 8-h urine output and the 8-h sodium excretion were increased significantly in IB and CRI groups. Similarly, those of CRI group were significantly higher than those of IB group. Plasma aldosterone concentration was elevated significantly in IB group but not significant in CRI group.

Our results indicate that action duration may be a predominant cause of loop diuretics-related diuretic differences. In addition, persistent diuresis may cause mild and long-acting effects with minimal activation of the RAA system.

ABSTRACT #211
CHANGES IN THE PLASMA ANP AND NT-PROBNP CONCENTRATIONS IN HEALTHY DOGS WITH AN ACUTE VOLUME OVERLOAD. Y Hori, N Sano, K Kanai, F Hoshi, N Itoh, S Higuchi. Kitasato University, Aomori, Japan.

This study investigated the difference in the secretory responses of plasma ANP and NT-proBNP to an acute volume overload in dogs.

Six healthy dogs were anesthetized. Lactated Ringer's was infused intravenously at 90–100 ml/kg/h for 60 min as a volume overload. Subsequently, furosemide was administered as a preload reduction. The left ventricular (LV) pressures and pulmonary capillary wedge pressures (PCWP) were monitored at baseline and at 20, 40, 60, 80, 100, and 120 min.The plasma atrial natriuretic peptide (ANP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations were determined using a radioimmunoassay and enzyme immunoassay, respectively.

Volume overload significantly increased LV peak systolic pressure (LVPs), LV end-diastolic pressure (LVEDP), and PCWP. Preload reduction decreased the LVEDP and PCWP significantly. Volume overload increased the plasma ANP and NT-proBNP concentrations significantly. Preload reduction decreased the plasma ANP concentration significantly, but not the plasma NT-proBNP concentration. The plasma ANP concentration was strongly correlated with the heart rate, LVPs, LVEDP, and PCWP. The plasma NT-proBNP concentration was significantly correlated with LVPs and LVEDP.

The different responses in the natriuretic peptides may reflect different mechanisms regulating their secretion. The plasma ANP level may be a more useful parameter for the non-invasive estimation of the changes in acute volume overload than the plasma NT-proBNP level, especially when the hemodynamic state changes rapidly.

ABSTRACT #212
ANALYTICAL VALIDATION OF A COMMERCIALLY AVAILABLE FELINE N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE ELISA. A Carrier[1], A Beardow[1], G Farace[1], K Yeung[1], SJ Ettinger[2], K Yee[2]. [1]IDEXX Laboratories, Inc., Westbrook, ME, [2]California Animal Hospital, Los Angeles, CA.

An assay for feline N-Terminal Prohormone Brain Natriuretic Peptide (NT-proBNP) has been available since 2007 initially as CardioScreen™ NT-proBNP (Guildhay Ltd., UK) and now as Cardiopet™ proBNP (IDEXX Laboratories Inc, USA). The assay was initially commercialized by Guildhay with a time to result (the start to finish time of the assay) of around 20 hours. However in the US the assay has always been offered with a time to result of around 7 hours and to our knowledge a complete validation of this faster protocol has not been presented to date.

An analytical validation of assay covers a number of areas including accuracy – how do samples run on the faster assay correlate to the same samples run on the original assay; dynamic range – what is the lowest and highest concentrations of feline NTproBNP that can be reliably detected by the assay; precision – how consistent is the assay both within a single run (intra-assay precision) and across multiple runs on different days (inter-assay precision); interferences – do lipids, hemoglobin or bilirubin effect the performance of the assay and finally the sample types that can be analyzed, i.e. can NTproBNP be successfully detected in both serum and plasma.

The accuracy of the new method compared to the old method is very good with a correlation of $r^2=0.95$ and a linear slope of 0.91, both of these show that there is little difference between the two protocols.

The dynamic range of the assay is determined by serially diluting spiked samples to determine the minimum and maximum concentrations that can be reliably measured. The dynamic range was determined to be between ~20 and 1500 pmol/L.

Inter- and intra-assay precision was determined by running a set of spiked samples both in a single assay (minimum $n=10$) and in multiple assays ($n=3$). Inter- and intra assay precision was found to be between 10 and 20% across the dynamic range of the assay.

Spiking samples with lipids, hemoglobin and bilirubin were all found to have no effect on the assay performance.

A set of 20 matched serum and plasma samples did not show good agreement. No consistent bias or trend, that would have allowed a mathematical correction to be applied, was observed. We thus concluded that interpretive guidelines generated for plasma should not be applied to serum.

Overall, the kit is robust and the shortened protocol is acceptable as there appears to be no effect on the overall assay performance.

ABSTRACT #213
MEASUREMENT OF CANINE B-TYPE NATRIURETIC PEPTIDE USING A HUMAN ANALYZER. K Cyr, A Beardow, G Farace. IDEXX Laboratories, Inc., Westbrook, ME.

Canine and human B-type Natriuretic Peptide (BNP) are only moderately homologous (68%) and so it would seem unlikely that a human analyzer could be used to detect canine BNP. However there are regions of significant homology that might provide epitopes large enough to allow these assays to act as a semi-quantitative assay that could detect severe heart disease. To test this hypothesis we took an I-stat instrument (Abbot Laboratories, USA) and attempted to measure canine BNP either in spiked canine plasma or in real patient samples.

Samples spiked with synthetic human BNP-32 (Phoenix Pharmaceuticals, USA) showed expected results while samples spiked with synthetic canine BNP-32 (Phoenix Pharmaceuticals, USA) could not be detected even when spiked at levels of over 1000 pg/ml.

Samples from 4 dogs with cardiac disease (ISACHC class 1b or worse) were tested pet-side and once again no measurable BNP was detected in any of the samples.

This combination of failures to measure canine BNP would indicate that the regions homology of the two peptides are not sufficient to allow the antibodies used in the I-Stat assay to detect canine BNP. This confirms the view that the only avenue for a canine BNP assay is to have canine specific reagents.

ABSTRACT #214
INTRADAY VARIATION IN FELINE N-TERMINAL PROHORMONE BRAIN NATRIURETIC PEPTIDE CONCENTRATION. K Yee[1], J Allen[1], SJ Ettinger[1], A Beardow[2], A Carrier[2], G Farace[2], K Yeung[2]. [1]California Animal Hospital, Los Angeles, CA. [2]IDEXX Laboratories, Inc., Westbrook, ME.

N-Terminal Prohormone Brain Natriuretic Peptide (NT-proBNP) is a neurohormone and like other hormones it is possible that its circulating concentration in the blood of the patient could be cyclical in nature. If this is the case, then it is important to understand the nature and shape of the cycle since it could have a profound effect on the timing and interpretation of blood sample values.

Five normal cats were recruited for the study. Physical exam, thoracic radiographs, echocardiogram, systolic blood pressure, BUN, creatinine, TT4, and urine specific gravity were evaluated to determine health status of the cat and confirm there was no significant disease. Plasma samples were obtained over 24 hours at 9am, noon, 3pm, 6pm, midnight, 6am, and 9am. NT-proBNP concentrations were determined for each time point.

Four of the five cats showed no change throughout the time-course study; one cat did show some variability. However a one-way analysis of variance (ANOVA) repeated-measures design revealed no significant difference between the times of the day that samples were taken from the cats with $F(6,24)=1.42$; $p=0.25$.

The major limitation with the study is that the low levels on NT-proBNP mean that the assay is operating at the very limit of sensitivity; any minor well-to-well differences could mask some subtle variations.

In conclusion, this study suggests that there is no significant, measurable, cyclic (diurnal) variation of NT-proBNP in normal cats.

ABSTRACT #215
PLASMA ADRENOMEDULLIN AS A BIOMARKER FOR SEVERITY OF PULMONARY HYPERTENTION IN DOGS. N Kanno, K Asano, K Teshima, M Seki, K Edamura, S Tanaka. Nihon University, Kanagawa, Japan.

Plasma adrenomedullin (AM) concentration has been shown to increase in human patients with chronic heart failure (CHF). In addition, plasma AM level has been demonstrated to elevate in human patients with pulmonary hypertension (PH). We have reported that plasma AM is higher in dogs with CHF due to mitral regurgitation (MR) than in normal subjects. In this study, plasma AM was measured in dogs with MR and/or tricuspid regurgitation (TR) to evaluate whether canine AM has the potential for a useful biomarker as PH.

In this study, 76 canine patients including 52 with MR and 24 with MR and TR, and 10 healthy Beagles (control group) were used. The patients were assessed by physical examination, thoracic radiography and echocardiography, and categorized by International Small Animal Cardiac Health Council (ISACHC) classification. Plasma concentrations of AM were measured by a radioimmunometric assay. In the echocardiographic examination, TR velocity $\geq$2.8 m/second was considered to indicate PH.

The plasma level of AM significantly increased in proportion to the severity of CHF in dogs with MR. Plasma AM level in dogs with PH (52.83 ± 29.93 fmol/ml; $n=12$) significantly increased compared with that in control (10.47 ± 3.78 fmol/ml). In ISACHC class II, plasma AM level in dogs with PH (53.99 ± 21.94 fmol/ml; $n=8$) significantly elevated compared with that in dogs without PH (37.79 ± 14.58 fmol/ml; $n=24$).

In conclusion, plasma AM has the potential to be a powerful indicator of PH in dogs with CHF.

ABSTRACT #216
PULSED TISSUE DOPPLER EVALUATION IN DOGS WITH IDIOPATHIC DILATED CARDIOMYOPATHY. GG Pereira, GT Goldfeder, VCM Oliveira, DG Prada, FL Yamaki, MHMA Larsson. Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, SP, Brazil.

Idiopathic dilated cardiomyopathy (DCM) in dogs is recognized by myocardial lesions and dysfunction. We hypothesize that a pattern of regional myocardial dysfunction can be stablished by pulsed tissue Doppler in dogs with spontaneous DCM.

Fourty four dogs were evaluated, being 22 with DCM and 22 healthy, with similar weight, for control purpose. Longitudinal myocardial velocities were obtained in mitral annular (basal), papillary muscle level (medial) and apical segments of left ventricular free wall (LVFW) and interventricular septum (IVS). Radial myocardial velocities were assessed in subendocardial and subepicardial regions of medial LVFW. Peak myocardial velocities resulting from ventricular systole (Sm), early (Em) and late (Am) ventricular filling were determined. According to cardiac rhythm, recordings of three (sinus) or five (atrial fibrillation) consecutive cycles were averaged. Unpaired *t*-test was employed to investigate differences between groups.

Peak radial subendocardial and subepicardial Sm velocities were lower in DCM group compared to control (0.068 ± 0.019 vs. 0.102 ± 0.020 m/s and 0.061 ± 0.016 vs. 0.099 ± 0.027 m/s, respectively; $p<0.0001$). Peak longitudinal Sm velocities were lower in basal and medial portions of LVFW (0.093 ± 0.033 vs. 0.166 ± 0.047 m/s and 0.091 ± 0.033 vs. 0.141 ± 0.044 m/s, respectively; $p<0.0001$) and IVS (0.063 ± 0.021 vs. 0.136 ± 0.039 and 0.066 ± 0.026 vs. 0.104 ± 0.032 m/s, respectively; $p<0.0001$). Peak diastolic waves were not significantly different.

Reduction in systolic longitudinal (basal and medial) and radial myocardial velocities represents the most significant pulsed tissue Doppler findings for DCM diagnosis in dogs.

ABSTRACT #217
EVALUATION OF LEFT VENTRICULAR SYSTOLIC AND DIASTOLIC FUNCTION USING PULSED TISSUE DOPPLER IN DOGS WITH CHRONIC MITRAL VALVE INSUFFICIENCY. LC Petrus; GT Goldfeder; VMC Oliveira, EC Soares, MHMA Larsson.

Thirty dogs with chronic mitral valve insufficiency, ISACHC class II and 30 healthy dogs were studied. The evaluation of the longitudinal myocardial function was assessed by means of the apical four chamber view, and the pulsed tissue Doppler was used to measure the velocity of six regions: apical (a), medial (m), and basilar or annular (b) of the interventricular septum (IVS) and of the left ventricular lateral wall (LVLW). From each studied segment the maximum myocardial velocity of the rapid filling (Em), atrial contraction (Am) and systolic (Sm) waves were obtained, as well as the ratio between the waves of the mitral inflow (E) and myocardial (Em), registered during the same cardiac cicle (Ef/Em ratio). Mean, median and standard deviation of the echocardiographic indices were calculated. Student t- test was used to compare each group of dogs. Therefore, this study aimed for determining the abnormalities of dogs with mitral valve insufficiency and investigating the influence of the ventricular filling pressure over the studied indices. Sm LVLWb; Sm LVLWm; Sm IVSb were lower in dogs with CMVI than in healthy ones., and Am LVLWb; Am IVSb; Em IVSm; Am IVSm; Am IVSa and Ef/Em ratio were higher in dogs with CMVI then in healthy ones. Statistical significant difference was not observed between both groups when the other echocardiographic indices were evaluated.

In conclusion, dogs with chronic mitral valve disease, ISACHC class II, may show abnormalities of the left ventricular systolic function, and this can be observed by means of the pulsed tissue Doppler, even in dogs with normal shortening fraction. Moreover, the high Ef/Em ratio of dogs with CMVI compared to the value obtained from healthy dogs assures that this indice is very trustful and representative of the left ventricular filling pressure, useful to rule out other causes of dyspnea. The velocity of Em and Am were higher in dogs with valve disease than in healthy dogs probably because, despite heart insufficiency class II of dogs with CMVI, there was not myocardial remodeling able to change the left ventricular diastolic function, even though it is a chronic disorder in advanced stage.

ABSTRACT #218
PATHOLOGICAL FINDINGS IN DOGS WITH MITRAL VALVE INSUFFICIENCY: BIOPSIES DURING OPEN HEART SURGERY WITH CARDIOPULMONARY BYPASS. M Nishida, M Uechi, T Mizukoshi, T Ebisawa, M Mizuno, T Mizuno, K Harada, M Fujiwara, T Nakayama. Nihon University, Fujisawa, Kanagawa, Japan.

The aim of this study was to examine histopathological characteristics of myocardial and lung biopsy specimens taken from dogs with mitral valve insufficiency (MVI). Twenty-nine dogs with mild to severe MVI, aged 5–14 years, underwent mitral valve plasty with cardiopulmonary bypass between October 2007 and November 2008, and samples for biopsy were taken during surgery from the left atrial wall, the left ventricular free wall (between the anterior and posterior papillary muscles), ruptured chordae and/or the lung (at the apex of the left middle lobe). Specimens were fixed in 10% phosphate-buffered formalin before histopathological analysis. Histopathological examination of the left atrium (n=25) showed no abnormalities in 16 cases but revealed interstitial edema in 7, eosinophilic degeneration in 6, connective tissue hyperplasia in 3, inflammatory infiltration in 4, and myocardial fascicle atrophy in 3. The left ventricular wall (n=26) showed no abnormality in 24 cases, but interstitial edema was noted in 2. In the ruptured chords (n=16), 11 cases showed myxomatous degeneration, while 3 had eosinophilic degeneration with tissue disruption. In the lung specimens (n=12), no abnormality was found in 5 cases, but disrupted alveolar structure was seen in 1, alveolar septal thickening in 2 and heart failure cells in 5, indicating previous episodes of pulmonary edema. These results suggest that the heart and lung tissue structures are relatively well maintained in MVI until shortly before death.

ABSTRACT #219
LONG-TERM SURVIVAL OF TWO DOGS AFTER MITRAL VALVE PLASTY USING EXPANDED POLYTETRAFLUOROETHYLENE CHORDS: PATHOLOGICAL STUDY. M Nishida, M Uechi, T Ebisawa, M Mizuno, T Mizuno, K Harada, M Fujiwara, N Nakayama. Nihon University, Fujisawa, Kanagawa, Japan.

Mitral valve plasty is one of the treatment options for mitral regurgitation (MR). Expanded polytetrafluoroethylene (e-PTFE) is a polymer, which has been widely used to make artificial chords. In this study, we report autopsy and histological findings in two dogs that underwent cardiopulmonary bypass for mitral annuloplasty using e-PTFE sheets and chordoplasty using e-PTFE sutures. Case 1 was a neutered, 7-year-and-5-month-old male Cavalier King Charles Spaniel with severe MR. Postoperative progress was favorable until 10 months later when the dog showed severe diastolic dysfunction with significantly decreased fractional shortening by echocardiography. The dog died unexpectedly at 23 months after surgery. Case 2 was a 10-year-and-3-month-old female Maltese with severe MR. Postoperative progress was also satisfactory, but the dog died at 26 months after surgery from respiratory failure caused by intrathoracic fibrosarcoma. By histopathological examination, the structural integrity of both atrial and ventricular muscle fibers was maintained in Case 1, with no evidence of degeneration, fibrosis or fiber disarray. In Case 2, mild fibrosis was noted at the base of the left ventricular papillary muscle, indicating an old myocardial infarct. In both cases, e-PTFE sheets and sutures were not damaged and well integrated in the surrounding, highly differentiated connective tissues. There was no evidence of reactive changes around e-PTFE. These results suggest that e-PTFE is excellent in tissue compatibility and durability and useful for canine mitral valve plasty.

ABSTRACT #220
MITRAL VALVE PLASTY IN 11 CAVALIER KING CHARLES SPANIELS. M Nishida, M Uechi, T Mizukoshi, T Ebisawa, M Mizuno, T Mizuno, K Harada, M Fujiwara. Nihon University, Fujisawa, Kanagawa, Japan.

This study aimed at retrospectively assessing the effectiveness of mitral valve plasty in Cavalier King Charles Spaniel (CKCS), a breed predisposed to mitral valve disease (MVD). Eleven CKCS (5 males and 6 females; bodyweight, 8.8±1.4 kg; age, 110±26.7 months) underwent cardiopulmonary bypass for mitral valve plasty between December 2006 and September 2008. Postoperative complications included 1 case of tricuspid valve insufficiency and 3 cases of left atrial thrombosis (one had a preexisting thrombus at the time of surgery). In 3 cases, neurological symptoms became evident after surgery due to preexisting syringomyelia. The mean survival time was 10.4±6.8 months. One dog died from a suspected cardiac cause at 22 months after surgery, and another from possible thromboembolism at 4 months after surgery. Nine dogs were still alive at the time of the report. At 1 and 3 months after surgery, the left atrial to aortic root diameter ratio (1.76±0.36 and 1.68±0.33, respectively; n=7) and the plasma atrial natriuretic peptide level (117.9±54.8 and 85.8±38.2 pg/mL, respectively; n=4) were lower than those before surgery (2.60±0.61 and 198.0±109.9 pg/mL). There were also significant improvements in the number of prescribed cardiovascular drugs 1 month after surgery (1.6±1.3 vs. 4.5±1.6 preoperatively, $p<0.05$; n=11) and in the cardiac murmur grade (2.5±0.8 vs. 5.1±0.6 preoperatively, $p<0.05$; n=9). These results suggest that mitral valve plasty is beneficial in CKCS with MVD.

ABSTRACT #221
INTRA- AND POST-OPERATIVE COMPLICATIONS IN 47 DOGS THAT UNDERWENT MITRAL VALVE PLASTY. T Mizuno, M Uechi, T Ebisawa, M Mizuno, T Mizukoshi, K Harada, M Nishida, M Fujiwara, T Nakayama. Nihon University, Fujisawa, Kanagawa, Japan.

We previously reported that mitral valve plasty (MVP) under cardiopulmonary bypass (CPB) is an effective treatment for mitral regurgitation (MR) in dogs (2007 ACVIM Forum). To assess the incidence of intra- and post-operative complications, we retrospectively reviewed 47 cases that underwent MVP with CPB at the Nihon University Animal Medical Center between August 2006 and September 2008. The subjects were 47 dogs [22 males and 25 females, age: 62–175 (123±25) months, bodyweight: 1.8–13.5 (5.7±3.0) kg]. The mean age of the 10 dogs that died within 4 months after surgery was 140±21 (range: 115–175) months, which was significantly higher compared to those that survived beyond 4 months postsurgery [119±25 (range: 62–157) months]. The 4-month postoperative mortality was 29% for dogs aged 10 years or older and 11% for those younger than 10 years. The causes of death were surgical technical problems (2 cases), thrombosis (4 cases), pancreatitis (2 cases), pulmonary hypertension (1 case) and unknown (1 case). Of the 37 cases that survived for 4 months or longer, 4 cases had postoperative complications (thrombotic cerebral infarction, pulmonary infarction, cerebellar infarction and aspiration pneumonitis; 1 case each). Thrombus formation (including those in the left atrium) was observed in 12 cases and was the most frequent causes of postoperative complication and/or death. These results suggest that prevention of thrombosis is an important strategy for improving the surgical outcome of MVP. For dogs over 10 years old, in addition, preoperative stabilization and postoperative management are critical, and earlier surgery is recommended.

ABSTRACT #222
EFFICACY OF METHYLPREDNISOLONE THERAPY FOR INFLAMMATION AFTER CARDIAC SURGERY. T Mizuno, M Uechi, T Ebisawa, H Kamiyama, H Yamada, M Nishida, M Fujiwara, K Harada, M Mizuno, T Mizukoshi, T Nakayama. Nihon University, Fujisawa, Kanagawa, Japan.

Previously, we reported that dogs develop systemic inflammatory response syndrome (SIRS), with elevations of the IL-6 and IL-10 levels, after cardiac surgery with cardiopulmonary bypass (CPB) (2007 ACVIM Forum). SIRS can lead to serious complications such as multiple organ failure. In this study, the anti-inflammatory effect of methylprednisolone (MP) after cardiac surgery with CPB was evaluated. Fourteen dogs (bodyweight, 4.4–7.3 kg) were randomly assigned to a placebo (n=8) or a MP group (n=6) and underwent cardiac surgery with CPB between April 2007 and December 2008. Serum cytokine levels (IL-6, TNF-α and IL-10) were measured before surgery, at 5 minutes after heparinization, at 5 minutes after the onset of CPB, immediately after heart rebeating, at 15 minutes after protamine administration, and at 3, 6, 12, 24 and 48 hours after the end of CPB. Saline (placebo) or MP (10 mg/kg) was administered

intravenously, concurrently with heparinization before the onset of CPB. Blood IL-6 peaked at 3 hours after the end of CPB in both groups, although its level was lower in the MP group than that in the placebo group (149±30 vs. 345±175 pg/mL). No intergroup difference was seen in IL-10 level at 3 hours after the end of CPB (placebo vs. MP, 57±126.8 vs. 74±128.1 pg/mL). There was no difference in TNF-α level between the groups. These results indicate that a single administration of methylprednisolone before CPB suppresses the elevation of IL-6 level following cardiac surgery.

ABSTRACT #223
RECOVERY OF ATRIAL FIBRILLATION IN THREE DOGS AFTER MITRAL ANNULOPLASTY UNDER CARDIOPULMONARY BYPASS FOR SEVERE MITRAL REGURGITATION. M Fujiwara, M Uechi, T Mizukoshi, T Ebisawa, M Mizuno, T Mizuno, K Harada, M Nishida. Nihon University, Fujisawa, Kanagawa, Japan.

In dogs with severe mitral regurgitation (MR), atrial dilatation and/or structural changes in atrial myocardium often lead to atrial fibrillation (AF). We report three cases of successful defibrillation following open heart surgery under cardiopulmonary bypass (CPB) performed for severe MR and cardiomegaly. Case 1 was a 7-year-old, male Cavalier King Charles Spaniel (CKCS) presenting with ascites. Body weight (BW) was 8.8 kg, systolic regurgitant murmur (SRM) was grade 5/6 (mitral area) and 4/6 (tricuspid area). Case 2 was a 12-year-old, female Miniature Schnauzer presenting with syncope. BW was 8.9 kg, SRM was grade 5/6 (mitral area). Case 3 was a 9-year-old, male CKCS with a history of ascites for 3 months. BW was 10.3 kg, SRM was grade 4/6 (mitral and tricuspid areas). AF was confirmed by ECG in all cases. All patients underwent CPB for mitral chordoplasty and annuloplasty. During surgery, significant enlargement in the left atrium and AF were confirmed. After return of spontaneous circulation, atrial and ventricular fibrillation occurred, but normal sinus rhythm was restored after defibrillation. AF was not observed for the following 3 months in all cases except for a transient recurrence in Case 1 at 1 week after operation. X-ray examination revealed reduced atrial and cardiac sizes in all cases. This study suggests that normal sinus rhythm can be restored by postoperative defibrillation in dogs with severe MR and AF, and that load reduction after mitral valve repair prevents the recurrence of AF.

ABSTRACT #224
LV 2D STRAIN ANALYSIS PRE- AND POST-BALLOON VALVULOPLASTY IN DOGS WITH PULMONARY STENOSIS. Y Fujii, H Takei, H Takano, Y Wakao. Azabu University School of Veterinary Medicine, Kanagawa, Japan.

Left ventricular (LV) deformation and paradoxical ventricular septal motion can alter coordination of LV regional dynamics in chronic right ventricular (RV) pressure overload. The purpose of this study was to assess the influence of right ventricular pressure overload on LV systolic motion using 2D strain parameters in dogs with spontaneous pulmonary stenosis (PS), and to see if these parameters change after successful balloon valvuloplasty.

Twenty-one clinically healthy beagles (control) and 9 dogs with PS were used. PS was severe in all PS dogs, based on Doppler pressure gradient (PG, 153.52±65 mmHg). Post-balloon PG was 54.05±30.9 mmHg. 2D strain parameters (radial strain [SR], strain rate [SrR], time to peak radial strain [SRT], radial displacement [DR], time to peak radial displacement time [DRT], radial displacement variation) were measured in the 6 segments of LV short-axis view. Eccentricity index (EI) and fractional shortening (FS) were also measured. FS, systolic and diastolic EI, DR, and SRT were significantly decreased in dogs with PS compared with control. In comparison of each segment, SR, SRT, DRT and radial variation in the septal segments were significantly decreased. No significant change was observed between pre- and post- balloon in 2D strain parameters except for segmental DRT. Based on these results, although LV dyssynchrony was improved, most of the 2D strain parameters did not change after successful balloon valvuloplasty. Impaired LV contraction dynamics would be due to not only pressure overload, but probably also other factors including right ventricular hypertrophy.

ABSTRACT #225
EFFECTS OF BISOPROLOL ON VENTRICULAR MYOCYTE ELECTROPHYSIOLOGY DURING CHRONIC HEART FAILURE. Y Nishijima[1], A Sridhar[1], RL Hamlin[1], G Beddies[2], CA Carnes[1]. [1]The Ohio State University, Columbus, OH. [2]Bayer Healthcare LLC, Shawnee Mission, KS.

Beta-blockers are known to reduce the occurrence of sudden cardiac death in patients with chronic heart failure; however, the electrophysiologic mechanisms remain poorly defined. We hypothesized that chronic treatment with bisoprolol, a beta-1 selective adrenergic blocker, would attenuate heart failure (HF)-induced remodeling of ventricular myocyte electrophysiology. The canine RV tachypacing model of heart failure was used. After 12 weeks of RV tachypacing (LVFS 15±2.5%) dogs were randomized to 5 weeks of bisoprolol or placebo. A separate group of normal controls were used for all comparisons. Myocytes from the LV mid-myocardium were studied. Compared to normal controls, the placebo-treated HF myocytes exhibited significant prolongation of APD_{50} and APD_{90} at both 0.5 and 1 Hz ($p<0.05$). Bisoprolol treatment significantly attenuated the HF-induced changes in APD_{50} and APD_{90}, to values that did not differ from normal controls. Heart failure did not alter the inward conductance of the inward rectifier current (I_{K1}) although HF significantly reduced peak outward I_{K1} ($p<0.05$). Bisoprolol treatment restored ($p<0.05$) peak outward I_{K1} to values which did not differ from control. Bisoprolol treatment did not alter the HF-induced reduction in the transient outward K^+ current (I_{to}). Chronic beta-1 selective adrenergic blockade normalizes the ventricular action potential in chronic HF. This effect may contribute to the reduced cardiac death observed with beta-adrenergic blockade during HF.

ABSTRACT #226
HEART RATE IN IRISH WOLFHOUNDS WITH LONE ATRIAL FIBRILLATION & ASSESSMENT OF DIGOXIN AS SOLE METHOD OF RATE CONTROL. M Rector[1], JM Bright[1], M Dentino[2]. [1]Colorado State University College of Veterinary Medicine & Biomedical Sciences, Ft. Collins, CO. [2]Nephrology Associates, Evansville, IN.

There is a high prevalence of lone atrial fibrillation (AF) in Irish wolfhounds (IWs), and it is possible that abnormally high heart rates over time may lead to dilated cardiomyopathy in affected dogs. Although digoxin is often prescribed for rate control in IWs with AF, it is not known whether this drug provides adequate rate control during typical daily activities. This study was done to determine whether average hourly heart rates (HRs) and peak hourly heart rates are higher in IWs with lone AF than in comparable IWs with normal sinus rhythm (NSR) and to determine whether administration of digoxin to IWs with lone AF would normalize HR. HR data was collected from 13 IWs with lone AF using 24 hour Holter monitor recordings obtained in the home environment. Comparable data was obtained from 13 healthy IWs of similar age and gender in NSR. All IWs with AF were then treated with digoxin (0.004–0.005 mg/kg q12 h), and Holter studies repeated after verifying therapeutic serum concentration (1.0–2.0 ng/ml) in each treated dog.

Average and peak hourly HRs were compared using a mixed model repeated measures ANOVA to evaluate differences between groups (NSR vs AF) and between baseline and digoxin treatment within the AF dogs. The peak hourly HRs did not differ significantly in IWs with AF compared to normal IWs (AF 164.8 [SE=13.3] bpm vs NSR 159.0 [SE+13.8] bpm). However, the average hourly HRs were higher in AF dogs than normal dogs (AF 98.6 [SE=5.1] bpm vs NSR 72.5 [SE=5.3] bpm; P=0.007). Both the peak and the average hourly HRs were significantly reduced in IWs with AF by treatment with digoxin (peak baseline 164.8 [SE=13.3] bpm vs peak digoxin 138.9 [SE=14.1] bpm; P=0.022 and average baseline 98.6 [SE=5.1] bpm vs average digoxin 82.6 [SE=5.4] bpm; P<0.001). After treatment with digoxin the average hourly HRs of IWs with AF were not significantly different than those of normal dogs (AF digoxin 82.6 [SE=5.4] bpm vs NSR 72.5 [SE=5.3] bpm).

These results indicate that IWs with lone AF have significantly greater average hourly HRs than IWs with NSR and that these HR differences are resolved by administration digoxin. This study does not evaluate whether administration of digoxin alone will have the same HR lowering effect in IWs with AF when dilated cardiomyopathy and congestive heart failure are present.

ABSTRACT #227

PREVALENCE OF THE MYOSIN-BINDING PROTEIN C MUTATION IN APPARENTLY HEALTHY CATS OF DIVERSE LINEAGE. CF Paige[1], JA Abbott[1], KM Meurs[2]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA. [2]College of Veterinary Medicine, Washington State University, Pullman, WA.

Hypertrophic cardiomyopathy (HCM) in Maine coon cats is associated with a mutation of the gene that codes for myosin-binding protein C; we sought to determine the prevalence of this mutation (MYBPC-MCC) in apparently healthy cats of diverse lineage.

Study subjects were identified during a community-based echocardiographic survey of apparently healthy cats. After DNA was extracted from buccal swabs, the region of the MYBPC gene containing the mutation was amplified by PCR and then sequenced. Based on identification of the constituent base pairs of codon 31 in exon 3, samples were classified as homozygous, heterozygous, or free of MYBPC-MCC.

One hundred three cats were echocardiographically examined. Within 10 months of echocardiographic examination, buccal swabs were obtained from 71 of these cats but it was not possible to isolate DNA from one of the samples. Of 70 cats for which results of DNA analysis were available, 66 (94%) were mix-breed, 2 (3%) were Siamese and 2 (3%) were Himalayan. Forty-two (60%) cats were neutered or sexually intact males. MYBPC-MCC was identified in DNA samples from 2 male and 1 female (4.3%; 95% Confidence Interval (CI): [0.9, 12.0]) young (< 5 years) mix-breed cats, all of which were heterozygous for the mutation. Two cases were domestic short-hair cats; one male was a domestic long-hair. The genetically affected cats were echocardiographically normal.

The MYBPC-MCC mutation is present not only in the Maine coon cat population but also in mix-breed cats. The prevalence of the mutation in apparently healthy cats is close to 4%.

ABSTRACT #228

THE USE OF STRAIN, STRAIN RATE, AND DISPLACEMENT BY 2D SPECKLE TRACKING FOR ASSESSMENT OF SYSTOLIC LEFT VENTRICULAR FUNCTION IN GOATS: APPLICABILITY AND INFLUENCE OF GENERAL ANESTHESIA. AJ Berli, R Jud, K Steininger, CC Schwarzwald. Vetsuisse Faculty, University of Zurich, Zurich, Switzerland.

Although cardiovascular disease is not commonly encountered in goats, this species often serves as an animal model for human cardiovascular disease. The goal of this study was to investigate the applicability of 2D speckle tracking (2DST) for assessment of segmental left ventricular (LV) function in goats, describe the techniques, and determine the influence of general anesthesia.

22 healthy, female Saanen-goats (age 3.7±1.1 y, weight 59.8±10.5 kg [mean±SD]) were studied. The goats underwent a complete echocardiographic examination in an unsedated state in the standing position (control). All goats then underwent a second echocardiographic examination under general anesthesia and in sternal recumbency, 7.4±3.4 days after the first examination (treatment). Echocardiography was performed using a GE Vivid 7 echocardiograph with a M4S probe (1.9/4.0 MHz). The LV was imaged in two-dimensional gray scale mode from a right-parasternal window using a long-axis, 4-chamber view (LAX) and a short-axis view at the level of the papillary muscles (SAX-PM). The recording frame rate ranged between 70.2 and 103.8 frames/second. Analyses were performed off-line (GE EchoPAC v6.1.2) and blinded to treatment. Measurements included longitudinal, circumferential, and radial peak systolic strain (SL, SC, SR), peak systolic strain rate (SrL-S, SrC-S, SrR-S) as well as longitudinal and radial peak systolic displacement (DL, DR). All measurements were reported by the software as average over each of 6 wall segments. Two-way RM ANOVA with a Holm-Sidak post-hoc test was used to assess the effects of segment and treatment on the variables. The level of significance was p=0.05.

2DST analyses were feasible in 256 of 264 recordings (97%). In LAX view, SL and SrL-S decreased gradually and DL increased gradually from the apical to the basal segments. SL was unaffected by treatment, while SrL-S was significantly reduced during anesthesia. DL was significantly higher during anesthesia in the lateral, but not in the septal segments. In SAX-PM view, SR was higher in the LV free wall segments compared to the septal segments, but it was unaffected by treatment. The interacting effects of segment and treatment on SC, SrC-S, and SrR-S did not show any consistent, identifiable pattern. General anesthesia increased DR in the LV free wall segments.

We conclude that 2DST can be used to characterize segmental LV wall motion in awake and anesthetized goats. Anesthesia influences some of the 2DST variables, probably related to alterations in cardiac contractility and loading conditions. Assessment of LV diastolic function by 2DST, derivation of indices of global function, assessment of LV synchrony using time intervals, and evaluation of the clinical and experimental value of 2DST will require further investigations.

ABSTRACT #229

BAROMETRIC WHOLE-BODY PLETHYSMOGRAPHY REFERENCE VALUES IN DIFFERENT AGE-RANGE CATS WITH NATURAL BRONCHIAL DISEASE. L Garcia-Guasch[1], A Caro-Vadillo[2], M Laporta[1], J Manubens[1], JA Montoya-Alonso[3]. [1]H.V.Molins, Barcelona, Spain. [2]Med. Cir. Anim., UCM, Madrid, Spain.[3]Int. Med., ULPGC, Las Palmas, Spain.

Feline bronchial disease (FBD) is characterized by inflammation of the lower airways without an obvious identifiable cause. Unfortunately there are no pathognomonic clinical signs or laboratory tests available in routine veterinary clinical practice for FBD. Barometric whole-body plethysmography (BWBP) is a noninvasive pulmonary function test (PFT) that allows a dynamic study of breathing patterns by placing the patient within an unrestrained Plexiglas chamber. BWBP is useful to study potential airway disease and the response to different treatments. The objectives of this preliminary study are (1) to establish a reference database for respiratory variables in cats with natural FBD using BWBP, (2) to evaluate if there are significant differences between healthy cats and cats with natural illness and (3) to evaluate if there are significant differences between healthy and affected cats in different age-range. The study was approved by the animal ethical committee of the University of Las Palmas (Spain). Twenty-three cats with natural FBD were included. Cats did not have a previous history of upper airway, cardiac or systemic diseases and had negative results when tested for FeLV/FIV diseases. Cats were arranged in three groups: young cats (n=5, from 0 to 2 years old), adult cats (n=11, from 2 to 8 years old), and old cats (n=7, more than 8 years old). Cats were placed in the chamber and after an adaptation period of time in a quiet and silent environment, four 3-minute periods were registered and data (tidal volume [TV], enhanced pause [Penh] and Pause) were shown as means with standard deviations. A p-value <0.05 was considered statistically significant.

	TV (mL)		Penh		Pause	
Age-range	Healthy N=26	FBD N=23	Healthy N=26	FBD N=23	Healthy N=26	FBD N=23
0–2	29,52± 11,74	43,25± 16,63	0,54± 0,15	0,81± 0,28	0,66± 0,12	0,83± 0,23
2–8	35,15± 12,24	60,65± 40,11	0,46± 0,17	1,74± 1,59*	0,57± 0,18	1,22± 0,59*
>8	34,75± 5,05	29,80± 9,59	0,44± 0,11	1,24± 0,71*	0,57± 0,07	0,17± 0,47*
*P<0,05	P= 0,037		P=0,02		P=0,001	

In conclusion: (1) measured results are firstly useful as baseline values for PFT by BWBP in cats with FBD and suggest that there are no significant differences in TV (p=0,120), Penh (p=0,349) and Pause (p=0,363) between different age-range groups of FBD cats, (2) there are significant differences for TV, Penh and Pause variables between healthy cats and cats with FBD (compared with reference values in healthy cats previously reported by authors), and (3) there are significant differences for Penh and for Pause between healthy cats and cats with FBD in adult (p_{Penh}=0,024 ; p_{Pause}=0,006) and old (p_{Penh}=0,05 ; p_{Pause}=0,03) cats.

ABSTRACT #230
CLINICAL APPLICATION OF BAROMETRIC WHOLE-BODY PLETHYSMOGRAPHY AND BRONCHOPROVOCATION TEST IN FELINE BRONCHIAL DISEASE. YA Jhuo, HP Huang. Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan.

The aim of this study was to evaluate the application of barometric whole-body plethysmography (BWBP) and bronchoprovocation tests in diagnosis of feline bronchial disease clinically.

Thirty nine client-owned cats were enrolled, 21 were clinically healthy and the other 18 were affected with bronchial disease. Diagnosis of feline bronchial disease was based on clinical manifestations (Feline Bronchial Disease Activity Scores), results of physical examinations, routine blood examinations, thoracic radiographs, electrocardiography and echocardiography in each cat. Baseline parameters of BWBP (respiratory rate, tidal volume, minute volume against body weight, peak inspiratory and expiratory flows, inspiratory and expiratory time, and relaxation time), and enhanced pause (Penh) were recorded over a 5-minute period. Bronchoprovocation tests were carried out using a jet nebulizer containing carbachol solutions at concentrations of 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, and 0.5%. Saline (0.9% NaCl) and increased concentrations of carbachol solutions were nebulized for 1 minute and followed by 5 minutes recording until the carbachol concentration induced bronchoconstriction (Penh exceeded 300% of baseline, C-Penh300). Clinically healthy (n=6) and FBD-affected cats (n=4) were then further selected to determine the repeatability of BWBP on two independent occasions. Repeating tests were performed following the same procedure as described above at an interval of 2 weeks.

Baseline BWBP parameters between clinically healthy cats and cats with bronchial disease were not significantly different. C-Penh300 was significantly higher in clinically healthy cats ($0.352\pm0.196\%$) comparing to cats with bronchial disease ($0.063\pm0.044\%$, $P<0.0001$). No correlation was found between the baseline BWBP variables and C-Penh300 in cats with bronchial disease. In the test of repeatability of BWBP, the concentrations inducing a 300% increase of Penh were similar (mean C-Penh300 Test 1: $0.326\pm0.225\%$; mean C-Penh300 Test 2: $0.258\pm0.212\%$; $P=0.50$). Results of two repeating tests were positively correlated ($r=0.76$, $P=0.00105$).

These findings suggested that the results of BWBP assessment could be repeatable, and bronchoprovocation test could be applied as a diagnostic protocol for feline bronchial disease.

ABSTRACT #231
THE QUALITY OF CANINE BRONCHOALVEOLAR LAVAGE FLUID CYTOLOGY IS PRESERVED FOR UP TO 72 HOURS WITH OR WITHOUT AUTOLOGOUS SERUM. MH Patrick, T Wills, RK Sellon. Washington State University College of Veterinary Medicine, Pullman, WA.

Cytological examination of bronchoalveolar lavage (BAL) fluid is the gold standard for diagnosing canine lower respiratory tract disease. BAL fluid is typically evaluated almost immediately to limit changes in cytological quality due to low protein medium, cellular release of enzymes, or delayed cell preparation. The preservation of BAL sample quality over time has not been previously evaluated. We hypothesized that addition of autologous serum to BAL samples immediately after collection would preserve cytological quality for at least 72 hours. Serum was collected from each of 14 healthy dogs prior to BAL. BAL samples were divided into 13 aliquots of 250 μL; one aliquot was analyzed immediately. Cells were counted via hemocytometer and cytological quality assessed by scoring the degree of nuclear swelling and frequency of broken cells on cytospin preparations. Autologous serum (50 μl; 5%) was added to 6/12 aliquots, and all aliquots were stored at 4 °C for 4, 24, 48, and 72 hours before analysis; the clinical pathologist was blinded to both serum treatment and storage time. Data were analyzed via ANOVA and paired t-test. There were no differences in cell count or sample quality at any of the time points between aliquots stored with or without serum. At 72 hours, the difference in the cell morphology between samples with serum versus those without serum approached significance. BAL samples remained of good diagnostic quality up to 48 hours. Adding serum may be useful for samples for which analysis will be delayed for more than 3 days.

ABSTRACT #232
CLINICAL AND LABORATORY CHARACTERISTICS OF 35 DOGS WITH PULMONARY THROMBOEMBOLISM. (2002–2008). SA Pumphrey, EA Rozanski, LM Freeman, SM Cunningham, JE Rush. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

Pulmonary thromboembolism (PTE) can be a life-threatening complication of critical illness. The purpose of this study was to evaluate clinical signs, underlying disease states, coagulation testing results, therapies, and outcome in dogs with confirmed PTE. The medical records and necropsy databases were searched for dogs with confirmed PTE between 2002 and 2008. Confirmation of PTE was made via necropsy, computed tomography (CT scan) with nonselective angiography, selective angiography, or visualization of a pulmonary thrombus on echocardiography (Echo). Dogs with suspected but not proven PTE were excluded as were dogs with PTE in association with heartworm infection. Information recorded from the medical record included history, presenting complaint, presence of key clinical signs, antemortem suspicion of PTE, therapies directed against PTE, co-morbidity, coagulation testing, and outcome.

Thirty-five dogs with confirmed PTE were identified. Twenty-six were confirmed at necropsy, while five had thrombi visualized on Echo, and 4 had PTE identified on CT scan. Thirty dogs presented with respiratory distress and 13 had cough, including 3 with hemoptysis. Seven dogs had right-sided congestive heart failure, 1 dog presented for collapse, and 1 experienced sudden death. Twenty dogs were treated with supplemental oxygen, including 4 who were mechanically ventilated. In 62% of cases, PTE was suspected antemortem. 12 of these dogs received anticoagulant or thrombolytic therapy, consisting of various combinations of unfractionated heparin, low molecular weight heparin, coumadin, clopidogrel, urokinase, tissue plasminogen activator and streptokinase. A variety of co-morbidities were identified, most commonly neoplasia, corticosteroid use, and protein-losing nephropathy. Prothrombin time (n=17) was elevated beyond the reference range in 8 dogs, and aPTT (n=19) was elevated in 7. Platelet count (n=27) was decreased in 15. D-dimer concentrations were measured in 13 dogs; 3 dogs had negative D-dimer concentration ($<$ 250 ng/ml). Five dogs had D-dimer levels of 250–500 ng/ml, 2 dogs had concentrations 500–1000 ng/ml, 2 dogs had concentrations 1000–2000 ng/ml and 1 dog had a concentration $>$ 2000 ng/ml. 30 dogs died or were euthanized, while 5 dogs were discharged. PTE was suspected antemortem in a higher percentage of dogs in this study than in previous reports. However, only 12 of 21 dogs with suspected PTE received anticoagulant or thrombolytic interventions. Additionally, in this population of dogs with confirmed PTE, D-dimers were not reliably elevated, with only 3/13 dogs having D-dimers $\geq$1000 ng/ml.

The clinical outcome associated with PTE remains poor. Future directions may include improving clinical suspicion for the disease in at-risk dogs, developing alternative means to aid antemortem diagnosis, and facilitating timely use of anticoagulant therapies.

ABSTRACT #233
COMPARATIVE STUDY OF THE TOLERANCE AND EFFICIENCY OF FOUR DIFFERENT TECHNIQUES FOR NEBULISATION DELIVERY IN CATS. Talavera J, Tudela A, Escobar M, Bayón A, Fernández del Palacio MJ. Departamento de Medicina y Cirugía Animal. 30100 Espinardo (Murcia) – Spain.

In humans, nebulisation/aerosolization is a well-established route to drug delivery to the respiratory tract and to provide airway humidification in patients with respiratory diseases. Protocols for nebulisation generation and delivery are well standardized. Because the inherent lack of cooperation of animals, most of these human protocols are not applicable to veterinary medicine. Successful therapy with nebulisation may depend more on adequate drug delivery than drug efficacy. The aim of this study was to compare the tolerance and efficiency of four different techniques for nebulisation delivery in cats.

Nine adult healthy cats were used. Tolerance to each nebulisation technique was subjectively evaluated. For objective evaluation of

the efficiency of the nebulisation delivery, a cough challenge test was used. Capsaicine was nebulised (ultrasonic nebuliser) following a dose-response protocol with increasing concentrations (3, 10, 30, 60, 90, 100, 300 and 500 μM) during 1 minute each and lasting 1 minute between concentrations. When more than 5 coughs were induced the response was considered positive. The test was always performed with the cats lightly sedated (acepromacine, 0.06 mg/kg+buprenorfine, 0.01 mg/kg, IM). In different days, the cough challenge was repeated in all cats once with each of the following techniques for nebulisation delivery: (1) direct approximation of the nebulisation outlet to the mouth/nose of the cat (flow-by method, FB); (2) using a face mask (FM); (3) using a plastic-covered Elizabethan collar (EC); and (4) using a plexiglas chamber (18.5 l) (PC). The number of positive responses (NPR) and the concentration of capsaicine that induced positive response (CCP) with the four techniques were statistically compared (Chi-square and Mann-Whitney test, respectively; $P < 0.05$).

The PC technique was the best tolerated, although the FB and FM techniques showed also acceptable tolerance. However, the EC technique resulted very stressful and the test must be suspended in 4/9 cats. The technique that induced positive responses at lower CCP was the FM (13.8±6.0 μM), followed by the FB technique (20.0±9.3 μM), the PC technique (23.8±10.2 μM) and the EC technique (40.0±9.4 μM), although the differences only were statistically significant when compared FM and EC techniques ($P=0.02$). The FM technique induced positive responses in greater number of cats (NPR=8), followed by the FB technique (NPR=7), the PC technique (NPR=5) and the EC technique (NPR=3). Again, the differences only were statistically significant when compared FM and EC technique ($P=0.016$).

In conclusion, FM is the most efficient technique for nebulisation delivery in cats, offering good tolerance and the best quantitative results. The FB nebulisation is less efficient but it may become practical if the FM is not tolerated. The PC technique offers a minor efficiency but it may be useful in very stressful or aggressive cats. The EC technique presents low efficiency and tolerance.

ABSTRACT #234

UTILITY OF TEPOXALIN, A DUAL LIPOXYGENASE AND CYCLOOXYGENASE INHIBITOR, IN CANINE CHRONIC AND/OR EOSINOPHILIC BRONCHITIS. CR Reinero, CH Chang, AE DeClue. University of Missouri, College of Veterinary Medicine, Columbia, MO.

Eicosanoids like leukotriene B4 (LTB4), prostaglandin E2 (PGE2) and thromboxane B2 (TxB2) are inflammatory mediators in human chronic bronchitis. We hypothesized that canine chronic bronchitis is caused at least in part by products of the lipooxygenase and cyclooxygenase-2 pathways, and by selective inhibition with tepoxalin (Zubrin®), a parallel decrease in the number/percentage of airway inflammatory cells and concentrations of LTB4, PGE2 and TxB2 would be observed.

Twelve dogs with naturally developing chronic/eosinophilic bronchitis were enrolled. For inclusion, dogs needed baseline thoracic radiographs, bronchoalveolar lavage fluid (BALF) with >370 cells/μl, >5% neutrophils and/or >5% eosinophils, negative BALF culture and owner consent. Tepoxalin was administered at 20 mg/kg PO once followed by 10 mg/kg daily for 2 weeks. At 2 weeks, thoracic radiographs and BALF collection were repeated. Concentrations of eicosanoid products were measured in BALF using commercial ELISAs. Data were analyzed using a paired t-test or a signed rank test with $p < 0.05$ considered significant.

At baseline, mean±SD BALF total cell number, % neutrophils and % eosinophils were 992±652 cells/μl, 30±18% and 33±32%; no significant difference was noted after Tepoxalin (1190±1233 cells/μl, 40±28% and 36±26%, respectively). BALF LTB4 was significantly greater [median (q1, q3); pre 0.84 (0.3, 1.2); post 1.2 (0.8,2.2); $p=0.02$] post-tepoxalin treatment but there was no significant difference in mean±SD PGE2 (pre 1.17±0.38 ng/ml; post 2.40±1.00 ng/ml; $p=0.22$) or TxB2 (pre 0.05±0.02 ng/ml; post 0.07±0.03 ng/ml; $p=0.54$).

In conclusion, in dogs with chronic and/or eosinophilic bronchitis Tepoxalin does not significantly diminish airway inflammation or reduce concentrations of local airway eicosanoid products.

ABSTRACT #235

TREATMENT OF TRACHEAL COLLAPSE WITH VET-STENTS® IN 36 DOGS: COMPLICATIONS AND LONG-TERM RESULTS. E De Madron. Alta Vista Animal Hospital, Ottawa, ON, Canada.

Dyspnea due to severe tracheal collapse in dogs can be alleviated by the insertion of intra-tracheal stents specifically designed for dogs: the Vet-Stent®. The purpose of this retrospective study is to review the long-term results and complications of this approach.

Thirty six dogs with severe tracheal collapse (grade 3 or 4) and dyspneic symptoms underwent a Vet-Stent® placement. The entire trachea was collapsing (94% of dogs), as well as the bronchi (81%). Duration of follow up was 2 to 822 days. Immediate post-operative complications included: ARDS (14%), death due to ARDS (11%), and pneumonia (17%). Cough was present in 69% of case weeks to months after stent placement. This cough proved intractable in 17% of dogs. The stent migrated in 8% of dogs due to improper sizing. Steroid responsive granulomas occurred in 6% of dogs. Stent rupture occured in 6% of dogs. A second stent had to be placed in 17% of dogs, and a third one in 6%.

Results were deemed very satisfactory in 25% of cases, acceptable but with lingering cough requiring medical treatment in 53% of dogs, poor due to severe persistent cough in 5% of dogs.

In conclusion, treatment of severe tracheal collapse with Vet-Stent® yields improvement in 88% of dogs. Medical control of cough is often necessary post stent placement. Proper measurement of the stent to place is primordial.

ABSTRACT #236

SUBACUTE ENDOTOXEMIA TEMPORARILY IMPAIRS INSULIN SENSITIVITY AND β-CELL FUNCTION IN CATS. M Osto[1], E Zini[2], M Franchini[3], M Ackermann[3], CE Reusch[2], TA Lutz[1]. [1]Institute of Veterinary Physiology; [2]Clinic for Small Animal Internal Medicine; [3]Institute of Virology; Vetsuisse Faculty, University of Zurich, Switzerland.

Systemic low-grade inflammation is a pathogenic component in chronic disease such as obesity and type 2 diabetes mellitus (T2DM). In humans and cats, chronic inflammatory conditions are associated with elevated proinflammatory cytokines. Activation of inflammatory signaling pathways are supposed to be linked to insulin resistance and to impairment of pancreatic β-cell function; the two most important features of T2DM. Because feline diabetes closely resembles human T2DM, we hypothesized that low-grade induced inflammation impairs insulin sensitivity and β-cell function in cats.

To test this hypothesis, 10 healthy cats were infused via the jugular vein with increasing doses (10, 200, 500 and 1000 ng/kg/h) of lipopolysaccharide (LPS; n=5) or saline (n=5) for 10 days. Body temperature, plasma glucose, insulin and α_1-acid glycoprotein levels were assessed each day during the infusion period. Before and at the end of the infusion, intravenous glucose tolerance tests (ivGTT) were performed. On day 10, tissue specimens were collected. To quantify neutrophils, tissue sections were immunostained with myeloperoxidase. Statistical differences between groups were determined with non parametric tests.

Infusion of LPS was well tolerated. Body temperature and glucose levels increased during the first five days of LPS infusion and then progressively decreased to baseline levels. Insulin was not different from controls at any time point. Based on the homeostasis model assessment, β-cell function and insulin sensitivity were significantly decreased in the LPS group on day 1 and on day 2 and 3, respectively. From day 1 to 10, plasma levels of α_1-acid glycoprotein were significantly increased in the LPS-infused cats as compared to controls. Based on the ivGTT at the end of the infusion, the insulin secretion pattern and insulin sensitivity were not different between groups. Compared to controls, on day 10 LPS-treated cats had increased number of neutrophils in the liver.

LPS induced an inflammatory response that lasted throughout the infusion. This was accompanied by reduced insulin sensitivity and β-cell function that were short-lasting. Because body temperature and glucose levels returned to normal, desensitization to LPS may have occurred. The underlying mechanisms are currently explored.

ABSTRACT #237

COMPARISON OF WIRELESS AND CONVENTIONAL CONTINUOUS GLUCOSE MONITORING SYSTEMS (CGMS) IN 4 DOGS. J. Rieder[1], J. Seipel[1], B. Vaske[2], K. Rohn[3], D. Simon[1], I. Nolte[1]. [1]Small Animal Clinic and[3]Institute for Biometry and Epidemiology, Hanover School of Veterinary Medicine, Germany, [2]Institute for Biometry, Medical School Hanover, Germany.

Diabetes mellitus is a common endocrine disorder in dogs and its therapeutic approach is still a challenge for veterinarians. Conventional and wireless Continuous Glucose Monitoring Systems (CGMS) are useful tools for an optimal insulin treatment in dogs.

The aim of this study was the comparison of the conventional and the wireless CGMS concerning their diagnostic value.

Three diabetic dogs and one healthy dog underwent simultaneous continuous glucose monitoring with a conventional and, concurrently, a wireless device. The measurement of one diabetic dog was continued for 5 days. Blood glucose values were determined at least three times a day and were used for the calibration for the CGMS. A comparison of both devices was performed using the Wilcoxon test, differences of CGMS and blood glucose values were analyzed by the Friedmann-Test. p-values $<0{,}05$ were considered to be significant.

Diabetic dogs were represented by a Labrador Retriever, a Cocker Spaniel and a Dalmatian. The healthy dog was a Beagle. The glucose values measured by the conventional CGMS were significantly different compared to the wireless CGMS. Glucose values measured by the conventional CGMS were higher in comparison to the wireless type. Furthermore, significantly lower numbers of values were recorded by the wireless CGMS if compared to the conventional device. This is probably due to an increased rate of discontinuations during the measurement of the wireless device. There were no significant differences between blood glucose values and glucose values measured by the conventional and the wireless CGMS.

Because of the increased rate of discontinuations the results suggest that to date a continuous glucose monitoring is probably more reliable if it is accomplished with the conventional CGMS. Further studies are needed to confirm this conclusion by a more detailed comparison of blood glucose values and glucose values recorded by both CGMS devices.

Previously presented at InnLab 2009, Berlin, Germany.

ABSTRACT #238

EXTENDED USE OF PROTAMINE ZINC RECOMBINANT INSULIN FOR TREATING DIABETES MELLITUS IN CATS. RW Nelson[1], K Henley[2], S. Peterson[2]. [1]School of Veterinary Medicine, University of California, Davis, CA. [2]Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO.

The study evaluated the long-term safety and efficacy of protamine zinc recombinant insulin (PZIR) on control of glycemia in diabetic cats. 145 diabetic cats that completed a 45 day field efficacy trial were enrolled for an additional 136 day period. All cats were treated with PZIR twice daily. Control of glycemia was assessed by physical examination, body weight and the investigator on Days 0 (entry into the extended study), 34, 68, 102, and 136, by serum fructosamine on Days 0, 68 and 136, and by owner assessment of clinical response weekly. Adjustments in PZIR dosage were made as needed to maintain control of glycemia.

113 cats completed the study. There was a significant ($p<0.0001$) decrease in serum fructosamine concentration and significant ($p<0.0001$) increase in body weight on Day 136, compared with Day 0. 95.6% of cats were in good clinical condition according to investigators and 95% of cats had the same or decreased water consumption and urination and 98% had the same or improved attitude according to owners at Day 136. Clinical signs of hypoglycemia were identified in 22 cats and resolved with appropriate therapy and insulin dosage adjustments.

Results of this study demonstrate that PZIR is safe and effective for control of glycemia in diabetic cats for at least 6 months.

ABSTRACT #239

URINARY MARKERS AND PLASMA IOHEXOL CLEARANCE IN HEALTHY DOGS AND DOGS WITH PITUITARY-DEPENDENT HYPERADRENOCORTICISM. P.M.Y. Smets[1], E. Meyer[1], B.E.J. Maddens[1], S. Croubels[1], H.P. Lefebvre[2] and S. Daminet[1]. [1]Ghent University, Ghent, Belgium. [2]Ecole Nationale Vétérinaire, Toulouse, France.

Proteinuria and systemic hypertension have been associated with progression of chronic renal disease in dogs. Forty-six % of dogs with untreated pituitary-dependent hyperadrenocorticism (PDH) have proteinuria and 50 to 80% have hypertension. Moreover, an increase in glomerular filtration rate (GFR) has been described in humans and several animal species receiving exogenous glucocorticoids. Still, the effect of PDH on renal function has not been documented.

Therefore, the aim of this study was to compare a selected set of glomerular and tubular markers, i.e. urinary protein to creatinine ratio (UPC), urinary albumin (uALB) and GFR, and urinary retinol binding protein (uRBP) and N-acetyl-β-D-glucosaminidase (uNAG), respectively, in healthy (H) dogs and in dogs with PDH.

Seven newly diagnosed PDH patients (age 6.7–11.6 years, bodyweight 20–42.8 kg) and eight H age-matched dogs (age 7.2–10.9 years, bodyweight 21.2–39 kg) were included. Urinalysis, bacterial culture and UPC were performed in all dogs. uALB was determined using a canine-specific ELISA, uRBP using a human RBP ELISA and uNAG activity using a colorimetric assay after validation for use in canine urine. Results were related to urinary creatinine (c) and expressed as ratios. In all dogs, GFR was calculated by means of plasma exo- (Cl_{exo}) and endo-iohexol clearance (Cl_{endo}) and expressed as ml/min/kg (WinNonlin® non-compartmental analysis).

In the H and the PDH dogs, the mean UPC±standard deviation (SD) was 0.1±0.05 and 2.1±1.3, the uALB/c 33.4±50.7 mg/g and 2385.8±3236.7 mg/g, the Cl_{exo} 1.8±0.2 and 2.6±0.7 and the Cl_{endo} 2.2±0.3 and 2.8±1.1, the uRBP/c 18.6±26.7 µg/g and 128.4±93.3 µg/g, the uNAG/c 2.3±1.3 U/g and 8.9±7.7 U/g, respectively. Based on a two sample t-test the UPC ($p=0.005$), uRBP/c ($p=0.012$), uNAG/c ($p=0.047$), Cl_{exo} ($p=0.015$) and Cl_{endo} ($p=0.004$) were all significantly higher in the PDH than in the H dogs. No significant difference was found for uALB/c, although increased ratios were observed in all PDH dogs.

In conclusion, results of this pilot study suggest the presence of glomerular and tubular renal dysfunction in PDH compared to H dogs, indicated by increases in UPC and GFR, and in uRBP/c and uNAG/c, respectively. Further research in a larger number of dogs is warranted to confirm these novel findings in order to gain a better insight into the effect of PDH on renal function.

ABSTRACT #240

EFFECT OF PHYTOESTROGENS AND MELATONIN ON ADRENAL STEROIDOGENESIS USING HUMAN ADRENAL CELLS AS A MODEL FOR CANINE HYPERADRENOCORTICISM. KA Fecteau, JW Oliver, H Eiler. Department of Comparative Medicine, College of Veterinary Medicine, University of Tennessee, Knoxville, TN.

Excessive secretion of adrenal sex hormones has been associated with clinical signs usually caused by cortisol in canine hyperadrenocorticism. Phytoestrogens and melatonin are effective in controlling hyperadrenocorticism in some dogs. The objectives of this study were to investigate: (1) the effectiveness of phytoestrogens, alone and in combination with melatonin, in blocking adrenal steroidogenesis at the cellular level and; (2) the effect phytoestrogens have on expression of aromatase which converts androgen to estrogen. Since human adrenal carcinoma cells but not canine adrenal cells are commercially available, human cells were used as a model for dog.

Cells were treated with 100 µM: cyclic AMP (cAMP), cAMP plus phytoestrogen enterolactone (ENL), cAMP plus phytoestrogen enterodiol (END), ENL plus melatonin, and END plus melatonin for 24 and 48 hours. Cell culture media were analyzed for estradiol, progesterone, 17-hydroxyprogesterone, androstenedione, aldosterone, and cortisol using radioimmunoassays. Treated-cell lysates were used for western blot immunoassay investigation of aromatase expression.

Results revealed a significant ($P<0.05$) decrease in cAMP-stimulated estradiol (207 vs 430 pg/ml), androstenedione (29.5 vs

112.6 ng/ml), and cortisol (15.5 vs 57.3 ng/ml) concentrations when ENL was combined with melatonin and a decrease in estradiol (143.6 pg/ml), androstenedione (35.9 ng/ml), and cortisol (16.7 ng/ml) when END was combined with melatonin. Hormones were not significantly decreased when ENL or END was used alone. Aromatase expression was decreased in phytoestrogen and melatonin treated cells. Results suggest phytoestrogens plus melatonin act directly on adrenal cells lowering estradiol by decreasing aromatase as well as lowering cortisol and androstenedione.

ABSTRACT #241
EFFECT OF LOW DOSES OF COSYNTROPIN ON CORTISOL CONCENTRATIONS IN CLINICALLY HEALTHY CATS. LG Martin[1], AE DeClue[2], EN Behrend[1], LA Cohn[2], DI Dismukes[2], R Cohen[2], HP Lee[1]. [1]Auburn University, College of Veterinary Medicine, Auburn, AL. [2]University of Missouri, College of Veterinary Medicine, Columbia, MO.

Use of low-dose ACTH stimulation testing may be important in diagnosing mild degrees of adrenocortical insufficiency and a new syndrome, relative adrenal insufficiency, which has been recognized in critically ill human and veterinary patients. In cats, the ACTH stimulation test utilizing a standard dose of cosyntropin (125 μg/cat, IV) is used for evaluation of adrenocortical function. A previous study has documented that lower doses of cosyntropin will stimulate maximal cortisol secretion in cats. However, this study used per cat dosing as opposed to per body weight dosing. The purpose of this study was to determine the lowest dose of cosyntropin on a per body weight basis that will produce maximal cortisol secretion in clinically healthy cats.

Six dose-response trials were performed in 7 clinically healthy cats (mean±SD weight 5.5±1.1 kg) instrumented with vascular access ports (Norfolk Vet Products). Each cat was randomly given 5 doses (125 μg/cat, 10 μg/kg, 5 μg/kg, 2.5 μg/kg, 1 μg/kg) of cosyntropin (Cortrosyn®) and saline (control) IV with a 2-week wash out period between doses. Blood samples for determination of serum cortisol concentrations were obtained before and at 15, 30, 45, 60, 75, and 90 minutes after cosyntropin administration. Samples were centrifuged after clotting, and the serum was separated and stored at −20 °C until analysis. Data were analyzed using a repeated measures ANOVA; post hoc comparisons were made using the Least Squared Means method. Significance was set at the $p \leq 0.05$ level.

Mean serum cortisol concentration increased significantly after administration of all 5 cosyntropin dosages when compared to baseline ($p < 0.0001$). After administration of 125 μg cosyntropin/cat, serum cortisol concentration peaked at 90 minutes. Mean serum cortisol concentrations peaked at 75, 60, 60, and 45 minutes following administration of 10, 5, 2.5, and 1 μg/kg cosyntropin, respectively. The peak serum cortisol concentrations subsequent to the 125 μg/cat, 10 μg/kg, and 5 μg/kg doses were not significantly different. Higher dosages of cosyntropin appeared to result in more sustained elevations in serum cortisol and a later time of peak response. No increase in cortisol concentration occurred after saline administration.

In conclusion, cosyntropin administered at a dose of 5 μg/kg IV produces maximal cortisol secretion in clinically healthy cats. Serum cortisol concentration was reliably increased in all cats after the administration of each dose of cosyntropin. These results can be used in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in healthy and critically ill cats.

ABSTRACT #242
DOSE RESPONSE OF ALDOSTERONE TO ACTH STIMULATION IN CLINICALLY HEALTHY CATS. LG Martin[1], AE DeClue[2], EN Behrend[1], LA Cohn[2], DI Dismukes[2], R Cohen[2], HP Lee[1]. [1]Auburn University, College of Veterinary Medicine, Auburn, AL. [2]University of Missouri, College of Veterinary Medicine, Columbia, MO.

ACTH is involved in regulation of aldosterone secretion. Administration of exogenous ACTH to dogs causes a reliable increase in serum aldosterone secretion and can be used for evaluation of adrenal function. However, the aldosterone response to ACTH administration, the dose of ACTH which will stimulate maximal adrenocortical aldosterone secretion, and the time of maximal response has not been evaluated in cats. The purpose of this study was to determine the aldosterone response to 5 doses of cosyntropin in clinically healthy cats.

Six dose-response trials were performed in 7 clinically healthy cats (mean±SD weight 5.5±1.1 kg) instrumented with vascular access ports (Norfolk Vet Products). Each cat was randomly given 5 doses (125 μg/cat, 10 μg/kg, 5 μg/kg, 2.5 μg/kg, 1 μg/kg) of cosyntropin (Cortrosyn®) and saline (control) IV with a 2-week wash out period between doses. Blood samples for determination of serum aldosterone concentrations were obtained before and at 15, 30, 45, 60, 75, and 90 minutes after cosyntropin administration. Samples were centrifuged after clotting, and the serum was separated and stored at −20 °C until analysis. Data were analyzed using a repeated measures ANOVA; post hoc comparisons were made using the Least Squared Means method. Significance was set at the $p \leq 0.05$ level.

Mean serum aldosterone concentration increased significantly after administration of all 5 cosyntropin dosages when compared to baseline ($p < 0.0001$). After administration of 125 μg cosyntropin/cat, serum aldosterone concentration peaked at 60 minutes. Mean serum aldosterone concentrations peaked at 60, 45, 45, and 30 minutes following administration of 10, 5, 2.5, and 1 μg/kg cosyntropin, respectively. The peak serum aldosterone concentration subsequent to 125 μg/cat was similar to all other doses, except the 1 μg/kg dose. Higher dosages of cosyntropin appeared to result in more sustained elevations in serum aldosterone and a later time of peak response. No increase in aldosterone concentration occurred after saline administration.

In conclusion, 125 μg/cat and 10 μg/kg cosyntropin stimulated the highest serum aldosterone concentrations at 60 minutes post administration. Cosyntropin administered at doses of 5 and 2.5 μg/kg also produce maximal aldosterone secretion in clinically healthy cats, but time of peak concentration occurred at 45 minutes. Serum aldosterone concentration was reliably increased in all cats after the administration of each dose of cosyntropin. These results can be used in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in healthy and adrenopathic cats, and cats with nonadrenal illnesses.

ABSTRACT #243
DETERMINATION OF THE OPTIMAL TIME FOR TESTING THYROID HORMONES DURING TREATMENT WITH METHIMAZOLE IN HEALTHY AND HYPERTHYROID CATS. Rutland BE[1], Nachreiner RF[2] & Kruger JM[1]. [1]College of Veterinary Medicine, [2]Diagnostic Center for Population and Animal Health (DCPAH); Michigan State University, East Lansing, MI.

Variation in the duration and magnitude of suppression of thyroid hormones, TT4 (total thyroxine), fT4 (free T4), TT3 (total triiodothyronine) and fT3 (free T3), over a 24hour period during methimazole treatment, has not been characterized in hyperthyroid cats. The purpose of this study was to determine the optimal sampling time to monitor therapeutic efficacy. Four healthy cats, in a crossover study, were treated with increasing doses of methimazole until a steady state of thyroid suppression was achieved. Additionally, thyroid fractions (including cTSH, canine thyroid stimulating hormone) were randomly and serially monitored for 24 hrs post medication. Thyroid profiles from 461 hyperthyroid cats treated with methimazole were retrieved from the DCPAH database and retrospectively reviewed. Wilcoxin signed rank tests and two-way ANOVA were used to evaluate the difference between thyroid fractions in treated and untreated groups. Linear regression analysis evaluated relationships of all thyroid fractions to time post-pilling in the retrospective study. Cats were divided according to dosage (>0.5 mg/kg/day), dosing interval (q24 vs q12 hrs) and TT4 < 55 nmol/L when correlations were performed.

All thyroid fractions were significantly suppressed and cTSH significantly elevated for the entire 24hours after once daily methimazole treatment in healthy cats (p-values < 0.05). In hyperthyroid cats, there was no significant relationship between time post-pill and thyroid fractions, even when dosing interval, dosage and TT4 < 55 nmol/L were taken into consideration. There was no correlation between dose rate and TT4. In conclusion, timing of blood sampling after methimazole administration does not appear to be a significant factor when assessing response to methimazole treatment.

ABSTRACT #244

THE EFFICACY AND SAFETY OF A NOVEL LIPOPHILIC FORMULATION METHIMAZOLE FOR THE ONCE DAILY TRANSDERMAL TREATMENT OF CATS WITH HYPERTHYROIDISM. KE Hill, MA Gieseg, DD Kingsbury, J Bridges, N Lopez-Villalobos & JP Chambers. Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, NZ.

Hyperthyroidism is a common disease in cats over 6 years of age. There are three treatment options for hyperthyroidism: thyroidectomy, radioactive iodine, or anti-thyroid drugs such as carbimazole (Neomercazole) or methimazole (Tapazole). Most cats are notoriously hard to medicate with oral drugs, so the use of gel formulations of drugs applied to the inner surface of cats' pinnae are practical and popular. For good transdermal absorption the drug needs to be highly lipophilic and applied in the correct vehicle. Previous studies on transdermal methimazole in cats have used pleuronic lecithin organogel (PLO) as the vehicle which may not be the most suitable vehicle for a lipophilic drug such as methimazole. Our study formulated a novel vehicle, highly suitable for lipophilic drugs. The purpose of our study was to determine if the once daily transdermal administration of this novel lipophilic gel formulation of methimazole is as safe and effective as orally administered carbimazole in treating spontaneous feline hyperthyroidism.

Forty-five cats with newly diagnosed, untreated, naturally occurring hyperthyroidism were randomly assigned to receive either carbimazole (5 mg PO, BID) or 10 mg (0.1 ml) of the lipophilic gel formulation of methimazole applied to the pinnae SID. Cats were examined on day 0 and after 1, 4, 8 and 12 weeks of treatment. The presence of clinical signs, body weight, systolic blood pressure, haematologic, serum biochemical and urine parameters, total serum thyroxine concentrations (TT4) and serum methimazole concentrations were recorded. At each visit owners were asked to complete a questionnaire on aspects of the health of their cat. Data were analysed with a linear mixed model that accounted for repeated measures on the same cat.

No significant differences between treatment groups were found at day 0. Both drugs were effective in treating hyperthyroidism as determined by a reduction in TT4, an increase in bodyweight and improvement in clinical signs. Repeated measurements of TT4, weight, blood pressure, heart rate, alkaline phosphatase, alanine aminotransferase, creatinine, urea and urine specific gravity showed no significant difference between treatment groups. The serum methimazole concentrations correlated poorly to the TT4 concentrations in both groups. No cats in the transdermal methimazole group developed pruritus or erythema of the ear.

The once daily application of a novel lipophilic formulation of transdermal methimazole applied to the pinnae is as effective as twice daily oral carbimazole in the treatment of feline hyperthyroidism. Once daily, transdermal application, compared to twice daily oral medications to cats has substantial, practical advantages to pet owners.

ABSTRACT #245

THE EFFECT OF A MULTI-STRAIN PROBIOTIC FORMULATION ON MARKERS OF GASTROINTESTINAL FUNCTION IN HEALTHY DOGS AND CATS. JS Suchodolski, JF Garcia, N Berghoff, N Grützner, CG Paddock, DJ Lanerie, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Probiotics are commonly recommended as an ancillary treatment for various gastrointestinal disorders. Administration of probiotics, due to their effect on intestinal microbial populations, could potentially lead to changes in concentrations of serum or fecal markers of gastrointestinal function. The aim of this study was to evaluate the effect of a commercially available multi-strain probiotic formulation (Proviable®, Nutramax Laboratories Inc.) on commonly used markers of gastrointestinal function in healthy dogs and cats.

Twelve healthy pet dogs and 12 healthy pet cats were enrolled in this study. The median age of the dogs was 3.5 years (range 1–10 years) and the median weight was 26.4 kg (range: 5.0–36.8 kg). The median age of the cats was 2.5 years (range 1–7 years) and the median weight was 4.9 kg (range 3.8–5.9 kg). Baseline serum and fecal samples were collected on day 0. A commercially available multi-strain probiotic formulation (1 capsule containing a total of 5×10^9 cfu *Bifidobacterium bifidum, Lactobacillus acidophilus*, *L. plantarum*, *L. rhamnosus*, *L. delbrueckii*, *Streptococcus salivarius*, and *Enterococcus faecium*) was administered daily for 21 days. Additional serum samples were collected on days 21 (last day of probiotic administration) and 42 (21 days after the last dose of probiotics). Follow-up fecal samples were collected every 3–4 days until day 42. Serum concentrations of cobalamin, folate, TLI, and PLI and fecal α_1-proteinase inhibitor concentrations were evaluated on days 0, 21, and 42. Probiotic species were detected by culture independent methods using denaturing gradient gel electrophoresis (DGGE) and genus specific 16S rDNA clone libraries. Quantitative changes in *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus* spp. were evaluated by quantitative real-time PCR using genus-specific 16S rDNA primers. Differences in gastrointestinal markers and bacterial groups between time points were determined using repeated measures ANOVA or Friedman's test where appropriate.

At least one of the probiotic species could be detected either by DGGE or by 16S clone libraries in 10 out of 12 dogs and in 11 out of 12 cats on day 21, but not on day 42. DGGE profiles revealed that probiotic bands appeared within 1–2 days of probiotic administration, but disappeared within a few days after discontinuation of administration. The number of *Lactobacillus* spp. (dogs and cats: $p<0.05$), *Bifidobacterium* spp. (dogs: $p=0.03$; cats: $p=0.07$), and *Enterococcus* spp. (dogs: $p=0.07$; cats: $p<0.05$) increased during probiotic administration, but returned to baseline values after administration of the probiotic was discontinued. None of the evaluated serum or fecal markers of gastrointestinal function changed significantly after treatment or discontinuation of treatment.

Probiotic species were detectable in the majority of treated dogs and cats. The results of this study would suggest that administration of this multi-strain probiotic for 21 days does not interfere with the interpretation of gastrointestinal function markers in healthy animals.

ABSTRACT #246

EFFECT OF *BIFIDOBACTERIUM ANIMALIS* AHC7 ON RESOLUTION OF ACUTE DIARRHEA IN THE CANINE. D. Minikhiem[1], R. Kelley[1], J. Park[1], T. Boileau[1], B. Kiely[2], L. O'Mahony[2,3]. [1]Procter & Gamble Pet Care, Lewisburg, OH, USA, [2]Alimentary Health Ltd., Cork, Ireland, [3]Alimentary Pharmabiotic Centre, National University of Ireland, Cork, Ireland.

The effect of a canine-derived probiotic, *Bifidobacteria animalis AHC7,* on idiopathic diarrhea was evaluated in adult dogs. Forty-five dogs (n=24 placebo, n=21 probiotic) were recruited but only 31 (n=18 placebo, n=13 probiotic) met the inclusion criteria used for subsequent analysis. The most common reason for exclusion was an initial stool score<4. Attending veterinarians prescribed metronidazole at their discretion. The mean age of dogs completing the trial was 1.58 years without difference between treatments. The probiotic *AHC7* (1×10^{10} CFU) and placebo were delivered twice daily for 14 days or until diarrhea resolved. Dogs were monitored daily for stool scores using a 4-point scale with 1 being ideal, and 4 being watery, liquid stool with little or no particulate matter. The mean time to diarrhea resolution was significantly reduced ($P=0.008$) for the *AHC7* group (3.9 days) compared to placebo (6.6 days). A subset analysis was performed for German Shepherd Dogs (n=10; n=6 placebo, n=4 AHC7) and for Labrador Retrievers (n=11; n=7 placebo, n=4 AHC7). In the German Shepherd Dogs, probiotic *AHC7* significantly ($P=0.026$) reduced the time to diarrhea resolution (3.3 days) compared to placebo (7.7 days). In Labrador Retrievers, probiotic *AHC7* also significantly ($P=0.048$) reduced the time to diarrhea resolution (3.3 days) compared to placebo (6.3 days). For all dogs, the percentage that were administered metronidazole was reduced in the probiotic group (38.5%) compared to placebo (50.0%). The results support that the probiotic, *B. animalis AHC7,* is an effective tool for the management of diarrhea in dogs.

ABSTRACT #247

EVALUATION OF INTRA-STOOL VARIABILITY OF THREE LACTIC ACID BACTERIAL GENERA IN DOGS BY QUANTITATIVE REAL-TIME PCR. JF Garcia, JM Steiner, JS Suchodolski. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Lactic acid bacteria are commonly used in probiotic formulations as they are thought to have beneficial effects by modulating the

intestinal microbiota. Quantitative real-time PCR (qPCR) allows detection and quantification of microbial genomic targets, and has been used for the quantification of bacterial groups in feces. To our knowledge, reproducibility of DNA extraction and intra-stool variation of lactic acid bacteria have not yet been described for qPCR from canine fecal samples. Therefore, the aims of this study were to evaluate the DNA extraction reproducibility and to determine intra-stool variability of three lactic acid bacterial genera assessed by qPCR in feces from dogs.

To test the reproducibility of the extraction method, DNA extraction was performed five times from four homogenized fecal samples. Additionally, feces from a total of eight dogs were used to evaluate intra-stool variability. Five sites were randomly sampled from each of the eight defecations (one from each dog). DNA was extracted from 100 mg of each sample using a phenol chloroform-based method. Separate qPCRs were performed for all samples using genus specific 16S rRNA gene primers for *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* spp. Standard curves using serial 1:10 DNA dilutions (range 2 to 0.0002 ng) containing species of these three bacterial genera were used to calculate the unknown bacterial genomic targets. Samples and standards were run in duplicates using a commercial real-time PCR thermocycler (iCycler iQ, Biorad).

Twenty samples were evaluated for testing the reproducibility of the DNA extraction for each genus. Coefficients of variation (%CV) were calculated from the five extractions for each of the 3 genera: *Bifidobacterium*: median: 5.2%CV, range: 2.7%CV to 6.2%CV; *Enterococcus*: median 7.1%CV, range: 5.9%CV to 8.4%CV; and *Lactobacillus*: median: 4.7%CV, range: 2.7%CV to 6.4%CV. Forty samples (eight defecations, five sites each) were analyzed to evaluate intra-stool variability for each genus. The %CV for the three genera: *Bifidobacterium*: median: 11.7%CV, range: 2.2%CV to 32.2%CV; *Enterococcus*: median: 6.3%CV, range: 1.8%CV to 21.1%CV; and *Lactobacillus*: median: 17.4%CV, range: 6.8%CV to 38.5%CV.

These results showed less than 10% variation among five different DNA extractions from the same fecal sample, thus showing that qPCR is a reproducible technique for the quantification of lactic acid bacteria in fecal samples from dogs. In this study we observed an intra-stool variability of up to 38.5%, which needs to be taken into account for future clinical studies.

ABSTRACT #248

MOLECULAR AND HISTOPATHOLOGICAL CHARACTERIZATION OF ENTEROADHERENT BACTERIA IN FAILURE TO THRIVE KITTENS. JL Nicklas[1], P Moisan[2], MR Stone[1], JL Gookin[1]. [1]North Carolina State University College of Veterinary Medicine, Raleigh, NC. [2]Rollins Animal Disease Diagnostic Laboratory, Raleigh, NC.

Diarrhea is a frequent cause of death or euthanasia of kittens, particularly those residing in rescue facilities. Bacterial culprits of diarrhea are particularly problematic to identify and it is likely that many have yet to be recognized. We sought to further characterize the molecular and histopathological features of failure-to-thrive kittens in which enteroadherent coccobacilli were observed on necropsy examination. Seven unrelated kittens aged 3–10 weeks from animal shelter or foster facilities died or were euthanized and submitted for necropsy to a state diagnostic laboratory. Clinical signs reported prior to death included: diarrhea (4), sudden death (1), upper respiratory infection (1), and unspecified (1). All kittens had histopathological examination findings of mild to moderate, subacute to acute, necrotizing enteritis with adherent colonies of coccobacilli. In each kitten, a diagnosis of attaching and effacing *E.coli* infection was presumed. Fecal bacterial culture results, performed in 4 kittens, were positive for *E.coli* in 3 cases, 2 of which were PCR positive for the attaching and effacing virulence factor. Concurrent infections (trichomonas, panleukopenia, and adenovirus) were identified in 3 kittens. Molecular characterization of the enteroadherent bacteria in each case was performed by fluorescence in-situ hybridization. Eubacterial (Eub338) and *E.coli*-specific fluorescence-labeled oligonucleotide probes were applied to formalin-fixed paraffin-embedded sections of intestinal tissue from each kitten. Only 2 of the 7 kittens demonstrated positive hybridization with Eub338 and *E.coli* probes. This was unexpected as all cases were presumed to be *E.coli* infections. Gram stains performed on fixed tissue from each kitten revealed Gram negative bacteria in the 2 cases positive for hybridization with Eub and *E.coli* probes, while the remaining 5 kittens demonstrated Gram positive infections. Hybridization conditions were optimized for lysozyme permeabilization of Gram positive bacteria and tissue sections re-probed using eubacterial (Eub338) and *Enterococcus* spp.-specific (Enc221) probes. Tissue sections from all 5 kittens with adherent Gram positive bacteria were positive for hybridization with eubacterial and *Enterococcus* spp. probes. Transmission electron microscopy of representative lesions from *E.coli* and *Enterococcus spp*. infected kittens revealed coccobacilli adherent to microvilli of intestinal epithelial cells.

Each of these kittens was histologically diagnosed as having attaching and effacing E.coli infection. In 2 of 7 kittens the adherent bacteria were molecularly identified in-situ as *E.coli*. In the majority of these cases however, adherent bacteria were identified as Gram positive Enterococci. Light microscopic and ultrastructural lesions resulting from these infections were highly similar. Enteroadherent *Enterococcus* spp. infection may be an unrecognized and important cause or co-morbid factor contributing to death or euthanasia in failure-to-thrive kittens.

ABSTRACT #249

ABSENCE OF A BACTERIAL ASSOCIATION IN YORKSHIRE TERRIERS WITH PROTEIN-LOSING ENTEROPATHY AND CYSTIC INTESTINAL CRYPTS. M Craven, GE Duhamel, NB Sutter, KW Simpson. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Yorkshire Terriers' (YT) are predisposed (OR 4.2–10.1) to protein-losing enteropathy (PLE). Intestinal pathology of YT-PLE typically includes lymphangiectasia and mucosal lymphocytic plasmacytic infiltrates. Lesions described as ''dilated intestinal crypts'' (JVIM 14:298–307, 2000) or ''mucoid cryptal ectasia'' (JAAHA 39:187–191, 2003) consisting of cystic crypts filled with mucus and necrotic cellular debris, and occasional crypt abscessation have been reported in PLE. We sought to further describe the clinical and pathological features of YT-PLE and to explore a possible relationship between mucosal histopathology and mucosal bacteria

14 YT with PLE were identified between 1999–2008 (8M, 6F: 4 prospective, 10 retrospective). Clinical features and outcome were available for 12 dogs and intestinal biopsies for 14 (8 endoscopic, 6 surgical). Mucosal histopathology was examined by a blinded pathologist (GED) and inflammatory infiltrates, lymphangiectasia and crypt abnormalities were scored as normal-0, mild-1, moderate-2 or severe-3. Fluorescence in situ hybridization (FISH) with a eubacterial probe was used to ascertain the presence and distribution of bacteria in duodenal biopsies.

The median age and bodyweight at presentation were 96 mo, and 3.1 kg, respectively. Vomiting (7), diarrhea (6) and inappetance (6) were the most frequent clinical signs. Bicavity effusions were present in 5 dogs, and ascites alone in 3. Hypoalbuminemia (< 3.1g/dl) was present in all 12 dogs (median 1.6g/dl), and hypoglobulinemia (<1.9g/dl) in 7 (median 1.7g/dl). Additional biochemical abnormalities included hypocalcemia (12), hypocholesterolemia (11) hypomagnesemia (9), hypokalemia (5) and hypochloremia (5). Hematological abnormalities included mild anemia (5), thrombocytosis (8), mature neutrophilia (6), and neutrophilia with a left shift (n=3). Anti-thrombin III was low ($<75\%$) in 4/6 dogs evaluated (mean 62%). Duodenal biopsies from all affected YT contained cystic intestinal crypts. Lymphangiectasia (median, range; 2,0–3), crypt hyperplasia (2,1–3) and villus blunting (4 dogs), were less consistent features. Mucosal infiltration of lymphocytes and plasma cells (villus 2,1–3; crypt 3,2–3) and eosinophils (1.5,1–2) was common. Empirical therapy with corticosteroids (11/12), azathioprine (2/12), antibiotics, plasma and diuretics was associated with a poor outcome. 7/12 cases died or were euthanased within 3 m of diagnosis. Long-term survival occurred in 3 dogs, (36, 24, and 8 m), and 2 are alive at 3 m and 4 m after diagnosis. FISH analysis showed no evidence of a bacterial association with crypt cysts or with mucosal inflammation.

We conclude that YT suffer a severe and often fatal form of PLE that is consistently associated with cystic intestinal crypts. The absence of a bacterial association suggests that this may be a primary morphogenetic disorder with or without a secondary environmental trigger.Further work is required to ascertain the etiopathogenesis of crypt lesions and their relationship to enteric protein loss.

ABSTRACT #250
SERUM fPLI AND SPEC fPL CONCENTRATIONS IN CATS WITH EXPERIMENTALLY INDUCED CHRONIC RENAL FAILURE. PG Xenoulis[1], DR Finco[2], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX. [2]College of Veterinary Medicine, University of Georgia, Athens, GA.

Although feline pancreatitis occurs frequently, its diagnosis can be challenging mainly because of its non-specific clinical presentation and the fact that until recently a sensitive and specific test for the diagnosis of this condition was not available. In the past decade, two immunoassays, feline trypsin-like immunoreactivity (fTLI) and feline pancreatic lipase immunoreactivity (fPLI) were developed and analytically validated as tests for pancreatic function and pathology. More recently, a new assay for the measurement of serum concentrations of feline pancreatic lipase, Spec fPL^{TM}, has been developed and validated. This assay shows the same performance characteristics as the original fPLI assay. Renal failure, a major differential diagnosis in cats with clinical evidence suggestive of pancreatitis, has previously been shown to be associated with significant increases of serum fTLI concentrations, therefore reducing the specificity of this assay for feline pancreatitis. The aim of the present study was to investigate the effect of experimentally induced chronic renal failure (CRF) on serum fPLI and Spec fPL concentrations.

Leftover serum samples (stored at −80 °C) from 18 cats with CRF induced by subtotal nephrectomy for an unrelated project were used for the present study. Serum fPLI concentration was measured in samples from all 18 cats and compared with those in 41 healthy cats. Serum Spec fPL was measured in 16 cats with experimentally induced CRF. To test the stability of feline pancreatic lipase in serum samples stored at −80 °C, nine unrelated samples were tested before and after 44 months of storage at −80°C.

There was no statistically significant difference of the mean serum fPLI concentrations for the 9 samples before and after 44 months of storage at −80 °C ($p=0.162$). All cats with experimentally induced CRF had an increased serum creatinine concentration (>1.5 mg/dL; median: 2.9 mg/dL; range: 2.2–5.4 mg/dL) at the time of the study. Median serum fPLI concentration was not significantly different between cats with induced CRF (median: 8.6 μg/L, range: 5.4–9.9 μg/L) and healthy cats (median: 7.4 μg/L, range: 5.0–15.2 μg/L, $p=0.124$). All cats with induced CRF had serum fPLI and Spec fPL concentrations within their respective reference ranges. There was no significant correlation between serum creatinine concentrations and fPLI concentrations (Spearman $r=0.037$; $p=0.884$) or Spec fPL concentrations (Spearman $r=0.127$; $p=0.639$).

In conclusion, there was no significant difference in serum fPLI concentrations between cats with experimentally induced CRF and healthy cats. These results suggest that serum fPLI and Spec fPL concentrations are not affected by CRF and that serum fPLI and Spec fPL concentrations can be used for the diagnosis of pancreatitis in cats with renal failure. Further studies involving cats with naturally occurring CRF or with acute renal failure are warranted to verify these results for these groups of patients.

ABSTRACT #251
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CANINE PANCREATA WITH LYMPHOCYTIC INFILTRATION. BM Bostrom[1], SJ Newman[2], JS Suchodolski[1], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX. [2]University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

Lymphocytic infiltration of the canine pancreas is more prevalent than previously thought. However, the significance of such lymphocytic infiltration of the canine pancreas is unknown. In order to further characterize the lymphocytic infiltrate, immunohistochemistry (IHC) for CD3+ (suggesting T cell lineage) and CD79+ (suggesting B cell lineage) was performed.

Pancreatic samples were collected from dogs during necropsy performed at either the Animal Medical Center in Manhattan, NY or at the College of Veterinary Medicine at Texas A&M University. Hematoxylin and eosin stained slides of the pancreatic samples were graded using a previously published canine pancreatic histologic grading scheme (Newman et al. J Vet Diagn Invest, 2006). Fifteen fixed samples of pancreatic tissue representing 15 individual dogs were selected based on a histologic grade of 2 or higher for lymphocytic infiltration, which is defined as 10–40% of the section being affected (grade 2) and more than 40% of the section being affected (grade 3). The 15 pancreatic samples were recut and stained with monoclonal antibodies against CD3+ and CD79+ antigens using standard IHC techniques. A board certified veterinary pathologist (SJN) examined the stained slides and estimated the percentage of lymphocytes that were CD3+ or CD79+.

Lymphocytes of the sections analyzed were mostly CD3+ (median: 60%, range: <5% to 95%), but not very commonly CD79+ (median: <5%, range: 0% to 50%), suggesting mostly lymphocytes of the T cell lineage.

The predominance of T lymphocytes could indicate a cell-mediated immune response similar to that found in human patients with chronic pancreatitis. Additional characterization of the T lymphocyte population is warranted to further study the pathophysiology of pancreatic lymphocytic inflammation in the dog.

ABSTRACT #252
PURIFICATION AND PARTIAL CHARACTERIZATION OF CANINE S100A12. RM Heilmann, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Canine S100A12 (cS100A12), also known as canine P6, MRP6, or calgranulin C, is a calcium-binding protein that is predominantly expressed in neutrophils and monocytes. Interaction of S100A12 with the receptor for advanced glycation end products (RAGE), a pattern recognition receptor with a central role in inflammation, has been shown to activate the NF-κB signal transduction pathway, leading to release of proinflammatory cytokines (e.g., TNF-α). A positive feedback mechanism involving RAGE expression has been suggested to result in amplification and perpetuation of inflammation in conditions such as inflammatory bowel disease (IBD) in humans. Moreover, the concentration of S100A12 in feces has been shown to be a sensitive and specific marker for gastrointestinal inflammation in humans with IBD. To date, only human, porcine, bovine, and rabbit S100A12 has been purified, and an immunoassay for the quantification of S100A12 is only available for humans. Therefore, the aim of this study was to develop a protocol for the purification of cS100A12 from canine whole blood and to partially characterize this protein as a prelude to the development of an immunoassay for its quantification in canine serum and fecal specimens.

Leukocytes were isolated from canine whole blood by dextran sedimentation. Canine S100A12 was extracted from the cytosol fraction of the cells by three successive freeze-thaw-sonication cycles, and was further purified by ammonium sulfate precipitation, hydrophobic interaction chromatography, strong cation- and anion-exchange column chromatography, and preparative native polyacrylamide gel electrophoresis. Purified cS100A12 was partially characterized by determination of the molecular weight using automated fluorescence-based reducing gel electrophoresis (Protein 80 assay) on an Agilent Bioanalyzer 2100, estimation of isoelectric point by isoelectric focusing, estimation of specific absorbance, and N-terminal amino acid (AA) sequencing by Edman degradation.

Canine S100A12 was successfully purified from canine whole blood. Based on comparison to known molecular weight standards using the Protein 80 assay, the molecular weight of cS100A12 was estimated at 7,730. Isoelectric focusing revealed an isoelectric point of 6.0. The approximate specific absorbance of cS100A12 at 280 nm was determined to be 1.78 for a 1 mg/ml solution. The N-terminal AA sequence of the first 15 residues of cS100A12 was Thr-Lys-Leu-Glu-Asp-His-X-Glu-Gly-Ile-Val-Asp-Val-Phe-His, and showed 100% homology with the predicted protein sequence available through the canine genome project. Sequence homology for the 14 N-terminal residues of cS100A12 identified with those of feline, bovine, and human S100A12 was 78.6%.

We conclude that canine S100A12 can be successfully purified from canine whole blood using the method described. These findings will facilitate the development of an immunoassay for the quantification of cS100A12 in serum and feces from dogs.

ABSTRACT #253
ASSOCIATION STUDY OF SINGLE NUCLEOTIDE POLYMORPHISMS WITH COBALAMIN DEFICIENCY IN THE CHINESE SHAR PEI. N Grützner, MA Bishop, JS Suchodolski, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Based on a genome-wide scan using the canine minimal screening set-2 (MSS-2), cobalamin deficiency in the Chinese Shar Pei (Shar Pei) has recently been linked to a genomic locus in close proximity to two microsatellite markers on canine chromosome 13. The canine MSS-2 contains 327 microsatellite markers with an average marker spacing of 9 megabases (Mb), but no gaps larger than 17.1 Mb. However, these studies do not conclusively narrow down the region on chromosome 13 as the major locus for a gene or genes responsible for cobalamin deficiency in the Shar Pei. Also, not all genes that have been associated with cobalamin deficiency in humans or genes encoding for cobalamin binding proteins have been identified in the dog. The aim of this study was to scan the whole genome using single nucleotide polymorphisms (SNP) to identify genes or regions that may be associated with cobalamin deficiency in the Shar Pei.

Whole blood and serum samples were collected from a total of 42 unrelated Shar Peis. Serum cobalamin concentration (reference range: 252–908 ng/L) was measured and DNA was extracted from whole blood. A total of 49,633 SNPs were genotyped using the Affymetrix v2 Platinum canine SNP array. The analysis of genotype data was conducted by a whole genome association analysis toolset (Plink v1.05). A Bonferroni correction for multiple statistical comparisons was used to evaluate the significance of any potential association. Significance was set at a p-value <0.000001 ($p<1.0\times10^{-6}$).

Undetectable serum cobalamin concentrations (<150 ng/L) were observed in 14 of 42 Shar Peis (33.3%), and these Shar Peis were considered to be severely cobalamin deficient. The remaining 28 Shar Peis had serum cobalamin concentrations within the reference range. The SNP analysis revealed a total of 4 markers with a significant p-value of $<1.0\times10^{-6}$. These markers were located between 26,440,885–28,178,693 Mb on chromosome 13.

In conclusion, SNP analysis revealed a cluster of SNP markers on chromosome 13, which were significantly associated with cobalamin deficiency in this group of Shar Peis. Interestingly, previous results from linkage studies using the MSS-2 have pointed to the same region of chromosome 13. Thus, the findings of this study provide further evidence that this region of chromosome 13 contains the causative gene or genes for this condition.

ABSTRACT #254
APOPTOSIS IN THE PATHOGENESIS AND TREATMENT OF CANINE INFLAMMATORY BOWEL DISEASE. AE Jergens, L Flagg, D Moore, R Denadel, MJ Wannemuehler. College of Veterinary Medicine, Iowa State University, Ames, IA.

Idiopathic inflammatory bowel disease (IBD) is a common cause for chronic gastrointestinal disease in dogs. Abnormal cell death by apoptosis may result in the persistence of activated intestinal T cells that contribute to chronic mucosal inflammation and possibly promote impaired therapeutic responsiveness. The aim of this study was to compare rates of apoptosis in different intestinal compartments between healthy dogs and dogs with IBD, and to investigate whether lymphocyte apoptosis might predict the success of medical therapy. Fifty four dogs with IBD and 6 healthy control dogs were evaluated prospectively in the context of a controlled drug trial. Intestinal biopsies obtained by endoscopy were analyzed for their densities and distribution of apoptotic cells in the duodenum (apical villous [AV] and base [BV]) and in the colon (C). Intestinal lymphocytes expressing pro-apoptotic caspase 3 (Casp3) and anti-apoptotic B-cell leukemia/lymphoma 2 (Bcl-2) were assessed by immunohistochemistry and quantitated using light microscopy. TUNEL assays confirmed detection of fragmented DNA in tissue specimens. Correlation between Casp3/Bcl-2 expression and clinical response (CIBDAI score) to medical treatment was also determined. Results indicate that immunolabelling for Casp3 and Bcl-2 was predominantly ($>90\%$) limited to the lamina proporia (LP). Bcl-2 positive cells were increased in the LP of IBD dogs as compared to control dogs, with the greatest numbers observed in AV and BV compartments of dogs having the greatest disease severity. Casp3 expression was greater in the AV and BV of healthy dogs versus IBD dogs. Most (17/20) moderate-to-severe IBD dogs responded to standardized medical therapy. In IBD dogs, Bcl-2 expression was greatest in a subset of dogs least responsive to drug therapy. We conclude that increased expression of Bcl-2 and decreased Casp3 expression in LP lymphocytes of dogs with IBD indicate a reduced susceptibility to apoptosis of lymphoid cells. Up-regulated Bcl-2 expression may be of value as a biomarker in predicting the response to therapy.

ABSTRACT #255
FEASIBILITY OF A MODIFIED USSING AIR SUCTION CHAMBER FOR THE EVALUATION OF INTESTINAL MUCOSAL FUNCTION IN ENDOSCOPIC BIOPSIES FROM DOGS AND CATS. I Ruhnke[1], JV DeBiasio[1], JS Suchodolski[1], MW Musch[2], JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Department of Medicine, University of Chicago, IL.

Chronic intestinal diseases in dogs and cats, such as inflammatory bowel disease, food hypersensitivity, and intestinal lymphoma are poorly characterized, and new diagnostic modalities are needed to better understand these disorders. Ussing chambers have been used to study intestinal membrane transport in many species. The conventional circulating chamber requires collection of large tissue specimens, and thus, such experiments have rarely been performed in small animal patients with spontaneous gastrointestinal disease. A modified Ussing air suction chamber (MUAS) allows the use of small tissue pieces, which would allow the study of clinical patients with gastrointestinal diseases. The aim of this study was to evaluate the feasibility of a MUAS for evaluation of intestinal membrane function in endoscopic biopsies from dogs and cats.

Six duodenal biopsies from two dogs and three duodenal biopsies from one cat were collected during diagnostic gastroduodenoscopy and analyzed. Biopsies were transferred into the MUAS within 15 minutes of collection. To simulate physiological conditions, the water reservoir was heated to 37 °C, oxygenated with 95% O_2 and 5% CO_2, and energy was provided by adding 10 mM glucose and 10 mM sorbitol. After 20 min of equilibration, the voltage applied through the tissue was clamped to zero. Voltage and the membrane-generated short circuit current (SCC) were constantly recorded. To determine absorptive function, the mucosal side of canine biopsies was exposed to 40 mM glucose, followed by 500 μM phloridizin and the feline biopsies were tested with 500 μM phloridizin. Secretory function was tested on feline biopsies with 200 μM histamine and 200 μM serotonin by adding those chemicals to the serosal side.

All biopsies showed reproducible responses to the applied stimuli. The mean±SD conductance for canine and feline duodenal biopsies was 47.8±2.8 mS cm^{-2} and 61.5±5.1 mS cm^{-2}, respectively. Addition of 40 mM glucose to the mucosal side of the dog tissue increased SCC by 55.0±36.2 μA/cm^2 in comparison to SCC before stimulation (ΔSCC). Application of 500 μM phloridizin was associated with a ΔSCC of −63.6±14.5 μA/cm^2 in canine biopsies and −22.0±8.7 μA/cm^2 in feline biopsies. Addition of 200 μM histamine caused a reproducible ΔSCC of 28.0±2.0 μA/cm^2 and that of 200 μM serotonin a ΔSCC of 30.6±1.1 μA/cm^2 in the cat duodenum.

These data demonstrate the feasibility of using a modified Ussing air suction chamber for evaluation of endoscopically collected canine and feline duodenal biopsies. Reproducible responses were observed after in-vitro exposure of biopsy specimens to glucose, phloridizin, histamine, and serotonin. Further studies to characterize tissue samples from small animal patients with spontaneous chronic intestinal disease with this technology are warranted.

ABSTRACT #256
COMPARISON OF GASTROINTESTINAL MOTILITY IN DOGS TREATED WITH IBEROGAST™ AND ONDANSETRON USING THE SMARTPILL™. AM Bradley, P Boscan, K Dowers, KW McCord, M Marquez and DC Twedt. Colorado State University, Fort Collins, CO.

There are a number of drugs used for the treatment of gastrointestinal (GI) motility disorders in humans but few have adequately been evaluated in the dog. Iberogast (stw 5) is a combination of a number of herbs and is reported to affect GI motility in humans.

Ondansetron is a 5-HT3 receptor antagonist used in dogs for antiemetic properties, but there are also a number of reports showing effects on GI motility in humans. The objective of this study was to determine the effects of Iberogast and ondansetron on GI motility using SmartPill technology. The SmartPill™ is a noninvasive wireless sensor capsule that is given orally and transmits data on gastric and small and large bowel pressures, transit time, luminal pH and temperature.

Ten healthy adult dogs were used in a cross-over placebo controlled design. All dogs received either ondansetron (0.5 mg/kg PO TID), Iberogast (1 ml PO TID) or placebo (saline 1 ml SQ TID) for three days prior to SmartPill administration and during motility recordings. On the fourth day a standard meal was fed, and the SmartPill was administered and motility data recorded until passage of the pill. There was a 7-day washout period between trials. Transit time (TT), pH, maximum pressure (MP), mean peak amplitude (MPA), contractions/minute (CPM) and motility index (MI, calculated AUC/time) for the stomach, small intestine (SI) and large intestine (LI). Data was analyzed using multivariable linear regression analysis with significance of $p < 0.05$.

Total TT was significantly longer for Iberogast and ondansetron compared to placebo. Treatment with ondansetron led to significantly higher pH in each GI segment. Treatment had no significant effect on MP, MPA, CPM or MI.

Both Iberogast and ondansetron increase TT, but given the lack of individual segmental parameter differences, the mechanism of action is unclear. Iberogast and ondansetron may be beneficial in the management of disorders in dogs associated with decreased GI transit time.

ABSTRACT #257
SUCCESSFUL TREATMENT OF BENIGN ESOPHAGEAL STRICTURES WITH BALLOON DILATION AND SUBMUCOSAL TRIAMCINOLONE INJECTION IN FIVE DOGS AND ONE CAT. B Mayer-Roenne, C Fraune, KA Ryan, FP Gaschen. Department of Veterinary Clinical Sciences, Louisiana State University, Baton Rouge, LA.

Development of benign esophageal strictures is an uncommon, but severe complication of general anesthesia, esophageal foreign bodies, severe prolonged vomiting and gastroesophageal reflux in small animals leading to regurgitation, weight loss and malnutrition. Successful treatment with balloon dilation has been described. Often multiple procedures are necessary for resolution of clinical signs.

Endoscopically guided submucosal triamcinolone injections at the stricture site were used in five dogs and one cat with esophageal strictures. In three dogs the injections were performed at the base of the stricture prior to balloon dilation. In one dog a small mucosal tear was noticed after injection. Subsequent dilation extended the tear both in length and depth, causing obvious bleeding. In two dogs and one cat triamcinolone was injected in the area of the stricture after balloon dilation without complications. All animals recovered well and were able to eat soft food without signs of regurgitation within twelve hours after the procedure. Three dogs had one ballooning procedure; two dogs had two procedures; the cat had four procedures with three triamcinolone injections. Two dogs continued to show regurgitation when eating regular food and were maintained on slurry. Four animals had recheck esophagoscopy revealing resolution of strictures; their signs had subsided completely.

Submucosal intralesional triamcinolone injections are a well tolerated adjunctive treatment for benign esophageal strictures in small animals. Controlled studies are necessary to assess whether this treatment improves outcome, and to evaluate the risks and benefits of performing injections prior to or after balloon dilation.

ABSTRACT #258
SMALL ANIMAL GASTROINTESTINAL ENDOSCOPY: A COMPUTER-AIDED INSTRUCTIONAL PROGRAM. AE Jergens, A Ginman, NS Rappa, T Leisen, A Staniger. College of Veterinary Medicine and Information Technology, Iowa State University, Ames, IA.

Gastrointestinal endoscopy is routinely performed in dogs and cats having clinical signs of chronic gastroenteritis. Endoscopy allows for direct visual inspection of mucosal surfaces of the alimentary tract and facilitates targeted biopsy for histopathologic interpretation. At present, experience with learning these advanced diagnostic techniques is sporadic and insufficient for most veterinary students to obtain prior to graduation. There is a real need for a concise, readily-accessible, and well illustrated teaching resource that can be quickly and conveniently reviewed by students prior to performing endoscopic procedures in the clinics. The aim of this project was to develop a novel, computer-aided instructional program for veterinary students which demonstrates performance of routine gastrointestinal endoscopic procedures in dogs and cats. The computer-aided instruction program (e.g., Flash, a multimedia authoring package) consisted of four individual lessons (e.g., esophagoscopy, gastroscopy, enteroscopy, colonoscopy) written for use on PC and MAC platform personal computers. Each lesson was designed using a standardized template to include clinical indications, instrumentation/patient preparation, normal endoscopic appearances, abnormal endoscopic appearances, biopsy technique with sample submission, and clinical case studies. Lessons were written for ease of learning and accompanied by digital endoscopic images, computer graphics, and short, real-time digital videostreams of actual gastrointestinal endoscopic procedures. Lesson integration with computer technology proved to be a seamless process. The completed lessons clearly and succinctly reviewed the key learning features of each endoscopic procedure. Performance assessment via WebCT is currently being developed to allow self-administration and immediate feedback by students in clinical rotations.

ABSTRACT #259
USEFULNESS OF CONTRAST-ENHANCED ULTRASONOGRAPHY FOR CHARACTERIZATION OF FOCAL LIVER LESIONS. K Nakamura, H Ohta, M Yamasaki, M Takiguchi. Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan.

In six normal beagles and twenty dogs with spontaneous focal or mutifocal hepatic lesions, contrast-enhanced ultrasonography was performed. Sonazoid, a newly developed second-generation contrast agent with the ability of real-time contrast imaging along with parenchymal imaging, was selected in this study. Appropriate protocol for the evaluation of all three phases (the arterial, portal and parenchymal phase) was established based on the results of normal beagles. The evaluation of echogenicity of hepatic nodules during the arterial, and parenchymal phase could differentiate malignant tumor from benign nodule with very high accuracy. In 17 of 18 dogs with malignant lesions, the nodule was hypoechoic to the surrounding normal liver parenchyma during the parenchymal phase. This finding was significantly ($P < 0.01$) correlated with malignancy with high sensitivity (100%) and specificity (94.4%). Moreover, the evaluation of the echogenicity during the arterial phase could differentiate nodular hyperplasia, hepatocellular carcinoma and hemangiosarcoma. Nodular hyperplasia, hepatocellular carcinoma and hemangiosarcoma showed characteristic echogenicity during the arterial phase (isoechoic, hyperechoic, and hypoechoic compared to the surrounding normal liver parenchyma, respectively). No characteristic finding was seen during the portal phase. Contrast-enhanced ultrasonography with Sonazoid appears to improve the characterization of canine focal or multifocal hepatic lesions.

ABSTRACT #260
HEPATIC COPPER CONCENTRATIONS IN LABRADOR RETRIEVERS WITH AND WITHOUT CHRONIC HEPATITIS (1980–2008): AN EMERGING SYNDROME OR OVER-SUPPLEMENTATION? AN Johnston, SA Center, SP McDonough, J Wakshlag, KL Warner. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Association between high hepatic copper and necroinflammatory liver injury is acknowledged. Transition metal status of copper imposes oxidant challenge during ordinarily innocuous hepatic responses and augments injury associated with chronic hepatitis (CH). Etiopathogenesis of hepatic copper accumulation remains enigmatic in breeds other than Bedlington terriers, while study of

related Labrador Retrievers (LR) implicated breed predisposition. We have recognized increased incidence of pathologically elevated hepatic copper in dogs (diverse breeds) from 1998–2008, coincident with a pet food industry recommendation to replace cupric/cuprous oxide in feed formulations because of low bioavailability.[1]

Hepatic copper concentrations in LR (used as a sentinel breed) biopsies collected between 1980–2008 were segregated into 1980–1997 (T1) and 1998–2008 (T2). Copper quantification (μg/gm [dry matter basis], atomic absorption spectrometry) in formalin-fixed-deparaffinized tissue was validated against fresh liver. Cases were identified (institutional software), and tissue sections stained with H&E, Masson's Trichrome, Reticulin, Prussian Blue, and Rhodanine to categorize CH (n=36) vs control (CT, n=36). Copper concentrations and age (median [range]) were compared using Wilcoxon Rank Sum. Gender was compared using two-by-two tables. $P \leq 0.05$ determined significance. Relatedness of dogs or dietary management were not investigated.

Copper concentrations of CHT2 (1,150[191–3,950]μg/gm) were significantly higher than CHT1 (441[166–3,300]μg/gm); likewise, copper concentrations of CTT2 (347[101–981]μg/gm) were significantly higher than CTT1 (156[104–398]μg/gm); $P=0.009$, $P=0.003$, respectively. There were no differences in age or gender.

Increased hepatic copper in LR with and without CH during T2 may reflect increased copper bioavailability and consequently increased copper delivery in revised feed formulations.

[1]Czarnecki-Maulden G, et al: FASEBJ;1993:7:A305.

ABSTRACT #261

REDUCTION OF CYTOKINE-INDUCED PROSTAGLANDIN E2 AND CHEMOKINE PRODUCTION IN CULTURED CANINE HEPATOCYTES BY NATURAL PRODUCTS. AY Au[1,2,3], JM Hasenwinkel[1], CG Frondoza[1,3,4]. [1]Nutramax Laboratories, Inc., Edgewood, MD. [2]Syracuse University, Syracuse, NY. [3]Johns Hopkins University, Baltimore, MD. [4]Mississippi State University, Mississippi State, MS.

Liver tissue is known to heal and regenerate with the critical participation of pro-inflammatory mediators and the growth factors that they induce. However, inflammation of the liver, which is a common ailment in dogs, can result in disease, fibrosis, and life threatening tissue damage when left uncontrolled. Hepatocytes that comprise the bulk of liver tissue have been documented to produce pro-inflammatory mediators and growth factors. How the production of pro-inflammatory mediators by hepatocytes is regulated is still unclear. Moreover, the mechanism by which hepatocytes play a role in liver repair and regeneration is also poorly understood. We have utilized a culture system which facilitates cellular adhesion, proliferation, and maintenance of the hepatocyte phenotype. Using this model, we tested the hypothesis that primary canine hepatocytes can be activated with interleukin 1-beta (IL-1β) to produce pro-inflammatory mediators. We also determined whether this activation can be counteracted by the hepatoprotective natural products Curcumin (NMXCC95™) and a melon-derived superoxide dismutase (MSOD) which is found in Oxstrin®.

Primary canine hepatocytes (5×10^5 cells) were plated in wells coated with 0.1% rat type I collagen. Cells were pre-treated for 48 hours with: (i) control media alone, (ii) NMXCC95 ™ (0.368, 3.68, or 36.8 μg/ml), or (iii) MSOD activity of 14000 IU/g (0.0143, 0.143, 1.43 μg/ml), and then activated for 72 hours with IL-1β(10 ng/ml). Phenotype analysis was verified by immunostaining for albumin and cytokeratin 8 (CK8). Production of pro-inflammatory mediators prostaglandin E2 (PGE_2), interleukin-8 (IL-8), and macrophage chemotactic protein-1(MCP-1) were assayed by ELISA. Statistical analysis was performed using one-way analysis of variance (ANOVA) with significance set at $p<0.05$ using Student-Neuman-Keuls post-hoc analysis.

Hepatocyte cultures continued to produce liver phenotype markers albumin and CK8. Activation with IL-1β significantly induced pro-inflammatory PGE_2, IL-8, and MCP-1 production 2- to 6-fold. Pre-treatment with both Curcumin and melon-derived SOD significantly reduced PGE_2 production by as much as 50% to 60% of the activated control, respectively. These agents also reduced IL-8 and MCP-1 production. The present study shows that production of pro-inflammatory mediators by hepatocytes can be reduced by the hepatoprotective natural products NMXCC95™ and MSOD.

ABSTRACT #262

SERUM PROTEIN ELECTROPHORESIS BETA-GAMMA BRIDGING IS A POOR PREDICTOR FOR HEPATIC DISEASE. MS Camus, PM Krimer, FS Almy, BE LeRoy. College of Veterinary Medicine, University of Georgia, Athens, GA.

Based on human studies conducted in the 1950's, beta-gamma bridging (β-γ bridging) on protein electrophoresis is presented in both the human and veterinary literature as virtually pathognomonic for hepatic disease. However, the criteria for β-γ bridging are not defined and very few veterinary publications exist to support a relationship between β-γ bridging and liver disease. The goal of this retrospective study was to confirm or repudiate an association between the two by measuring the positive predictive value of β-γ bridging for liver disease. All electrophoretograms generated at the University of Georgia between 1994 and 2008 were evaluated for the presence of β-γ bridging. β-γ bridging was identified if there were 1) an albumin to globulin ratio below the established reference interval; 2) indistinct separation/demarcation between all β and γ globulin fractions or between the β_2 and γ fractions, with a negative shoulder slope of $<5\%$; and 3) predominance of γ proteins. There were 237 electrophoretograms examined, of which 25 cases (11 dogs, 11 cats, 3 horses) met the inclusion criteria for a β-γ bridge. Of these cases, 8/25 (32%) had hepatic disease based on biochemistry, cytology, histopathology, and/or necropsy findings, while 9/25 (36%) had a variety of infectious diseases. The positive predictive value of β-γ bridging for hepatic disease was determined to be 32.0% with a 95% confidence interval of 15.0–53.5% ($p<0.000$). The positive predictive value of this phenomenon for infectious disease was determined to be 36.0% with a 95% confidence interval of 18.0–57.5% ($p<0.000$). Though β-γ bridging can be found in some cases of hepatic pathology, it is not "pathognomonic" for liver diseases and is as frequently found with infectious diseases.

ABSTRACT #263

IN VITRO COMPARISON OF PLAIN RADIOGRAPHY, DOUBLE CONTRAST CYSTOGRAPHY, ULTRASONOGRAPHY, AND COMPUTED TOMOGRAPHY FOR MEASURING UROCYSTOLITH SIZE USING A BLADDER PHANTOM. K Byl, JM Kruger, J Kinns, N Nelson, J Hauptman, CA Johnson. Michigan State University College of Veterinary Medicine, East Lansing, MI.

Accurate estimation of stone size is a critical factor in determining whether urocystoliths are amenable to removal by minimally invasive procedures. The purpose of this study was to compare the ability of plain radiography (PR), double-contrast cystography (DCC), ultrasonography (US), and computed tomography (CT) to accurately estimate the size a single urolith in an in vitro bladder phantom model.

The bladder phantom model consisted of a rubber balloon distended with a standard volume of 1% saline to simulate a urinary bladder. The balloon was positioned inside a 4-quart storage container on a 2 cm cushion of 2% gelatin and covered with 2 cm of water to approximate the soft tissue density present in a medium sized dog. Thirty canine urocystoliths of varying size (1–11 mm) and mineral composition (calcium oxalate, struvite, and ammonium urate) were individually placed in the bladder phantom and imaged by the four imaging modalities. Two radiologists, blinded to the stone size and mineral content, measured each stone at its greatest diameter using computerized calipers. Size estimates were then compared to actual stone size as determined by digital calipers.

Ultrasonography significantly overestimated urolith size by a mean of 2.95 mm±SEM 0.36 mm ($p<0.01$) compared to other imaging modalities, regardless of mineral composition. Size estimates obtained by PR, DCC, and CT were not significantly different. In conclusion, US consistently overestimated sizes of solitary urocystoliths; PR, DCC, or CT should be utilized whenever urolith size is a critical factor for determining treatment options.

ABSTRACT #264

INFLUENCE OF FOOD CONSUMPTION ON VARIATION OF URINARY VOLUME, PH, ANALYTES, AND RELATIVE SUPERSATURATION FOR CALCIUM OXALATE AND STRUVITE IN HEALTHY ADULT MALE HOUND DOGS.

B Young, JW Bartges, S Cox, T Moyers. University of Tennessee, College of Veterinary Medicine, Knoxville, TN.

In healthy human beings, there is diurnal variation to urinary excretion of calcium and pH; however, this circadian rhythm has been found to be absent in some human beings that form calcium oxalate uroliths. We hypothesized that healthy, adult, male hound dogs will exhibit a diurnal variation for urinary mineral excretion, pH, and relative supersaturation for calcium oxalate and struvite.

Six healthy adult male hound dogs weighing 18.6 to 26.8 kg were evaluated. They were fed an adult maintenance, dry-formulated dog food at 8:00AM each day. Dogs ate all food within 2 hours; fresh tap water was available at all times. Dogs were housed in individual metabolism cages for urine collection. The urinary bladder of each dog was emptied by transurethral catheterization to begin the study, and at 12 hours and 24 hours after food consumption. Urine from each 12-hour period (AM=8AM to 8PM and PM=8PM to 8AM) was measured, and pH and concentrations of sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), phosphorous (PO_4), oxalic acid (Ox), citric acid (Cit), and ammonia (NH_4) were determined. Results were entered into a computer program (EQUIL 1.51b, College of Medicine, University of Florida) for estimation of relative supersaturation for calcium oxalate monohydrate (COMrss), calcium oxalate dihydrate (CODrss), and magnesium ammonium phosphate hexahydrate (struvite, MAPrss). Paired *t*-tests were used to compare results.

Differences were not found for urine volume, pH, or analytes between AM and PM: however, urine volume, pH, Na, K, Ca, NH_4, Ox, Cit, and Cl tended to be greater and urine Mg and PO_4, tended to be lower in PM when compared with AM. COMrss (AM: 5.25 vs PM: 3.27; $p=0.025$) and CODrss (AM: 2.27 vs PM: 1.39; $p=0.025$) were significantly greater and MAPrss (AM: 0.31 vs PM: 0.72; $p=0.05$) was significantly lower in AM when compared with PM.

Results of our study are similar to previous studies in healthy beagles and calcium oxalate urolith-forming Miniature schnauzers (Lulich, et al 1991); however, those studies utilized pooled 24-hour urine samples. Although urinary concentrations of analytes were not significantly different between AM and PM, COMrss and CODrss were significantly higher after food consumption and MAPrss was significantly higher when food was withheld; therefore, these dogs exhibited diurnal variation in urine saturation for calcium oxalate and struvite. Our results suggest dietary conditions be considered when measuring urinary concentrations of analytes and urinary saturation for calculogenic minerals, and provide a basis for comparing results with calcium oxalate urolith-forming dogs.

ABSTRACT #265

ALPHA 1-ACID GLYCOPROTEIN, SERUM AMYLOID A, AND MONOCTE CHEMOATTRACTANT PROTEIN-1 IN CATS WITH IDIOPATHIC CYSTITIS.

B Gerber, CE Reusch, E Zini. Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Switzerland.

Feline idiopathic cystitis (FIC) is the most common diagnosis in cats with clinical signs of irritative voiding. The etiology of the disease is not known. It is suspected that FIC is a systemic disease rather than a disease restricted to the bladder. This is supported by findings like increased plasma catecholamine levels or an association with gastrointestinal tract signs in cats with FIC. In humans with interstitial cystitis, for which the cat is a model, similar associations were recognized. We hypothesized that in cats with non-obstructive FIC levels of the acute phase proteins alpha 1-acid glycoprotein (AGP) and serum amyloid A (SAA) and the chemokine monocyte chemoattractant protein-1 (MCP-1) are increased and show a similar pattern of increase as cats with obstructive lower urinary tract disease (LUTD). The aim of the study was to evaluate AGP, SAA and MCP-1 in serum of cats with FIC and to compare the values with healthy control cats and cats with obstructive LUTD.

FIC was diagnosed by excluding other causes of LUTD using urinalysis with culture, evaluation of the lower urinary tract by radiographs and/or ultrasound and hematology and serum biochemistry parameters. Cats were considered obstructed if the bladder was distended and they were unable to urinate. AGP was measured with a commercial radial immunodiffusion test. SAA was measured with a commercial multi-species ELISA assay and MCP-1 was measured with a commercial canine CCL2/MCP-1 ELISA kit which was validated for cats. Results were compared using the Mann–Whitney-U test. Differences were considered significant at $P < 0.05$.

Nine cats with FIC, 9 cats with obstructive LUTD of any cause and 9 control cats were included in the study. AGP was 360 ug/ml (200–600 ug/ml) (median and (range)) in control cats, 500 ug/ml (140–1400 ug/ml) in cats with FIC and 780 ug/ml (420–3200 ug/ml) in cats with obstructive LUTD. SAA was 0 ng/ml (0–5.2 ng/ml) in control cats, 0.5 ng/ml (0–23.4 ng/ml) in cats with FIC and 91.2 ng/ml (0–417.6 ng/ml) in cats with obstructive LUTD. MCP-1 was 56 pg/ml (21–73 pg/ml) in control cats, 204 pg/ml (54–806 pg/ml) in cats with FIC and 320 pg/ml (133–823 pg/ml) in cats with obstructive LUTD. All measured inflammatory proteins were significantly higher in cats with obstructive LUTD compared to control cats. Compared to control cats, AGP and SAA in cats with FIC were not significantly different, but MCP-1 was significantly higher. There was no significant difference in MCP-1 between FIC cats and cats with obstructive LUTD.

While AGP and SAA are not increased in cats with FIC, MCP-1 is increased similar to cats with obstructive LUTD, indicating that a systemic inflammatory reaction is present in FIC.

ABSTRACT #266

OXIDATIVE STRESS IN CATS WITH CHRONIC RENAL FAILURE.

R Keegan and CB Webb. Colorado State University, Fort Collins, CO.

Oxidative stress is an important component in the progression of chronic renal failure (CRF) in humans, and neutrophil function may be impaired by oxidative stress in these patients. This study was designed to test the hypothesis that cats with CRF have increased oxidative stress and decreased neutrophil function when compared to Control cats.

A biochemical profile, complete blood count, urinalysis, blood pressure, plasma antioxidant capacity, erythrocyte lysate superoxide dismutase enzyme (SOD) level, whole blood reduced-to-oxidized glutathione ratio (GSH:GSSG), and neutrophil phagocytosis and oxidative burst were measured in 20 cats with CRF and 10 Control cats. Statistical comparisons (two-tailed t-test) are reported as mean±standard deviation.

There was no difference in age or body weight between Control and CRF cats. The CRF cats had significantly greater serum BUN, creatinine, and phosphorus concentrations than Control cats, and significantly lower PCV and urine specific gravity than Control cats. The GSH:GSSG ratio was significantly greater in the CRF group (177.6±197, 61.7±33; $p<0.02$), while the SOD level and antioxidant capacity were not significantly different between groups. Neutrophil oxidative burst following E. coli phagocytosis, measured flow cytometrically as an increase in mean fluorescence intensity (a unitless measure), was significantly greater in CRF cat then Control cats (732±253, 524±54; $p<0.05$).

The significantly greater GSH:GSSG ratio in CRF cats is consistent with an activation of antioxidant defense mechanisms in this disease state. It remains to be determined if supplementation with antioxidants beyond the level of Control cats would be of benefit in cats with CRF.

ABSTRACT #267

ERYTHROCYTE ZINC PROTOPORPHYRIN EVALUATION IN CATS WITH CHRONIC RENAL DISEASE.

CS Prosser, MM Kogika, MF Waki, AGT Daniel, DMN Simões, A Alves, MB Silva, BM Teixeira. School of Veterinary Medicine – University of São Paulo, Brazil.

Erythrocyte zinc protoporphyrin (ZPP) is used in human medicine as a screening method to detect iron deficiency. ZPP is produced instead of heme and, thus, increased concentrations of ZPP may be observed in cases of iron deficiency prior to detectable anemia. In cats, no studies concerning the evaluation of ZPP have

been reported yet. Cats with chronic renal disease (CRD) may develop anemia as the disease progresses; iron deficiency may be one of the causes. Therefore, the aim of this study was to investigate whether ZPP may be altered in anemic and non-anemic cats with CRD.

Thirty-two client-owned domestic cats with CRD in stages II, III and IV were placed in one of two groups: group I (n=12) – CRD with normocytic, normochronic anemia (PCV<29%) and group II (n=20) – CRD without anemia (PCV>29%). Patients that received iron, vitamin B, blood transfusion or rHuEPO were excluded, as well as those with neoplasia or cardiac disease. Results were compared to a control group of 36 healthy cats (16 cats aging from 1 to 6 y-old and 20 cats older than 6 y-old). All cats of all groups were negative for FIV and FeLV, with normal serum levels of bilirubin (hyperbilirubinemia interferes with ZPP measurement). Blood was collected in EDTA tubes and hematofluorometric method was used to determine the ZPP (ProtoFluor® Z -Helena Laboratories); ZPP values were obtained three times/sample prior to 6 hours post-collection.

The mean ZPP value of the control group was 38.39 µmol ZPP/mol heme (SEM±1.98; min=18.67; max=72.33), with no statistically significant difference between younger and older normal cats (P=0.680). Mean ZPP measurements were 60.41 µmol ZPP/mol heme (SEM±6.76; min=35.33; max=117.33) and 45.62 µmol ZPP/mol heme (SEM±2.97; min=30.00; max=77.33) in groups I and II, respectively. Difference was detected between control group and group I (*t* test; P=0.013), as well as between control group and group II (P=0.008); however there was no difference between groups I and II (P=0.11).

Anemic cats of group I showed significant increase of erythrocyte ZPP levels, suggesting that ZPP may have been produced instead of heme in consequence of iron deficiency. Similarly, non-anemic CRD cats (group II) showed higher level of ZPP than clinically normal cats; ZPP concentrations remained altered despite normal PCV and erythrocyte indices, indicating a degree of iron depletion. Development of iron-deficient erythropoiesis (microcytic anemia) may result only in late stages in cats, therefore ZPP may be an important tool to detect early iron depletion before anemia. Further studies are necessary to investigate the role of ZPP, serum iron and ferritin to confirm whether ZPP values correlate with the degree of iron deficiency in cats, and, therefore, recommended as a diagnostic tool to guide iron supplementation in clinical practice.

ABSTRACT #268

SEQUENTIAL EVALUATION OF ERYTHROCYTE ZINC PROTOPORPHYRIN IN CATS WITH CHRONIC RENAL DISEASE. CS Prosser, MM Kogika, MF Waki, BM Coelho, KK Kanayama, VABF Wirthl, MB Silva, PRE Mosko. School of Veterinary Medicine – University of São Paulo, Brazil.

Anemia may develop during the progression of chronic renal disease (CRD) in cats, and iron deficiency may be one of the causes. In the absence of iron, erythrocyte zinc protoporphyrin (ZPP) is formed during the final step of heme synthesis; in human medicine, ZPP has been used as a diagnostic indicator of iron deficiency. The aim of this study was to evaluate whether erythrocyte ZPP levels alter during the evolution of CRD in cats. Twelve domestic cats with CRD were evaluated for at least 50 days (range of 50 to 140) after the initial diagnosis. Animals were divided in three groups based on initial presentation: group I (#1–6; non-anemic CRD cats in stage II), group II (#7 and 8; anemic CRD cats, one in stage III and the other in IV, both receiving iron supplementation), and group III (#9–12; anemic CRD cats in stage III, treated with rHuEPO and iron). All cats were negative for FIV and FeLV, and had normal serum levels of bilirubin (hyperbilirubinemia interferes with ZPP measurement). Blood was collected in EDTA tubes and ZPP determinations were obtained three times/sample prior 6 hrs post-collection by hematofluorometry method (ProtoFluor® Z -Helena Laboratories).

Initial PCV values ranged from 30% to 39% and remained relatively consistent throughout the evaluation period for all group I cats, except cat #6 that developed progressive decrease in PCV, declining to 23% after 85 days. Regarding to ZPP levels of group I, it ranged consistently from 30.7 to 73.7 µmol ZPP/mol heme (previous study, ZPP in normal cats was 38.39±1.98 [mean±SEM; min=18.7 and max=72.3]), however cat #6 presented increase of ZPP levels (128.33 µmol ZPP/mol heme) as PCV was decreasing. The findings in cat #6 suggest that a degree of iron depletion may be also involved as one of the causes of anemia. Cat #7 (group II) developed a concomitant 16.5% decrease in ZPP value and 29.4% increase in PCV from initial levels following iron supplementation; similarly, cat #8 decreased ZPP levels by 26.5% and increased PCV values by 4.8%. These data may suggest that supplemental iron availability was adequate, since ZPP levels decreased. In group III, cat #9 responded during the treatment with increased PCV (120%) and ZPP (87.2%) values; ZPP probably increased due to increased iron demand secondary to rHuEPO stimulus, which may have resulted in iron depletion, and probably iron supplementation was not sufficient. In cat #10, ZPP values also increased and PCV values decreased, showing that there was not a good response for rHuEPO and other causes of anemia, other than iron deficiency, may have been also involved. Cats #11 and #12 showed decrease on ZPP (PCV increased in cat #11, but not in cat #12); probably iron supplementation may have been adequate. The detectable variation in ZPP levels along the progression of CRD suggests that iron may be involved in the mechanism; further studies are necessary to investigate the role of ZPP and iron depletion in the development of anemia in CRD cats.

ABSTRACT #269

ENDOGENOUS ERYTHROPOIETIN LEVELS AND IRON UTILITY IN CATS WITH CHRONIC KIDNEY DISEASE. S Yamano[1,2], K Tanaka[2], M Nishida[1], K Harada[1], M Fujiwara[1], T Ebisawa[1], M Uechi[1]. [1]Nihon University, Fujisawa, Kanagawa. [2]Kanie Animal Clinic, Kanie, Aichi, Japan.

The aim of this study was to assess the changes in endogenous erythropoietin (EPO) and serum iron levels in cats with chronic kidney disease (CKD). The study included 14 healthy cats and 27 cats with CKD [stage 2 (n=8), stage 3 (n=7) and stage 4 (n=12), according to the International Renal Interest Society (IRIS) Staging]. Investigated parameters were complete blood count, serum creatinine, EPO, serum iron, total iron binding capacity, unsaturated iron binding capacity, and transferrin saturation (TSAT). The EPO level in healthy, stage-2, stage-3, and stage-4 cats was 15.6±4.25, 14.6±4.61, 17.6±7.41 (n=4), and 10.6±2.50 mIU/mL (n=9), respectively. There was a negative correlation between the logarithm of EPO (Log_{10} EPO) and serum creatinine level (r=–0.488, p=0.029). Stage 3 and stage 4 cats showed significantly lower levels of hematocrit, hemoglobin and red blood cell count than healthy and stage 2 cats ($p<0.05$ for comparison with stage 3, $p<0.01$ for comparison with stage 4). The serum iron level in stage-4 (136.8±63.2 µg/dL) was significantly higher than that in the healthy group (74.6±20.4 µg/dL; $p<0.01$). TSAT in stage 4 was significantly higher than that in the healthy group ($p<0.05$). These results indicate that erythropoiesis is compromised, as indicated by reduced iron utility, in cats with advanced CKD.

ABSTRACT #270

IS CADMIUM EXPOSURE ASSOCIATED WITH CHRONIC KIDNEY DISEASE IN CATS? N. Finch, H. Syme, J. Elliott. Royal Veterinary College, London, UK.

Chronic cadmium exposure in human patients has been associated with kidney damage and the development of tubulointerstitial nephritis, the predominant pathological lesion associated with chronic kidney disease (CKD) in cats. There are no published studies investigating any association between chronic cadmium exposure and CKD in cats.

Urine samples were collected by cystocentesis from clinically healthy, non-azotaemic (plasma creatinine <2.0 mg/dl), geriatric (<9 years old) cats and geriatric cats with previously confirmed CKD (persistent azotaemia and urine specific gravity <1.035). Any cats with concurrent diseases such as hyperthyroidism were excluded. Urinary cadmium concentrations were determined at a commercial laboratory using inductively coupled plasma mass spectrometry (ICP-MS) and urine creatinine concentration was measured using the kinetic jaffe method. The urinary cadmium: creatinine ratio was calculated to account for the concentration of the urine. Statistical analysis comparing the healthy and azotaemic groups was performed using the Mann-Whitney U test following

assessment of the data for normality. Data are reported as median (range).

Thirty seven cats were recruited into the study (19 normal, 18 CKD). Twenty-five of the cats were classified as domestic shorthair, 5 as domestic longhair and 7 as purebred. The study included 17 male neutered cats, 19 female neutered cats and 1 female entire cat. Of the cats with CKD 11 were in IRIS stage II CKD and 7 in stage III CKD. The plasma creatinine concentration for the normal cats was 1.46 (1.06–1.73) mg/dl and for the cats with CKD was 2.63 (1.77–4.59) mg/dl. The urinary cadmium: creatinine ratio in normal cats and cats with CKD was 0.11 (0.02–0.63)$\times 10^{-6}$ and 0.14 (0.04–0.46)$\times 10^{-6}$ respectively. The urinary cadmium: creatinine ratio did not differ significantly between the groups of cats ($P=0.261$).

This preliminary study does not provide any evidence to support the hypothesis that chronic exposure to cadmium from the environment plays a role in the pathogenesis of CKD in cats. However, the limitations of the study are that it involves spot urine samples, the content which may not reflect cadmium accumulation within the kidney tissue. Further work is required to determine whether cadmium accumulation in the kidney is associated with feline CKD.

ABSTRACT #271

GLOMERULAR FILTRATION RATE IN HEALTHY ENGLISH POINTER, ENGLISH SETTER AND GERMAN SHEPHERD DOGS. J Seguela[1], Y Queau[1], P Murgier[1], P Mimouni[2], D Concordet[1], C Duperron[1], HP Lefebvre[1]. [1]UMR 181 Physiopathologie et Toxicologie Expérimentales INRA-ENVT, National Veterinary School, Toulouse. [2]Clinique Vétérinaire, L'Isle Jourdain, France.

A breed effect on glomerular filtration rate (GFR) has been hypothetized in dogs (Lefebvre et al., JVIM, 2004;18:415). The aim of this study was to compare GFR in healthy populations from 3 different breeds.

44 English Pointer (EP), 50 English Setter (ES), and 55 German Shepherd (GS) healthy dogs were recruited. GFR was measured by plasma exogenous creatinine clearance test. Intravenous administration of exogenous creatinine (40 mg/kg) followed by repeated blood sampling was performed. Effect of age, body weight (BW) and breed was analyzed by ANOVA.

The mean±SD age and body weight (BW) were for 4.3±2.9 y. and 21.1±2.5 kg for EP, 3.5±2.7 y. and 18.4±3.2 kg for ES, 4.9±2.8 y. and 29.4±4.0 kg for GS. The age was similar between breeds and the BW was higher for GS ($P<0.001$). GFR was 3.5±0.6, 3.4±0.8 and 2.5±0.7 mL/min/kg for EP, ES and GS, respectively. GFR was lower ($P<0.001$) in GS than in EP and ES. The GFR distribution was normal for EP and ES, and after inverse transformation, for GS. Tentative reference intervals were 2.3–5.1, 1.8–5.0 and 1.7–3.8 mL/min/kg for EP, ES, and GS, respectively.

In conclusion, establishment of breed-specific reference intervals for GFR in dogs is needed, although BW could be a confounding factor.

ABSTRACT #272

24-HOUR WATER CONSUMPTION IN HEALTHY CATS USING A FOUNTAIN VERSUS A BOWL. DC Grant. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Increasing water consumption has been recommended to reduce urine concentration and recurrence of urolithiasis and idiopathic cystitis in cats. Water fountains are being advertised and some advocate their use to increase water consumption. This study evaluated the affect of a fountain versus a bowl on 24-hour water consumption and urine concentration.

Thirteen client-owned cats had a normal physical examination, hematocrit, biochemistry profile, and urinalysis. Cats were kept in their home environment and consumed their regular diet. Cats were allowed 7 days to acclimate to the fountain. Cats were randomized to be offered water from a fountain or their own bowl first, followed by the other, and 24-hour consumption was measured. The volume of water evaporated and spilled in 24 hours from the fountain or bowl was subtracted from the amount offered. Following each 24-hour period urine osmolality was measured. Analysis of variance was performed with significance set at a p-value <0.05.

One cat refused to drink from the fountain. For the remaining cats, there was no significant difference in water consumption from a bowl (22.9±10.2 ml/kg/day) or from a fountain (31.6±13.5 ml/kg/day), $p=0.091$. Similarly, there was no difference in urine osmolality when water was offered form a bowl (2469±367.3 mOsm/kg) or a fountain (2538±440.8 mOsm/kg), $p=0.682$.

This refutes the theory that water fountains increase water consumption in cats.

ABSTRACT #273

CLINICAL AND GENETIC CHARACTERIZATION OF A CONGENITAL ENTLEBUCHER MOUNTAIN DOG URINARY TRACT SYNDROME. C North[1], JM Kruger[2], PJ Venta[2], JM Miller[3], DS Rosenstein[2], EK Randall[2], SD Fitzgerald[2]. [1]Estero, FL. [2]College of Veterinary Medicine Michigan State University, East Lansing, MI. [3]University of Michigan Health System, Ann Arbor, MI.

The "Entlebucher urinary syndrome" (EUS) was first recognized in North American Entlebucher mountain dogs in 1996. The purpose of this study was to characterize clinical and genetic features of EUS. Eleven female and 4 male client-owned Entlebucher mountain dogs were evaluated. Six female dogs had histories of urinary incontinence, recurrent urinary tract infection, or hydronephrosis; the remaining dogs were asymptomatic. Thirteen dogs were evaluated with a hemogram, serum biochemistries, urinalysis, urine culture, excretory urography, ultrasonography, and urethrocystoscopy. Two other dogs had partial evaluations that included necropsy. Five candidate genes (*PAX2, EYA1, FOXC2, HOXB7, and NPHP4*) were selected and evaluated by SINE insertion polymorphism or microsatellite marker-based exclusion analysis of DNA obtained from affected dogs. Clinical and pedigree histories from an additional 138 North American Entlebuchers were compiled for analysis.

Abnormalities identified in clinically affected dogs included bilateral ectopic ureters (6 dogs), hydroureter/hydronephrosis (4 dogs), and urinary tract infection (2 dogs). Both necropsied dogs had bilateral hydroureter/hydronephrosis with ureterovesical junction obstruction caused by chronic granulation tissue or lymphoplasmacytic inflammation. Bilateral intravesicular ectopic ureters also were identified in 3 asymptomatic female dogs. Probable EUS was identified in 12% of dogs in pedigree analyses; all affected dogs were female. Because of incomplete penetrance, mode of inheritance could not be determined. Exclusion analyses did not associate any candidate genes with EUS.

In conclusion, clinical features of EUS include ureteral ectopia (asymptomatic or with urinary incontinence), hydroureter/hydronephrosis, and urinary tract infection. Further analyses are necessary to confirm and characterize the hereditary nature of EUS.

ABSTRACT #274

PROTEOMIC ANALYSIS OF URINE FROM MALE DOGS IN EARLY STAGES OF PROGRESSIVE TUBULOINTERSTITIAL INJURY DUE TO X-LINKED HEREDITARY NEPHROPATHY (XLHN). MB Nabity, GE Lees, LJ Dangott, SK Ramaiah. Texas A&M University, College Station, TX.

Chronic kidney disease (CKD) is a major cause of morbidity and mortality in dogs, and methods to reliably identify tubulointerstitial disease in its early stages currently are lacking. The objective of this study was to identify predictive biomarkers of early tubulointerstitial injury in urine of male dogs with XLHN using 2-dimensional gel electrophoresis. Dogs with XLHN develop glomerular lesions due to a *COL4A5* mutation that causes the type IV collagen in their glomerular basement membranes to be abnormal. Affected males first develop glomerular proteinuria, but progressive tubulointerstitial injury is associated with the subsequent development of end-stage CKD during adolescence.

Urine samples from male dogs ($n=6$) were analyzed using 2-dimensional differential gel electrophoresis (DIGE), which uses 3 fluorescent dyes (Cy2, Cy3, and Cy5) for sample labeling. Samples were compared at two time points: 1) onset of urine protein:creatinine (UPC) persistently >2; and 2) onset of serum creatinine ≥ 1.2 (mildly azotemic). Albumin was removed from all samples by affinity chromatography, and samples were concentrated with

ultrafiltration (10-kDa cut-off). Equal amounts of each sample were then precipitated with acetone, denatured, and fluorescently labeled. Proteins were focused on 13 cm IPG strips (pH 4-7) followed by separation on 12% SDS gels. Gels were scanned and spots analyzed using DeCyder software. Fifty-seven protein spots showed statistically significant changes between the time points. Of these, 20 were identified by mass spectrometry and included retinol binding protein (RBP), clusterin, alpha1-microglobulin, fetuin A, hemopexin, apolipoprotein A1, gelsolin, immunoglobulin, collagen type XXVII, complement C3, and haptoglobin.

RBP showed the greatest statistical significance and was subsequently evaluated by Western blot in the urine of affected male dogs (n=25) and their normal siblings (n=19). Urine samples were evaluated every 2–4 weeks starting two weeks prior to UPC persistently >0.5 in affected males and at several corresponding time points in their normal littermates. Samples were normalized to a creatinine of 20 mg/dl. RBP was detected approximately 2 months prior to the onset of azotemia (serum creatinine >1.2) in the urine of the affected male dogs and typically increased throughout the course of disease independently of the UPC value. RBP was not detected in the urine of the normal males.

In summary, these results suggest that 2-D gel electrophoresis can be successfully employed for biomarker identification in chronic kidney disease in the urine of dogs and that urine RBP may be a potential early marker of kidney disease and of CKD progression in dogs.

ABSTRACT #275
METABOLOMIC PROFILING OF PLASMA IN ARTHRITIC VS NON-ARTHRITIC DOGS. N.Z. Frantz, R.M. Yamka, and J. Brockman. Hill's Pet Nutrition, Inc., Topeka, KS.

Sixty-three neutered/spayed beagles (average age=10.9±2.3 years) were identified for this study. Dogs were radiographed to confirm changes consistent with degenerative joint disease and lameness in the arthritic dogs or absence in the non-arthritic dogs. Metabolomic profiling of plasma samples was performed by Metabolon (Durham, NC). T-test analysis was applied to log-transformed, day normalized data. Metabolites having a $P<0.05$ (following a false discovery rate adjustment) were considered different among the two groups. KEGG and HMDB identifiers used were from commercial databases. Analysis of the plasma found differences in 10 metabolites between the two groups. Of the metabolites identified, 2 had increased fold-changes and 8 had decreased fold-changes in the arthritic group when compared to the non-arthritic group. The metabolites with increased fold changes in arthritic dogs were dimethylarginine and beta-sitosterol. The metabolites with decreased fold changes in arthritic dogs were glycine, trans 4-hydroxyproline, mannose, glucose, myo-inositol, threonate, 4-hydroxymandelate, and phenol sulfate. In summary, metabolites associated with increased oxidative stress were elevated while metabolites associated with glycosylation of proteins, Vitamin C metabolism, and collagen turnover were decreased in arthritic dogs.

ABSTRACT #276
METABOLOMIC PROFILING OF PLASMA IN ARTHRITIC VS NON-ARTHRITIC CATS. N.Z. Frantz, R.M. Yamka, and J. Brockman. Hill's Pet Nutrition, Inc., Topeka, KS.

Sixty American short-hair cats (average age=11.3±2.7 years) were identified for this study. Cats were radiographed to confirm changes consistent with degenerative joint disease and lameness in the arthritic cats or absence in the non-arthritic cats. A plasma sample was collected to determine metabolomic differences between the two populations. Of the sixty cats identified, 30 were classified as arthritic and 30 as non-arthritic based on radiographic evidence and visual appraisal. Metabolomic profiling of plasma samples was performed by Metabolon (Durham, NC). T-test analysis was applied to log-transformed, day normalized data. Metabolites having a $P<0.05$ (following a false discovery rate adjustment) were considered different among the two groups. KEGG and HMDB identifiers used were from commercial databases. Analysis of the plasma found differences in 20 metabolites between the two groups. Of the metabolites identified, 11 had increased fold-changes and 9 had decreased fold-changes in the arthritic group when compared to the non-arthritic group. The metabolites with increased fold changes in arthritic cats were kynurenine, dimethylargnine, N-acetylornithine, homocitrulline, 2-aminobutyrate, 3-hydroxydeconate, 3-hydroxybutyrate, allantoin, 5-methylcytidine, pseudouridine, and trigonelline. The metabolites with decreased fold changes in arthritic cats were tryptophan, 3-indoleproprionate, creatinine, carnosine, gamma-glutamylfelinylglycine, glycerol 3-phosphate, campesterol, alpha-ketogluterate, and hippurate. In summary, plasma metabolomic analysis indicates differences of increased metabolites of oxidative stress and inflammation in arthritic compared non-arthritic cats.

ABSTRACT #277
METABOLOMIC PROFILING OF PLASMA IN LEAN VS. OBESE DOGS. RM Yamka, NZ Frantz and SC Zicker. Hill's Pet Nutrition, Inc., Topeka, KS.

Fifty-two neutered/spayed beagles (average age=8.6±3.1 years) were identified for this study. Dogs were weighed, given a body condition score (BCS; 1=lean, 3=ideal and 5=obese) and a plasma sample was collected. Of the fifty-two dogs identified, 24 were classified as lean (BCS=2.8±0.5; BW=11.2±1.9 kg) and 28 as overweight/obese (BCS=3.9±0.7; BW=14.4±3.6 kg). Metabolomic profiling of plasma samples was performed by Metabolon (Durham, NC). T-test analysis was applied to log-transformed, day normalized data. Metabolites having a $P<0.05$ (following a false discovery rate adjustment value of 0.1) were considered different among the two groups. KEGG and HMDB identifiers were from commercial databases. Analysis of the plasma found differences in 20 metabolites between the two groups. Of the metabolites identified, 15 had increased fold-changes and 5 had decreased fold-changes in the overweight group when compared to the lean group. The metabolites with increased fold changes in overweight dogs were glutamine, 3-methylhistidine, lysine, isoleucine, valine, citrulline, oxidized glutathione, glutamylvaline, pyroglutamylvaline, gamma-glutamylleucine, gamma-glutamylmethionine, gamma-glutamylglutamine, stearoylglycerol (monostearin), 3-hydroxybutyrate and citrate. The metabolites with decreased fold changes in overweight dogs were p-cresol sulfate, 1,5-anhydroglucitol, glycerate, docosahexaenoate and threonate. There were minimal differences in metabolites for gender as all dogs were spayed or neutered. In summary, amino acid metabolism, lipid metabolism, carbohydrate metabolism and oxidative stress were different between overweight and lean dogs.

ABSTRACT #278
DETERMINATION OF MAINTENANCE ENERGY REQUIREMENT OF CATS AFTER SPAYING. Y Mitsuhashi[1], AJ Chamberlin[1], KE Bigley[1], JE Bauer[1,2]. [1]Comp. An. Nutr. Lab., [2]Intercollegiate Faculty of Nutrition, Texas A&M Univ., Col Stn, TX.

Several cat studies have reported decreased energy expenditure after spaying. Spayed cats, therefore, tend to increase their body weights (BW) without changing calorie intake, which often results in obesity. The recently revised National Research Council (NRC) publication recommends a maintenance energy requirement (MER) for cats based on a 9-point body condition score (BCS) as follows: $100\times BW^{0.67}$ for BCS$\leq$5 and $130\times BW^{0.40}$ for BCS$>$5. Although weight gain of spayed cats is widely recognized, the MER for these animals has not been clearly defined. Therefore, this study aimed to determine the MER for spayed cats whose BW began to increase shortly after surgery. Twenty-two short-haired adult female cats (2 yrs old, on average) were used. Three complete and balanced diets (diets A, B, and C) containing similar nutrient compositions but varying in fatty acid type had been fed in an amount to maintain their BW and BCS prior to the study. To begin the study, all cats were spayed and diet C was fed once daily for 9 weeks using the same MER before the study. The cats were then fed an amount of diet based on NRC recommendations for 2 additional weeks. During these 11 weeks, all cats gained weight. Beginning on wk 12 a weight loss regimen was initiated until each cat achieved a BCS of 5.

Cats were fed approximately $65\times BW^{0.67}$ to achieve a 1–2% body weight loss per week during this period. After each cat obtained a BCS of 5, it was then fed an appropriate amount of diet to maintain its body weight for at least 4 weeks. BW and BCS were monitored weekly and bi-weekly before and during weight loss, respectively. Daily food consumption was recorded and used to determine an allometric factor (AF) calculated by dividing calories consumed by metabolic body weight ($BW^{0.67}$) based on the BW of cats at BCS of 5. Blood was collected at the time of surgery and after weight loss for plasma biochemistry profiles. Statistical analyses were performed on data both prior to the study and during weight gain. Weekly BW and AF were analyzed by repeated measures ANOVA using a general linear model. Weekly BCS data were analyzed by Friedman test followed by Wilcoxon signed ranks test with Bonferroni adjustment. All blood chemistries were within normal limits. BW and BCS did not change prior to spaying but showed significantly incremental increases thereafter such that a 16% increase in BW and nearly 1 point increase of BCS was observed ($p<0.01$). It should be noted that the AF was constant ($AF=101.5\pm6.6$ (SD) kcal/$BW^{0.67}$, $p=0.16$) both prior to surgery and thereafter during which time all cats significantly gained weight. The AF needed to maintain BW of each cat after obtaining a BCS of 5 was 75.0 ± 5.6 kcal/$BW^{0.67}$. This AF is 25% lower than the current NRC recommendation and was statistically significantly lower than cats before surgery by paired t-test ($p<0.05$). In conclusion, spaying significantly increased body weight when using MER values for intact cats. Thus, $75\times$(ideal $BW)^{0.67}$ is proposed for the MER of spayed cats.

ABSTRACT #279

ASSESSMENT OF METABOLIC SYNDROME OCURRENCE IN OBESE CLIENT-OWNED DOGS

MM Jericó[1], FB Fusco[2], FC Chiquito[1], F Lorenzini[1], CF Pinto[2], CB Ferreira[2], RM Sousa[2]. [1]Anhembi Morumbi University, Laureate International Universities, SP, Brazil. [2]Santo Amaro University Veterinary Hospital, SP, Brazil.

The metabolic syndrome, a morbid condition well-described in humans, is defined as a set of risk factors such as obesity (mainly visceral obesity), arterial hypertension, dyslipidemia and insulin resistance; at least three of these events must occur simultaneously in the same individual for the diagnosis to be confirmed. It is known that the binding factor among these alterations is insulin resistance (characterized by hyperinsulinemia) and that the clinical relevance of the metabolic syndrome is related to its role in the development of atherosclerosis and onset of diabetes mellitus. In dogs, the metabolic syndrome has been well recognized in experimental conditions, where obesity is induced by the exaggerated administration of fat-rich diets. We aimed at assessing the metabolic syndrome occurrence in obese client-owned dogs (n=34), through the measurements of serum cholesterol, triacylglycerols, glycemia, amended insulin to glucose ratio (AIGR) and the homeostasis model assessment (HOMA). Normal values were established based on the results (mean value+2 SD) obtained in a group of normal dogs (n=18). When the results were analyzed alone, none of the obese dogs presented hyperglycemia. On the other hand, 10 dogs (29.4%) presented absolute hyperinsulinemia (>18.8 uUI/ml), 11 (32.4%) presented relative hyperinsulinemia as demonstrated by the amended insulin to glucose ratio (AIGR>37.7), 10 (32.4%) presented increased HOMA index (>4.82), 7 (20.6%) presented hypercholesterolemia (>307 mg/dL) and 9 (26.5%) presented hypertriacylglyceridemia (>140 mg/dL). The glycemia values in the normal dogs did not differ from those in the obese dogs (T-test, $p>0.05$). On the other hand, the cholesterol, triacylglycerol, insulin, AIGR and HOMA values in obese dogs were higher than those in normal animals (Mann-Whitney test; $p<0.05$). Finally, when all the data were considered, 8 of the obese dogs presented, simultaneously, hyperlipidemia and hyperinsulinemia (absolute and relative), in addition to visceral obesity. We conclude that a significant number of animals in the obese client-owned dog group assessed in the present study presented metabolic syndrome, which is an important risk factor for the development of diabetes mellitus, as well as atherosclerosis, a morbid condition that is uncommon in the canine species, but that has been described in the presence of endocrine or metabolic disorders.

ABSTRACT #280

A VEGETARIAN DIETARY OIL SOURCE OF γ-LINOLENIC ACID RESULTS IN ARACHIDONIC ACID ACCUMULATION IN FELINE UTERINE TISSUES: EVIDENCE OF DELTA-5 DESATURATION

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Beyond the fact that cats express low delta-6 desaturase activities, lipid metabolism in this species remains to be fully explored. Essential fatty acid requirements of cats include a preformed source of arachidonic acid (AA) especially during growth and reproduction. Consequently, the ability of reproductive tissues to sustain healthy pregnancies depends on AA availability. The possibility exists that γ-linolenic acid (GLA, 18:3n6) may serve as a precursor of AA in such tissues especially if coupled with chain elongation and a functionally active delta-5 desaturase. Delta-5 desaturase activity has been found to be low in cats but it was undetermined whether it might be sufficient to result in AA synthesis. In the present study, it was hypothesized that uterine and ovarian tissues of cats fed GLA would accumulate AA and other fatty acid metabolites compared to a control diet. In addition, the appearance of dihomogammalinolenic acid (DGLA, 20:3n6), the delta-5 desaturase substrate, was predicted to accumulate in adipose tissue under this condition as a reservoir for conversion. A group of 19 adult female cats were divided into two groups and fed either a high (n=10) or low (n=9) GLA diet (4.2 vs<0.1 g/kg diet). Both diets contained similar amounts of LA and minimally adequate AA. The diets were fed for 300 days prior to ovariohysterectomy at which time EDTA plasma and ovarian, uterine, and subcutaneous adipose tissues were collected. Homogenates of each tissue were prepared and frozen in aliquots at −80 C. Total lipids were extracted from the plasma and tissue homogenates followed by phospholipid (PL) fractionation via thin layer chromatography and fatty acid analyses by gas chromatography. The Shapiro-Wilks test was used to determine normal distribution of fatty acid data followed by Student's t test ($p<0.05$). Plasma PLs were significantly increased in both GLA and DGLA in the high GLA group. Uterine tissue homogenates were found to have statistically significant increased amounts of DGLA and AA. However, ovarian tissue showed an increase of only DGLA. It is concluded that a high GLA diet results in increased AA in uterine, but not ovarian, tissues and thus may supply eicosanoid precursors in support of reproduction. Furthermore, the increase in DGLA may provide an adipose storage reservoir for additional conversion under times of metabolic need. These data support the presence of a functionally active delta-5 desaturase in uterine, but not ovarian, tissues. The findings also suggest that increased dietary GLA may be used to meet the AA requirements for reproduction in cats in the absence of an animal based pre-formed source of AA.

ABSTRACT #281

EFFECT OF PARENTERAL L-ALANYL-L-GLUTAMINE SUPPLEMENTATION ON PHAGOCYTIC RESPONSES OF CANINE PERIPHERAL BLOOD POLYMORPHONUCLEAR NEUTROPHILIC LEUKOCYTES EXPOSED TO METHYLPREDNISOLONE SODIUM SUCCINATE

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The objective of this study was to examine the effects of parenteral L-alanyl-L-glutamine (Ala-Gln) supplementation on functions of canine peripheral blood polymorphonuclear neutrophilic leukocytes (PMNs) exposed to methylprednisolone sodium succinate (MPSS). The experimental design involved administration of a high dose of MPSS, which is the recommended protocol for dogs with acute spinal cord injury. Fifteen healthy Beagles were randomly assigned into 3 groups and administered an IV infusion with 0.9% NaCl solution (Treatment A), admixture of 0.9% NaCl solution with 8.5% amino acids (2.3 g/kg/day) (Treatment B), or admixture of 0.9% NaCl with Ala-Gln solution (Dipeptiven) (0.5 g/kg/day)-supplemented 8.5% amino acids (1.8 g/kg/day) (Treatment C). The two admixtures were isonitrogenous and isocaloric. The infusion started with the first injection of MPSS and continued until 12 hours after MPSS injections completed. To evaluate PMNs functions, blood samples were collected before IV injections of MPSS (time 0) and 2, 12, and 24 hours after injections ceased. Phagocytic capacity,

oxidative burst activity (OBA) and filamentous actin (F-actin) polymerization were measured by use of flow cytometry.

Relative to preinfusion values, the phagocytic capacity, OBA and F-actin polymerization of PMNs in Treatment A and B groups were suppressed by MPSS injections, while the PMNs functions in Treatment C group were not reduced. The PMNs values suppressed by MPSS in Treatment A and B groups were restored 12 hours after the injections concluded. In conclusion, these results suggest that the parenteral Ala-Gln supplementation can modulate the immune functions of canine PMNs affected by MPSS treatment.

ABSTRACT #282
THE PHARMACOKINETICS OF MIRTAZAPINE IN HEALTHY CATS. JM Quimby, DL Gustafson, BJ Samber, KF Lunn. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

This study was designed to determine the pharmacokinetics of mirtazapine, an appetite stimulant, in healthy cats after oral administration of a single 3.75 mg high dose (HD), or 1.88 mg low dose (LD).

Ten cats with normal CBC, chemistry and urinalysis were used. Blood samples were collected prior to and 0.25, 0.5, 1, 4, 8, 24, 48, and 72 hours after oral administration of 3.75 mg (5 cats), or 1.88 mg (5 cats) of mirtazapine. Serum was collected, frozen and analyzed by LC/MS/MS. Mirtazapine, 8-hydroxymirtazapine and glucuronide metabolite concentrations were measured. Non-compartmental pharmacokinetic modeling was performed.

Increased vocalization and affection were the only side effects noted. Mean half-life was 15.4±4.7 hours (HD) and 10.2±2.2 hours (LD). Mean peak plasma concentration was 156.5.0±92.4 ng/ml (HD) and 73.1±45.5 ng/ml (LD). Mean clearance was 18.0±3.1 ml/min/kg (HD) and 10.5±3.6 ml/min/kg (LD). The Mann-Whitney test was used to compare the groups and a statistically significant difference in half-life and clearance was found ($p=0.03$ for both). The lack of dose proportionality may be due to delay in metabolism at the higher dose, as there was no significant difference between groups in the amount of glucuronidated metabolite. The limited glucuronidation ability of cats may explain this observation.

A single low dose of mirtazapine was well tolerated and resulted in a half-life that is compatible with 24 hour dosing intervals in healthy cats. Higher doses may result in delayed metabolism of the drug.

ABSTRACT #283
BIOAVAILABILITY OF A NOVEL, BIOENHANCED PREPARATION OF CURCUMIN IN DOGS. B Antony[1], RK Butchin[2], DW Griffin[2]. [1]Arjuna Natural Extracts Ltd, [2]Nutramax Laboratories, Inc., Edgewood, MD.

Curcuminoids, the biologically active components of tumeric, have been shown to have high antioxidant, and anti-inflammatory activity. Both in vitro and in vivo studies on model systems in multiple species have demonstrated a wide range of effects that support the use of curcumin in a wide range of conditions. However, these applications are constrained by the relatively poor bioavailability of curcumin. A comparison was made in dogs of a standard commercial curcumin extract and a unique highly bioavailable enhanced formulation of curcumin extract (NMXCC95™ Nutramax Laboratories, Inc.). Two groups of 3 dogs, weighing 12–15 kg were dosed with 2 gram equivalents of curcumin either as curcumin powdered extract or NMXCC95™ following a 12 hour fast. After a one week washout dogs received the other formulation. Plasma curcumin levels were determined for 0–8 hr samples by HPLC method. The standard curcumin extract had a shorter T_{max} compared to the NXMCC95™ formulation (1.5 hrs vs 3.17 hrs), but the enhanced formulation reached a 3-fold higher C_{max} (296.4 vs 98.6 ng/g) and a 7-fold higher AUC over 8 hrs (1381 vs 199 ng•hr/g). Furthermore the plasma levels with NMXCC95™ remained elevated at 8 hrs (107.1±62.7 ng/g) while levels from standard curcumin extract returned close to zero (6.3±8.2 ng/g). These data show that the enhanced formulation of curcumin NMXCC95™ has a substantially higher bioavailability in dogs than standard powdered curcumin extract and has the potential to serve as an effective control of conditions with an underlying inflammatory basis.

ABSTRACT #284
BIOAVAILABILITY OF A NOVEL FORMULATION OF S-ADENOSYLMETHIOINE IN BEAGLE DOGS. DW Griffin, MO Whalen, CR Filburn. Nutramax Laboratories, Inc., Edgewood, MD.

S-adenosylmethionine (SAMe) is used to support liver function both in companion animals and humans, and is also used in humans for mental and joint health. Tablets of SAMe are typically enteric-coated to protect from moisture and enhance stability. In a blinded crossover study a new Denosyl® (Nutramax Laboratories, Inc.) chewable tablet containing a novel, non-hygroscopic, protected formulation of SAMe (NMXSS75™) was administered to beagle dogs to assess its bioavailability relative to an enteric-coated Denosyl SAMe tablet formulation. Six male and 6 female beagles were dosed after an overnight fast with 225 mg SAMe ion (mean 18.75 mg/kg) in one formulation of the supplement, followed by the other formulation 6 days later. Plasma values for SAMe 2 days before dosing and at 0–24 hrs were determined using an LC-MS/MS assay. Plasma SAMe increased substantially to C_{max} values of 1,577±920 and 1,964±ng/ml for the enteric coated and chewable tablets, respectively. Enteric coated and chewable tablet mean values for AUC_{0-24} (5,741±2,729 and 7,099±2,313 ng•hr/ml) and t 1/2 (2.06±1.17 and 2.01±1.19 hrs) were comparable for both formulations. However, the time course of uptake with the chewable tablet was highly reproducible with an earlier, significantly shorter T_{max} of 2.00±0.0 hrs compared to the more variable uptake of the enteric coated tablet (3.42±2.0 hrs, $p<0.01$). Unlike the enteric coated tablet, in the chewable tablet formulation SAMe was detected in the blood at 30 minutes. Fasting prior to dosing resulted in higher absorption than dosing with a meal, regardless of the type of formulation. These data indicate that this chewable formulation of SAMe can be used in dogs with equivalent bioavailability and substantially improved reproducibility to enteric coated SAMe tablets.

ABSTRACT #285
IN VIVO EFFECTS OF FIROCOXIB, MELOXICAM AND TEPOXALIN ADMINISTRATION ON EICOSANOID PRODUCTION IN TARGET TISSUES OF NORMAL CATS. LA Goodman, BT Torres, LR Reynolds, SC Budsberg. University of Georgia College of Veterinary Medicine, Athens, GA.

The purpose of this study was to investigate the in vivo activity of firocoxib, meloxicam and tepoxalin in normal cats by measuring eicosanoid production within target tissues. Eight normal adult neutered male cats were used in this blinded, randomized, crossover study. Cats were treated with firocoxib (1 mg/kg PO q 24 h), meloxicam (0.05 mg/kg PO q 24 h), tepoxalin (5.0 mg/kg PO q 12 h), or placebo for 8 days. Blood samples and gastric and duodenal mucosal biopsies were collected on days 0 (baseline), 3, and 8 of each dosing period. Thromboxane B 2 (TXB2) concentrations were measured in serum and prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) levels were measured in plasma. PGE1 and PGE2 synthesis, and LTB4 levels were determined in the mucosal biopsy specimens. A 21 day washout period was observed between treatments. Repeated measures analyses were performed with significance set at $p<0.05$.

In the blood, firocoxib and meloxicam decreased plasma PGE2 levels compared to baseline on both days. Firocoxib and meloxicam decreased PGE2 compared to placebo on day 3. Tepoxalin did not decrease PGE2 in the plasma compared to baseline or placebo on either day. Firocoxib showed no difference in serum TXB2 compared to baseline or placebo on both days. Tepoxalin decreased TXB2 compared to baseline, placebo and firocoxib on both days. No decrease was noted in plasma LTB4 concentrations in cats administered placebo, firocoxib, meloxicam, or tepoxalin at any time. Firocoxib decreased pyloric PGE1 synthesis on day 8 compared to baseline. Meloxicam showed no significant differences from baseline or placebo on either day. Tepoxalin decreased duodenal PGE1 synthesis on both days compared to baseline. Neither firocoxib or meloxicam decreased pyloric PGE2 synthesis compared to baseline or placebo on days 3 and 8. Tepoxalin decreased pyloric PGE2 synthesis compared to baseline on days 3 and 8 and decreased pyloric PGE2 synthesis compared to placebo on day 3. Furthermore, tepoxalin decreased pyloric PGE2 synthesis compared to firocoxib

and meloxicam on days 3 and 8. Tepoxalin decreased duodenal PGE2 synthesis compared to baseline on days 3 and 8. Meloxicam decreased pyloric LTB4 on day 3 compared to baseline and placebo. Tepoxalin decreased pyloric mucosal LTB4 on days 3 and 8 compared to baseline.

Firocoxib and meloxicam spared the cyclooxygenase-1(COX-1) enzyme while tepoxalin exhibited COX-1, COX-2 and lipoxagenase-5 (LOX-5) inhibition. Meloxicam may have LOX-5 inhibition capabilities at the dosages used in this study.

ABSTRACT #286

INTESTINAL PARASITES OF DOGS ON THE GALAPAGOS ISLANDS. EN Gingrich[1], AV Scorza[1], MR Lappin[1], EL Clifford[2]. [1]Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO. [2]Animal Balance, San Francisco, CA.

Dogs on the Galapagos Islands are a unique population created by isolation from the mainland and regulations prohibiting further importation. The effect of infectious agents of these domestic dogs on the indigenous fauna is largely unknown. The purpose of this study was to determine the prevalence of intestinal parasites in dogs on the Galapagos Islands.

Fecal samples were collected from 97 dogs presented during neutering campaigns on Santa Cruz (n=51), San Cristobal (n=17), and Isabela (n=29) islands. Feces were evaluated for parasites by microscopic examination after zinc sulfate centrifugation flotation as well as by a commercially available IFA for *Cryptosporidium* spp. and *Giardia* spp. Polymerase chain reaction for *Cryptosporidium* spp. DNA and *Giardia* spp. DNA was performed on all positive samples to provide the infecting genotypes.

Ancylostoma caninum (57.7%) and *Toxocara canis* (16.5%) were most commonly detected, followed by *Giardia* spp. (5.2%), *Isospora canis* (4.1%), *Sarcocystis canis* (3.1%), and *Cryptosporidium* spp. (1%). Adequate DNA for sequencing was available for one *Giardia* spp. which was shown to be assemblage D.

Despite being isolated, the dogs on the Galapagos have many of the same enteric parasites detected on the mainland of South America. These dogs are not routinely administered anthelmintics or other drugs, but are often allowed to roam the streets and live in close proximity to humans. Parasite prophylaxis is necessary to decrease the parasite burden within the population and to lessen the risk of spread to humans or other animals also inhabiting the islands.

ABSTRACT #287

CANADIAN PREVALENCE AND DIAGNOSIS OF GIARDIA INFECTION IN DOGS AND CATS USING A FECAL ANTIGEN TEST AND FECAL SMEAR. Merle E Olson. Bow Valley Research Inc. Calgary, Alberta and the Department of Biomedical Engineering, University of Alberta, Edmonton Alberta.

Giardiasis is a common gastrointestinal infection in dogs and cats but it remains a diagnostic challenge due to intermittent shedding and the small size of the cysts. A fecal antigen test (SNAP® *Giardia* Test kit, IDEXX Laboratories, Inc.) was utilized in Canadian veterinary clinics to determine the prevalence of *Giardia* in dogs (134 clinics) and cats (94 clinics) with gastrointestinal clinical signs. The fecal antigen test is an in-clinic ELISA which detects a highly specific, soluble cyst wall antigen that is released at the time the trophozoites encyst. Results of the fecal antigen test were compared to those of a fecal smear which was the method used by many veterinary clinics. A total of 1871 dogs and 389 cats were enrolled in the study. The presence of fecal antigens were observed in 299 (16.0%) and 30 (7.7%) symptomatic dogs and cats, respectively. Loose or watery diarrhea with increased frequency was the predominant presenting clinical sign of both dogs and cats. *Giardia* cysts were not observed in the fecal smears of 131 (68.2%) fecal antigen positive dogs and 11 (73.3%) fecal antigen positive cats. The fecal antigen test for *Giardia* appears to be a valuable tool in the diagnosis and ultimate control of giardiasis in dogs and cats.

ABSTRACT #288

PREVALENCE AND MOLECULAR ANALYSIS OF *GIARDIA* SPP. INFECTIONS IN DOGS IN CHIANG MAI, THAILAND: PRELIMINARY FINDINGS. S Tangtrongsup, AV Scorza, LR Ballweber, JS Reif, MD Salman, MR Lappin. Colorado State University, Fort Collins, CO.

Giardia spp. is a common cause of waterborne diarrhea in human, pets and wildlife animals worldwide. However, the prevalence of this organism in dogs in Thailand and the potential for dogs to serve as a reservoir host is unknown. This study was conducted to explore the prevalence of *Giardia* infection in dogs in this province. The results will be used to determine prevention strategies in Chiang Mai.

A cross-sectional study was designed and 82 canine fecal samples were obtained from client owned dogs, breeding farms and a shelter during June and July 2008. Demographic and geographic data were recorded. Fecal samples were scored using a standardized system. *Giardia* infections were diagnosed using a PCR targeting the GDH gene. Associations of age category (<1 year, 1–7 years and >7 years), type of housing (client-owned, breeding farm, and shelter), fecal score (1 to 7), and *Giardia* positive results were analyzed using Fisher's exact test and odds ratios were estimated.

The prevalence of *Giardia* infections was 15.9%. Positive results were significantly associated with the age categories (p=0.04). When compared to dogs >7 years, the odds ratio of dogs <1 year was 11.90 (95%CI: 0.61–230.84) and the odds ratio of dogs 1–7 years was 3.36 (95%CI: 0.18–64.1). Fecal score and type of housing were not significantly associated with *Giardia* positive results. The PCR products were genotyped and all isolates were *Giardia intestinalis* assemblages C or D.

In conclusion, *Giardia* infection in young dogs in Chiang Mai was common with dog specific assemblages.

ABSTRACT #289

SEROTYPES AND PATHOGENICITY ASSOCIATED WITH ANTIMICROBIAL-INDUCED RESISTANCE IN FECAL *ESCHERICHIA COLI* OF HEALTHY DOGS. N. Debavalya, and DM Boothe. Auburn University, College of Veterinary Medicine, Auburn University, AL.

The purpose of this study was to examine the relationship between resistance and virulence in canine fecal *E coli* isolates multidrug resistance (MDR) versus those not expressing multidrug resistance (NMDR) in response to antimicrobial therapy. We have previously demonstrated that close to 100% of fecal *Escherichia coli* in healthy dogs develop NMDR versus multidrug MDR resistance when treated with amoxicillin (10 mg/kg bid) or enrofloxacin (5 mg/kg sid), respectively, with no resistance emerging untreated controls (n=8 per treatment group).

Isolates from each treatment group (n=10) were subjected to phenotyping using microbroth dilution susceptibility testing to 17 antimicrobial drugs using a customized plate and following CLSI guidelines. Isolates were genotyped based on PFGE. Serotypes (O and H) and virulence factors (7 toxins and 1 adhesin) were then determined in one isolate from each phenotype and genotype in each treatment group.

Amoxicillin treatment resulted in 12 genotypes and 7 phenotypes among resistant isolates (NMDR) whereas enrofloxacin treatment (MDR isolates) resulted in only 1 genotype and 3 phenotypes. Only one genotype and phenotype was yielded from the controls (no resistance). Seven serotypes were detected in 11 NMDR isolates from amoxicillin treatment, whereas one serotype was detected in 3 MDR isolates from enrofloxacin treatment. The MDR serotype (H30:O9) was not similar to those of NMDR (H31:O83, H30:O8, H43:O138, H12:O-negative, H32:O-negative, H11 or 47:O11, and H-positive:O-negative). Control (susceptible) isolates exhibited serotype H4:O5 which also was different from both NMDR and MDR serotypes. Among the virulence genes tested, only Cytotoxic Necrotizing Factors (*cnf*) 1 and 2 were detected (indicating necrotoxigenic *E. coli*) and only in association with 3 phenotypes: one control isolate (*cnf*1), and two in the amoxicillin (NMDR) isolates that had the same serotypes; *cnf*1 in H31:O83 isolates (resistant to beta-lactams and ceftiofur) and *cnf*2 in H-positive (O-negative) isolates (resistant to beta-lactams, ceftiofur and tetracycline). No other toxins or adhesins were expressed in isolates of this study.

These data demonstrate that antimicrobial resistance induced in fecal *E coli* by either amoxicillin or enrofloxacin was not associated with an increase in pathogenicity.

ABSTRACT #290
COMPARISON OF QUANTITATIVE PCR AND CONVENTIONAL ENDPOINT PCR FOR AMPLICATION OF PARVOVIRUS DNA IN BLOOD FROM NATURALLY INFECTED AND RECENTLY VACCINATED DOGS. JK Veir, AL Duffy, SW Dow, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Canine parvovirus (CPV) can be detected in feces by antigen ELISA and in blood and feces by PCR. We previously demonstrated that CPV DNA can be readily amplified from the blood of healthy puppies via conventional endpoint PCR (cPCR) as early as day 2 and through day 14 after modified live CPV vaccination. Because viremia is present one to two days after infection in clinically infected dogs as well, differentiation of clinically affected and vaccinated dogs using endpoint PCR is impossible. We hypothesize that vaccination with modified live CPV should produce lower levels of circulating CPV DNA as compared to natural infection, thereby allowing differentiation between vaccinates and diseased dogs. Real time quantitative PCR (qPCR) allows for accurate and reliable quantification of viral load. The objectives of this study were to compare results of cPCR and qPCR assays using blood from vaccinated dogs and to compare the CPV DNA load between vaccinated dogs and naturally infected dogs.

A modified live CPV vaccine was administered SQ to twelve, six week old puppies on day 0. Presence of CPV DNA in whole blood taken from the puppies at days 0, 2, 5, 7, 10, and 14 was evaluated using both cPCR and qPCR. Additionally, the CPV viral load in peripheral blood collected on the day of presentation from naturally infected, fecal CPV antigen positive, clinically ill dogs was determined via qPCR. Results of the log transformed quantitative data were compared via the Kruskal-Wallis test with Dunn's post analysis and sensitivity and specificity calculated via a receiver operator characteristic curve (ROC). Agreement between the two methods was evaluated using the Kappa coefficient.

No parvoviral DNA was detected via either method in any vaccinated dog samples from day 0. Canine parvoviral DNA was detected via both methods in all samples taken on day 14. Kappa values for days 2, 5, 7, and 10 were 0.667, 0.657, 0.824, 0.833, indicating substantial agreement between the two methods in the samples from vaccinated dogs. There were statistical differences in CPV viral load between the vaccinated dogs and the naturally infected dogs at all time points except days 0 and 14 post vaccination ($p < 0.05$) with the naturally infected dogs having higher values. The area of the ROC curve was 0.9646 with a p value of < 0.0001 when all samples except day 0 from the vaccinates were defined as the control group and the clinical animals were defined as the diseased group.

Canine parvoviral DNA is detectable by both cPCR and qPCR after vaccination. Additionally, quantitation of viral load in the peripheral blood was able to differentiate between naturally infected, diseased dogs and dogs that were recently vaccinated with a single modified live CPV vaccine. However, viral load following vaccination using other commercially available vaccines must be investigated prior to widespread clinical use of the assay.

ABSTRACT #291
THE EPIDEMIOLOGY OF *STREPTOCOCCUS CANIS* INFECTIONS IN DOGS AND CATS. EF Kruger, BA Byrne, PA Pesavento, KF Hurley, JE Sykes. University of California Davis William R. Pritchard Veterinary Medical Teaching Hospital, Davis, CA.

Little is known regarding the degree of genotypic relatedness between *Streptococcus canis* isolates from dogs and cats. The purpose of this study was to determine whether correlations existed between the genotypes of canine and feline *S. canis* isolates as determined using pulsed field gel electrophoresis (PFGE) and different clinical manifestations of disease. Eighty-nine isolates of *Streptococcus canis* that caused specific manifestations of disease in dogs and cats were collected from the University of California, Davis between 1998–2005. Associated clinical manifestations included sepsis, otitis, pyometra, skin infections, respiratory disease, and urinary tract infections. Ten isolates were also collected from a Northern California shelter which experienced an outbreak of *S. canis* infections manifesting as chronic respiratory infections and septic arthritis in cats. Bacterial isolates were characterized by PFGE analysis using the restriction enzyme *SmaI*. The relationships between banding patterns were analyzed using BioNumerics software combined with visual interpretation. Results demonstrated that the feline shelter isolates of *Streptococcus canis* were 99% similar in bacterial PFGE profile suggesting a clonal origin. The remainder of samples differed significantly, with less than 80% similarity in PFGE banding patterns. Thus in the latter group of isolates, there appeared to be no relationship between clinical manifestation of infection and PFGE profile.

ABSTRACT #292
BACTERIAL AND FUNGAL COLONIZATION OF PERIPHERAL INTRAVENOUS CATHETERS IN DOGS AND CATS. JP Pages[1], J Seguela[2]. [1]Clinique Vétérinaire Croix du Sud, Saint Orens, [2]Dept Clinical Sciences, National Veterinary School, Toulouse, France.

Infections associated with intravenous (IV) catheters have been recently shown to be a potential concern in an intensive care unit (ICU) population of 151 dogs and cats, the prevalence of positive catheter-tip culture rates reaching 25% (Marsh-Ng et al, JAAHA 2007;43:13–20). However, this issue in non ICU small animal patients has not been documented. The aim of this prospective study was to assess, in routine clinical settings, the prevalence of positive catheter-tip cultures associated with peripheral IV catheters in dogs and cats.

100 peripherally placed IV catheters (20-gauge 1.1×30 mm and 22-gauge 0.9×25 mm) from 13 cats and 78 dogs were used for infusion therapy for various surgical and medical indications. They were aseptically removed and the distal two-thirds were cut and submitted to bacterial and fungal culture. Antimicrobial susceptibility of each isolate was determined.

17/100 bacterial cultures were positive. *Staphylococcus* spp. was the most common isolate (61%). The other isolates were *Klebsiella*, *Citrobacter*, *Escherichia*, *Pseudomonas* and *Enterobacter* spp.. The most frequent antimicrobical resistance were for amoxicillin (13/17), lincomycine (12/17), doxycycline (11/17), flumequine (8/17), tylosin (8/17), enrofloxacin (8/17). Inversely, resistance to gentamicin (3/17), amoxicillin-clavulanate (3/17) and cefalotin (2/17) were less common. *Candida glabrata* was also isolated in one catheter.

In conclusion, colonization of IV catheters with bacteria is frequent but its potential clinical consequences require further investigations.

ABSTRACT #293
DETERMINATION OF FOUR IgG SUBCLASSES IN ASYMPTOMATIC DOGS WITH VISCERAL LEISHMANIASIS AND IN ANIMALS VACCINATED AGAINST THE DISEASE. FA Ikeda-Garcia[1], MJ Day[2], VMF Lima[1], FA Rosa[1], LSV Sobrinho[1], AAD Gomes[1], JP Vides[1], SHV Perri[1], M Marcondes[1]. [1]São Paulo State University, Araçatuba, São Paulo, Brazil. [2]Department of Pathology and Microbiology, University of Bristol, Bristol, United Kingdom.

Visceral leishmaniasis in Brazil has been showing a geographical spread since the 80 s, reaching big urban areas. In this context, a vaccinal immunoprophylaxis applied to dogs may represent an important control measure of the infection through the implementation of immune protective mechanisms. However, due to the fact that vaccinated animals have the ability of performing seroconversion, routine serological tests cannot differentiate an infected dog from a vaccinated one. This present study aimed to evaluate the four IgG subclasses in asymptomatic dogs naturally infected with *Leishmania chagasi* and in animals which were vaccinated against the disease. In order to meet the aforementioned purpose, three dog groups were used, as such: control (n=45), asymptomatic with visceral leishmaniasis (n=45) and vaccinated against the disease (n=37). The presence of anti-*Leishmania chagasi* IgG in serum was

determined by the ELISA technique. In order to determine the four IgG subclasses, monoclonal antibodies were employed. In vaccinated animals the predominant subclass was IgG1, followed by IgG3, IgG2 and IgG4. In asymptomatic dogs the only subclass that was stimulated was IgG1. The IgG1 and IgG2 subclasses did not allow differentiation between dogs vaccinated against visceral leishmaniasis from naturally infected asymptomatic dogs. On the other hand, IgG3 and IgG4 allowed differentiation of dogs from the two groups because the vaccinated animals had an increase of the two subfractions, whereas the same immunoglobulins from asymptomatic ones were not stimulated.

ABSTRACT #294

PERFORMANCE EVALUATION OF SPECIES-SPECIFIC PEPTIDE-BASED ASSAYS FOR DETECTION OF CANINE ANTIBODIES TO *E. CANIS* AND *E. CHAFFEENSIS*.

J. Saucier[1], D. Daniluk[1], R. Krah[1], R. Chandrashekar[1], S. Gaunt[2], T. O'Connor[1]. [1]IDEXX Laboratories, Inc., Westbrook, ME. [2]Louisiana State University, Baton Rouge, LA.

Canine ehrlichiosis is caused by several species of tick-borne intracellular bacteria that are distributed worldwide. Infection is characterized by fever, lethargy, weight loss and thrombocytopenia and can lead to death. Early diagnosis and treatment provides the best opportunity for a favorable outcome and typically leads to complete recovery. Species-specific differentiation of *E. canis* and *E. chaffeensis* infection is important as it can lead to different approaches for disease management and treatment.

The goal of the project was to identify peptides that can be used in place of full-length proteins in diagnostic assays for *E. canis* and *E. chaffeensis*. Generally, we have found that peptides were more amenable for use in rapid-format assays and were typically more specific than full-length proteins. We synthesized and compared the reactivity of peptides from several regions of the p16 and p140 proteins of *E. canis* and from the variable length PCR target (VLPT) and p120 proteins of *E. chaffeensis*.

In a series of *E. canis*-experimentally infected dogs, the p16 peptide-based ELISA detected antibody between 13 and 17 days post infection which typically coincided with the appearance of clinical symptoms. The full length p16 recombinant protein ELISA and the synthetic peptide ELISA had identical results for 69 of the 71 dogs (31 positive, 40 negative) in a population of dogs from an *E. canis*-endemic region of the United States.

In *E. chaffeensis*-experimentally infected dogs the VLPT peptide-based ELISA detected antibody 7 days post infection which coincided with the detection of antibody in the IFA and recombinant protein-based ELISA. Twenty-eight of 29 samples reactive in the VLPT-recombinant protein ELISA were reactive in the synthetic peptide ELISA. Samples from *E. canis*-infected dogs and from *E. chaffeensis*-infected dogs did not react in the VLPT and p16 peptide assays, respectively. Samples from *E. ewingii*-experimentally infected dogs did not react in either assay.

These peptides can be used to develop diagnostic assays capable of simultaneous but specific detection of antibody to *E. canis* and *E. chaffeensis* in canine serum, plasma and whole blood.

ABSTRACT #295

EHRLICHIA/ANAPLASMA SPP INFECTION AND EXPOSURE RATES IN DOGS FROM OKLAHOMA AND ARKANSAS.

T. O'Connor[1], J. Saucier[1], S.E. Little[2], R. Chandrashekar[1]. [1]IDEXX Laboratories, Inc, Westbrook, ME. [2]Dept of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK.

Several species of *Ehrlichia* and *Anaplasma* are capable of infecting dogs resulting in a condition characterized by fever, lethargy, weight loss and anemia that can lead to death. Species-specific identification of *Ehrlichia* is important because it can lead to different approaches for disease management and treatment.

The purpose of this study was to determine the prevalence of *Ehrlichia/Anaplasma* spp. by microscopy and PCR and the seroprevalence of antibodies reactive to *Ehrlichia* spp. by ELISA in a population of febrile and healthy dogs from the Ozark Plateau region of northeastern Oklahoma and northwestern Arkansas. A total of 143 samples were collected from veterinary practices (V3 and V4) (n=88) and shelters (n=55) in the study area. Among the samples from the veterinary practices, 11 (12.5%) were collected from febrile (T > 103.4 F) dogs; ticks were noted on 9 (10.2%) of the dogs.

Results are detailed in the table below. PCR results for *E. canis* (*Eca*), *E. chaffeensis* (*Ech*) and *E. ewingii* (*Ee*) are based on conventional nested PCR; serology results for *E. canis*, *B. burgdorferi* (*Bb*), and *A. phagocytophilum* (*Ap*) antibody were obtained using the SNAP® 4Dx® test. Results for *E. ewingii* antibody were obtained using a peptide-based microtiter plate ELISA.

	Serology				PCR		
Group	***Eca***	***Bb***	***Ap***	***Ee***	***Eca***	***Ech***	***Ee***
V3 (n=39)	20.5%	0	2.6%	59.0%	0	2.6%	23.1%
V4 (n=49)	8.2%	0	0	55.1%	0	4.1%	4.1%
Shelter, (n=55)	1.8%	0	7.3%	25.5%	0	1.8%	3.6%
Total	9.1%	0	3.5%	44.8%	0	2.8%	9.1%

Morulae were not found in any of the blood smears (N=143). Overall, 9.1% of the dogs were reactive in the *E. ewingii* PCR and 44.8% in the *E. ewingii* ELISA, supporting previous studies suggesting that *E. ewingii* may be the primary agent of canine ehrlichiosis in this region. The *E. ewingii* ELISA detected 11 of 13 (84.6%) of the PCR positive samples. Differences in positive rates between groups of dogs are likely due to timing of samples collection and relative exposure to *A. americanum* ticks.

ABSTRACT #296

EFFICACY OF DOXYCYCLINE TREATMENT IN DOGS NATURALLY INFECTED WITH *ANAPLASMA PHAGOCYTOPHILUM*.

Diniz, PPVP[1], Correa, MT[1], Chandrashekar, R[2], Beall, M[2], Breitschwerdt, EB[1]. [1]Intracellular Pathogens Research Laboratory, North Carolina State University, College of Veterinary Medicine, Raleigh, NC, USA. [2]IDEXX Laboratories, Westbrook, ME, USA.

A. phagocytophilum is a tick-transmitted bacterium capable of infecting dogs, cats, horses, human beings and other species. Clinical observations of selected cases, as well as preliminary data from an experimental study, have suggested that doxycycline may not clear the infection in all treated dogs. Since differences in antibiotic susceptibility could occur between experimental strains and wild strains of this organism, this study evaluated the efficacy of doxycycline treatment for canine granulocytic anaplasmosis (CGA) in 11 naturally infected dogs. Dogs presenting signs compatible with CGA at two private clinics in the Northeastern United States (Baxter, MN and Plover, WI) were evaluated every 30 days over a 2-month period: at the initial consultation (day 0); after doxycycline treatment (day 30) and roughly 2 months after the initial consultation (day 60). Each enrolled dog was treated with doxycycline at a dose of 5 mg/kg twice a day for 28 days. Blood-EDTA and serum samples were collected at each evaluation date, and lymph node aspirates (LNA) were collected at days 0 and 60. Additionally, clients and veterinarians answered a clinical and epidemiological questionnaire at each visit. Finally, complete blood cell counts were performed from all enrolled dogs at each evaluation. DNA from blood-EDTA and LNA was extracted and *A. phagocytophilum* DNA was amplified targeting the msp2 gene. Antibodies against *Anaplasma* spp. were detected by Snap® 4Dx®. All 11 dogs were re-evaluated at the first follow-up (range of 24–47 days from day 0) and 10 dogs were evaluated for the second follow-up (range of 57–94 days). *A. phagocytophilum* DNA was not detected from blood-EDTA or LNA from any of the 11 dogs at day 30 nor from any of the 10 evaluated dogs at day 60. Six of 11 infected dogs seroconverted between day 0 and day 30, while 5 dogs were seroreactive on day 0. Platelet numbers significantly increased from day 0 to days 30 and 60 of the study (mean±SD: day 0=98.3±69.3×10^3/mm^3, range 11–250×10^3/mm^3; day 30=255.7±89.6×10^3/mm^3, range 147–420×10^3/mm^3; day 60=294.6±92.3×10^3/mm^3, range 205–451×10^3/mm^3; $p<0.001$). Body temperature significantly decreased after treatment (mean±SD: day 0=104±1.3°F, range 101.3–105.4°F; day 30=101.8±0.5°F, range 101–102.8°F; day 60=101±1.2°F, range

98–102°F; $p < 0.001$). After doxycycline treatment, all dogs fully recovered according to clients' assessment. Our results suggest that doxycycline is effective in controlling *A. phagocytophilum* infection and in reversing clinical signs in dogs in selected areas of the USA; however, a larger population of naturally-infected dogs from several regions needs to be evaluated to confirm these results.

ABSTRACT #297

BARTONELLA SPP. ASSOCIATED ENDOCARDITIS IN DOGS IN COLORADO AND WYOMING. A Fenimore[1], M Lappin[1], M Varanat[2], R Maggi[2], E Breitschwerdt[2]. [1]Department of Clinical Sciences, Colorado State University, Fort Collins, CO. [2]North Carolina State University, School of Veterinary Medicine, Department of Clinical Sciences, Raleigh, NC.

Since the mid 1990's, several species of *Bartonella*, including *B. vinsonii*, *B.quintana*, and *B. clarridgeiae* have been identified in dogs diagnosed with infectious endocarditis (IE). The prevalence of *Bartonella* spp. infection in dogs in the referral area for Colorado State University is unknown. The purpose of this study was to evaluate the heart valves of dogs with suspected IE for the presence of *Bartonella* spp. DNA by polymerase chain reaction (PCR).

The medical records system at the Veterinary Medical Center was searched for dogs with a clinical diagnosis of endocarditis that had been admitted from January 1990 to June 2008. DNA was extracted from the available formalin fixed valvular tissues and assessed for *Bartonella* spp. DNA by three PCR methods. The *Bartonella* spp. was determined by genetic sequencing or fluorogenic PCR.

Of the 119 patients with a presumptive diagnosis of endocarditis, 22 had IE based on histopathology results. The histopathology blocks and medical records were available from 9 dogs. *Bartonella henselae* DNA was amplified from the tissues of seven dogs; *B. vinsonii* DNA was amplified concurrently from three dogs. Of the seven dogs, six were from Colorado and one was from Wyoming (*B. henselae* only) and all were greater than five years of age. The dogs were not known to have left the region and fleas or ticks were reported on some dogs.

This is the first report of *B. henselae* associated IE in dogs. The results suggest that *Bartonella* spp. infection can occur in dogs in the western states and these agents should be on the differential list for dogs with suspected IE in the region.

ABSTRACT #298

DETECTION OF _BARTONELLA HENSELAE_ IGM IN CATS WITH EXPERIMENTALLY INDUCED BARTONELLOSIS. J Ficociello, C Bradbury, A Morris, MR Lappin. Colorado State University, College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

The temporal appearance of *Bartonella henselae* DNA in blood and *B. henselae* antibodies in serum has usually been studied in cats after parenteral inoculation. However, little is known about the progression of *B. henselae* test results in cats exposed to *B. henselae* infected *Ctenocephalides felis*.

Cats (n=6) were inoculated with a field strain of *B. henselae* IV or were exposed to fleas allowed to feed on *B. henselae* PCR positive cats (n=6). Blood and serum samples were collected on week 0, week 2 or 3, and then weekly until week 13. A *Bartonella* spp. IgM ELISA was validated using samples from experimentally inoculated cats. All samples were then assayed in the optimized IgM ELISA, a previously reported IgG ELISA, and a previously reported conventional PCR assay that detects DNA of *B. henselae*.

For cats inoculated IV, the first positive *Bartonella* IgM, IgG, and PCR assay result was detected on week 4 (four cats), week 3 (three cats), and week 3 (six cats), respectively. For cats exposed to fleas, the first positive *Bartonella* IgM, IgG, and PCR assay result was detected on week 8 (one cat), week 4 (one cat), and week 7 (one cat), respectively. All cats in both groups eventually became IgM positive (week 12 for flea cats; week 4 for IV cats) and PCR positive (week 11 for flea cats; week 3 for IV cats). Five IV inoculated cats were IgM negative week 13 and all cat exposed to fleas were IgM positive week 13. All cats infected IV became IgG positive by week 4 and maintained positive titers throughout the study. Of the cats exposed to fleas, five of six were IgG positive by week 12; the sixth cat never developed detectable IgG and died of *B. henselae* myocarditis during week 13.

Differences may exist in *Bartonella* spp. test results in cats based on the route of inoculation. These findings may relate to the infective dose administered or on effects imparted by the vector.

ABSTRACT #299

PREVALENCE OF _BARTONELLA_ AND HAEMOPLASMA DNA IN SAMPLES FROM NON-OWNED FREE-ROAMING CATS ADMITTED TO ANIMAL SHELTERS IN COLORADO AND FLORIDA. JN Eucher, M Brewer, MR Lappin. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Bartonella spp. and the haemoplasmas (*Mycoplasma haemofelis, 'Candidatus* M. turicensis*', 'Candidatus* M. haemominutum') are common feline pathogens. *Ctenocephalides felis* has been shown to be the vector for *Bartonella* spp.. It is hypothesized that *Bartonella* spp. and haemoplasmas are transmitted between cats by bites or scratches. To further elucidate the mechanisms of transmission of these organisms, the prevalence of *Bartonella* spp. and haemoplasma spp. in blood, salivary glands, and tonsils was compared among free-roaming cats admitted to animal shelters in states where fleas are rare (Colorado) or common (Florida).

Blood, salivary glands, and palatine tonsils were collected from non-owned, free-roaming shelter cats in Colorado and Florida within 24 hours after euthanasia. DNA was extracted by use of commercially available kits and then assessed for *Bartonella* spp. and haemoplasma spp. DNA using previously published polymerase chain reaction assays.

The prevalence rates for *Bartonella* spp. DNA in blood (CO=0 of 20 [0%]; FL=10 of 18 [55.6%] $p < 0.0001$), salivary gland (CO=0 of 24 [0%]; FL=5 of 18 [27.8%]; $p=0.017$) and tonsil (CO=0 of 20 [0%]; FL=6 of 18 [33.3%]; $p=0.006$) were statistically different between the two states. Haemoplasma spp. DNA was detected in similar numbers of blood samples (CO=1 of 20 [5%]; FL=2 of 18 [11.1%]), salivary glands (CO=3 of 24 [12.5%]; FL=2 of 18 [11.1%]), and tonsils (CO=3 of 24 [12.5%]; FL=2 of 18 [11.1%]).

The presence of *Bartonella* spp. and haemoplasma DNA in salivary glands or tonsils may merely reflect the presence of infected erythrocytes within the tissues. Alternately, the results may support the hypothesis that the organisms are passed in these tissues as a mode of transmission.

ABSTRACT #300

ASSOCIATION OF PATHOGENICITY AND RATE OF COINFECTION OF THREE FELINE HEMOTROPIC MYCOPLASMA STRAINS WITH PRESENCE AND SEVERITY OF ANEMIA. C.M. Leutenegger, Thao M, Estrada M, Robertson J, Cornwell D. IDEXX Laboratories, West Sacramento, CA.

Three species of epierythrocytic mycoplasmas (Feline Hemotropic Mycoplasma, FHM) are known to infect cats: *Mycoplasma haemofelis* (Mhf), *Candidatus Mycoplasma haemominutum* (CMh) and *Candidatus Mycoplasma turicensis* (CMt). Infection with Mhf has been associated with severe hemolytic anemia in immunocompetent cats. In contrast, infection with Mhm, a smaller organism, has only rarely been associated with disease in immunocompetent cats and is dependent on the presence of cofactors such as co-infections with other hemotropic mycoplasma strains or retrovirus infections. With intermediate pathogenicity, CMt, a smaller organism similar to CMh, has been described to induce anemia in immunocompetent cats. The purpose of this study was to evaluate the association between strain presence, presence of coinfections and severity of anemia.

Whole blood samples from 500 cats submitted to the IDEXX Reference Laboratory system throughout the US were analyzed using Real-time TaqMan® PCR assays. These assays were developed with high analytical sensitivity and specificity for each strain and results are quantitative. The prevalence of cats mono-infected with each of the three hemotropic strains and the prevalence of cats co-infected with more than one strain was determined. Mycoplasma load for each infection was also determined.

Cats were divided into 3 groups according to hematocrit values: Group 1: <26% (moderately to severely anemic); group 2: 26–28% (mildly anemic); group 3: >28% (non-anemic). The overall prevalence of FHM in group 1 was 29%. Monoinfections accounted for:

MHf: 6.9%, CMh: 13.2%, CMt: 1.7%. Coinfections: 26.1%. Group 2: overall prevalence 28.6%. Monoinfections accounted for: MHf: 5.5%, CMh: 16.5%, CMt: 1.1%. Coinfections: 19.2%. Group 3: overall prevalence was 13.5%. Monoinfections accounted for MHf: 0%, CMh: 10.8%, CMt: 2.7%. Coinfections: 0%.

In this study, the presence and severity of anemia correlated with a) the degree of coinfections and b) the presence of Mhf and CMt and c) the Mycoplasma load present in cats. Monoinfections with the least pathogenic strain CMh in group 2 were associated with anemia if the Mycoplasma load exceeded 20,000 Mycoplasma organisms per ml blood.

ABSTRACT #301
ANALYTICAL SENSITIVITY AND SPECIFICITY OF A REAL-TIME PCR ASSAY DETECTING PATHOGENIC LEPTOSPIRA IN DOGS BASED ON THE HAP-1 GENE. C.M. Leutenegger, Palaniappan R, Elsemore D, Estrada M, Thao M, Cornwell D. IDEXX Laboratories, West Sacramento, CA.

Leptospirosis is a zoonotic bacterial disease of worldwide importance in both human and veterinary medicine. Disease is caused by serovars of the pathogenic species Leptospira interrogans sensu latu. The consequences of infection in dogs range from subclinical to peracute disease, with typical presentations including acute renal failure sometimes combined with acute hepatic disease. Currently available microscopic agglutination tests (MAT) cannot provide an early diagnosis since MAT relies on antibodies to leptospiral antigens and cannot detect infection until 10–15 days post-exposure. Immunofluorescence and immunoperoxidase staining in the clinical samples are not sensitive and culturing the organisms is difficult and slow due to their fastidious nature.

Renal carriage and urine shedding remains a key objective in the diagnostic workup to determine implication in active disease in dogs and to determine the zoonotic potential from a public health perspective. Serology is commonly described as a poor predictor of leptospiruria or health status. Molecular tools such as real-time PCR have been described to reliably detect pathogenic Leptospira in the urine, even in healthy carrier animals. This study aimed to define the diagnostic sensitivity and specificity of a real-time TaqMan® PCR assay targeting the gene encoding for the hemolysin adapted protein (hap1). This gene has been mapped to pathogenic Leptospira and is absent in non-pathogenic strains.

A total of 72 paired blood and urine samples in duplicate obtained from experimentally infected dogs were utilized in this study. Dogs were infected with Leptospira sensu lato, serovar pomona strain. Genomic DNA (gDNA) was extracted from whole blood and urine samples using an automated extraction system and analyzed in parallel using controls to monitor the amount and quality of the extracted gDNA as well as extraction efficiency using a spiked internal positive control (IPC). DNA samples were free of PCR inhibiting substances as determined by IPC and dilution experiments. Using MAT as the gold standard, real-time PCR had 92% diagnostic sensitivity and 99% diagnostic specificity in this sample set. Advantages of a molecular diagnostic approach are the detection of all pathogenic strains as opposed to those used for a particular MAT procedure, the acute phase and urine diagnostic application, and a limited risk for vaccine interference.

ABSTRACT #302
PREVALENCE OF INFECTIOUS AGENTS AND ANTI-ERYTHROCYTE ANTIBODIES IN CATS WITH ANEMIA. KL Dowers, AG Miller, CB Webb, RF Keegan, AC Avery, PK Kiser, MR Lappin. Colorado State University, Fort Collins, Colorado.

The most common causes of feline immune-mediated hemolytic anemia (IMHA) are infectious agents and neoplasia. Primary IMHA is considered less common, but the Coombs' test is the only available assay to detect anti-erythrocyte (RBC) antibodies and it has low sensitivity and specificity in cats. The study's purpose was to determine the prevalence of infectious agents and anti-RBC antibodies using the Coombs' test and flow cytometry in anemic cats.

Blood collected from 35 anemic cats was evaluated with the Coombs' test at 37C and 4C, and flow cytometry. Infectious agent assays included FeLV antigen, FIV antibody, *Bartonella* spp. antibody, and PCR assays for *Mycoplasma hemofelis*, 'Candidatus *M. haemominutum*', 'Candidatus *M. turicensis*', *Bartonella* spp, *Ehrlichia* spp and *Anaplasma phagocytophilum*.

Of the cats, 57.1% (20/35) were positive for an infectious agent. Of these, 10 cats were positive for anti-RBC antibodies in at least one of the assays. Anti-RBC antibody positive cats by flow cytometry (5/5), Coombs' test at 37C (2/3), or Coombs' test at 4C (9/13) were usually concurrently positive for an infectious agent. There was 88.6% agreement between flow cytometry and the Coombs' at 37C and 66.7% between flow cytometry and the Coombs' at 4C.

Anti-RBC antibodies were detected in 50% of the cats with evidence of exposure to an infectious agent and 88.6% of anti-erythrocyte antibody positive cats were concurrently positive for an infectious agent. Therefore the presence of anti-RBC antibodies did not distinguish between infectious and primary immune-mediated causes of anemia.

ABSTRACT #303
***BARTONELLA HENSELAE* ANTIGEN RECOGNITION PATTERNS IN THE SERUM AND AQUEOUS HUMOR OF CATS WITH AND WITHOUT UVEITIS**. CL McInnis, CC Powell, JR Hawley, EJ Ehrhart, MR Lappin. Colorado State University, Fort Collins, CO.

Uveitis is a common ocular syndrome of cats that has multiple causes, including *Bartonella* spp. However, the organisms are host-adapted and so *Bartonella* spp. antibodies and bacteremia are common in both normal cats and cats with uveitis, making it difficult to prove a clinical association with uveitis using currently available blood tests. The importance of accurately diagnosing ocular bartonellosis lies in the public health significance of this zoonotic disease, as well as in determining the optimal appropriate medical therapy for the individual cat. The objective of this study is to assess serum and aqueous humor *Bartonella* spp. antigenic recognition patterns as a diagnostic method for ocular bartonellosis.

Paired serum and aqueous humor samples were collected from cats with and without uveitis (healthy feral shelter cats from Florida). All samples were assayed in an IgG Western blot immunoassay, using culture grown *B. henselae* as the antigen preparation. The density and apparent molecular masses of any detectable band was determined with a computerized system by an individual blinded to the sample source. A serum or aqueous humor sample was considered positive if a previously defined immunodominant antigen was recognized with an average intensity greater than a predetermined cutoff value. The primary criterion for documenting intraocular production of *Bartonella* spp. antibodies was recognition of an immunodominant antigen by the aqueous humor sample but not the corresponding serum sample. In addition, if an immunodominant antigen was recognized by both the serum and aqueous humor sample and the average band intensity was greater in the aqueous humor sample, intraocular antibody production was documented.

Bartonella spp. antibodies were detected in serum of 61 of 84 cats (72.6%) with uveitis and 19 of 19 (100%) of the normal shelter cats. *Bartonella* spp. antibodies were detected in aqueous humor of 20 of 84 cats (23.8%) with uveitis and 0 of 19 (0%) of the normal shelter cats. Based on the criteria described, 10 of 84 cats (11.9%) with uveitis had evidence of intraocular production of antibodies against *Bartonella* spp.

The results of this study suggest that Western blot analysis on paired serum and aqueous humor samples from cats with uveitis may aid in the diagnosis of ocular bartonellosis. Whether these test results can be used to predict clinical response to appropriate medical therapy remains to be proven.

ABSTRACT #304
EFFECT OF ORBIFLOXACIN OR DOXYCYCLINE ADMINISTRATION ON *BARTONELLA* SPP. AND HEMOPLASMA TEST RESULTS FROM NATURALLY-EXPOSED CATS. W Miller, AK Morris, M Lappin. Department of Clinical Sciences Colorado State University, Fort Collins, CO.

Bartonella spp. and hemoplasmas are differential diagnoses for cats with fever. Little is known concerning the effects of antibiotic treatment on clinical responses or diagnostic test results in naturally-exposed cats. The purpose of this study was to monitor clinical

signs, *Bartonella* spp. IgG titers, and PCR assay results for *Bartonella* spp. and hemoplasmas after antibiotic treatment.

Veterinary clinics in states considered high risk for *Ctenocephalides felis* infestation (TX, FL) participated. Adult cats with a body temperature of > 102.5F without an obvious cause on physical examination were eligible for the study. All cats were assessed by complete blood cell count, FeLV antigen assay, FIV antibody assay, *Bartonella* spp. IgG ELISA, and PCR assays that amplify DNA of *Bartonella* spp., *Mycoplasma haemofelis*, '*Candidatus* M. haemominutum', and '*Candidatus* M. turicensis.' Each cat was administered doxycycline (50 mg, PO, daily for 28 days) or orbifloxacin (22.7 mg, PO, daily for 28 days). Owners were requested to return for followup testing one week and four weeks after finishing antibiotic treatment.

Of the 18 cats entered to date, 15 were administered the entire treatment course and both followup testing and clinical information were available. All cats were negative for FeLV and FIV but 14 had laboratory evidence of *Bartonella* spp. or hemoplasma infection. On day 0, three cats were *Bartonella* PCR +, *Bartonella* IgG +, five cats were *Bartonella* PCR +, *Bartonella* IgG -, five cats were *Bartonella* PCR -, *Bartonella* IgG +, and two cats were negative in both tests (one of these cats seroconverted by day 35). DNA of *M. haemofelis* (one cat) and '*Candidatus* M. haemominutum' (two cats) were amplified from some cats with concurrent evidence of *Bartonella* spp. infection. Clinical signs resolved within days in seven of nine cats (77.8%) administered orbifloxacin and five of six cats (83.3%) administered doxycycline. Two of eight cats with *Bartonella* PCR positive results became negative (one orbifloxacin and one doxycycline). One of eight cats with *Bartonella* spp. IgG in serum became negative (orbifloxacin). Two of three haemoplasma spp. infected cats became negative (one orbifloxacin and one doxycycline).

The combination of serology and PCR assay results is needed to provide the most information concerning the *Bartonella* infection status of individual cats. If these cats were clinically ill from *Bartonella* or hemoplasma spp. infections, both orbifloxacin and doxycycline had similar clinical effects. Administration of these antibiotic protocols does not consistently eliminate *Bartonella* spp. or hemoplasma spp. DNA from the blood of cats.

ABSTRACT #305

Abstract Withdrawn

ABSTRACT #306

ALPHA ENOLASE AND ANNEXIN A2 ANTIBODIES IN CATS. J Whittemore[1], JR Hawley[2], MR Lappin[2]. [1]University of Tennessee, Knoxville, TN. [2]Colorado State University, Fort Collins, CO.

Feline herpesvirus 1, calicivirus, and panleukopenia (FVRCP) vaccine antigens are grown on cell lines. In previous studies, we had shown that cats inoculated with FVRCP vaccines grown on the Crandell-Reese feline kidney (CRFK) cell line developed anti-CRFK antibodies that also cross-reacted with feline renal cell extracts. In addition, some cats that were hypersensitized with CRFK proteins developed interstitial nephritis. By use of immunoprecipitation and protein sequencing, we showed the immunodominant antigens recognized by vaccinated cats or CRFK hypersensitized cats to be alpha-enolase or annexin A2. Spontaneously occurring antibodies to these two enzymes are associated with a variety of autoimmune disorders in people. The purpose of this study was to validate ELISA for detection of anti-alpha enolase and anti-annexin A2 antibodies in cats.

The protein structures of alpha-enolase and annexin A2 are thought to be conserved among species. Commercially available alpha-enolase and annexin A2 were purchased and used as the antigens in indirect ELISAs. Checkerboard titrations using different microELISA plates, binding buffers, antigen concentrations, and secondary antibody concentrations were performed in preliminary experiments. Using serum from vaccinated cats and CRFK hypersensitized cats assayed in six different ELISAs, the coefficient of variation for the optimized alpha-enolase and annexin A2 ELISAs were shown to be 9.7% and 10.1%, respectively. It was determined that a %ELISA value of > 10% was positive for both alpha-enolase and annexin A2 antibodies. To determine whether alpha-enolase and annexin A2 antibodies develop in FVRCP vaccinated cats, serum was collected from young adult cats housed in a SPF facility without vaccination (n=20) and young adult cats (n=20) three weeks after the administration of three doses of a commercially available modified live FVRCP vaccine.

While only one of the 20 SPF cats (5%) without FVRCP vaccination developed an alpha-enolase %ELISA value > 10%, 11 of the 20 cats (55%) of FVRCP vaccinated cats were positive (p=0.0012). While only one of the 20 SPF cats (5%) without FVRCP vaccination developed an annexin A2 %ELISA value > 10%, 13 of the 20 cats (65%) of FVRCP vaccinated cats were positive (p=0.0001).

Results of this study document that cats administered this FVRCP vaccine developed antibodies against alpha-enolase and annexin A2. Whether these antibodies are associated with clinical diseases in cats remains to be proven.

ABSTRACT #307

PREVALENCE OF FELINE IMMUNODEFFICIENCY VIRUS ANTIBODY AND FELINE LEUKEMIA VIRUS ANTIGEN IN SAMPLES SUBMITTED TO IDEXX REFERENCE LABORATORIES FOR FELINE HEMOTROPIC MYCOPLASMA TESTING. K. Curtis, C. Leutenegger, R Chandrashekar. IDEXX Laboratories, Westbrook, ME.

Three feline hemotropic mycoplasma (FHM) species are the causative agents for infectious anemia in cats; *Mycoplasma haemofelis* (MHf), *Candidatus Mycoplasma haemominutum* (CMh) and *Candidatus Mycoplasma turicensis* (CMt). Studies have indicated old age, male gender and outdoor activity as risk factors for FHM infection. FHM infection has also been found to be associated with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infection. The aim of this study was to evaluate the prevalence of FIV and FeLV infection in cats with suspected cases of hemoplasmosis.

Samples from anemic cats (n=185) submitted to the IDEXX Reference Laboratories for FHM testing by polymerase chain reaction (PCR) were obtained for this evaluation. FIV and FeLV testing was performed using the SNAP® Feline Triple™. In addition, reference laboratory data was obtained for each sample where available. FHM positive PCR results were obtained for 44 (23.8%) of the 185 samples. CMt infection was limited to cats co-infected with CMh; co-infection with MHf and CMh was found in 6 of the 44 FHM positive samples. FHM positive cats ranged in age from 7 months to 16 years (median of 7 years). FIV and FeLV infections were prevalent in these FHM suspect cases, 14/185 (7.6%) and 41/185 (22.2%) respectively. More than a third of the FHM positive cats were co-infected with FIV and/or FeLV.

FHM		FIV Positive	FeLV Positive
Positive	44	8	11
Negative	141	6	30

The findings in this study are consistent with previous reports suggesting an association between FHM and retrovirus infection in cats. Because of this association and the potential for similar symptoms, testing for retrovirus as well as FHM is recommended in cases where infectious anemia is suspected.

ABSTRACT #308

AN UPDATED EPIDEMIOLOGICAL SURVEY OF FELINE IMMUNODEFICIENCY VIRUS INFECTION IN JAPAN. Y Nakamura[1], Y Nakamura[1], A Ura[1], M Hirata[1], K Nishigaki[2], H Tsujimoto[3], A Setoguchi-Mukai[1] and Y Endo[1]. [1]Kagoshima University, Kagoshima, Japan. [2]Yamaguchi University, Yamaguchi, Japan. [3]University of Tokyo, Tokyo, Japan.

Feline immunodeficiency virus (FIV) is distributed in domestic cats worldwide. The prevalence of FIV is thought to range from approximately 10 to 30%. The subtypes A, B, C and D of FIV are present in Japan. An epidemiological and molecular based survey covering all over Japan had not been undertaken since an

FIV vaccine was introduced to Japan in 2008. In this study, we evaluated the prevalence and distribution of subtypes of FIV in Japan.

Blood samples were collected from cats admitted at 47 hospitals located in each prefecture from March to October in 2008. Cats venturing outside at least once a week were included, however cats stating completely indoors were excluded. Age, gender and chief complaint were recorded at each hospital. The status of FIV infection was confirmed using two methods; a serological test using a SNAP FeLV/FIV combo kit (IDEXX Laboratories) for detection of anti-FIV antibody, and polymerase chain reaction for detection of *env* (V3-V5) and *gag* regions of FIV proviral DNA. The nucleotide sequence of amplified FIV *env*-derived DNA fragments were determined by direct sequencing and phylogenetic analysis was performed by a Neighbor-Joining method to estimate subtypes of FIV.

1,770 cats were finally included in this study; 411 (23.3%) were sero-positive. All kittens less than 6-month old were sero-negative for FIV; however, juvenile cats (0.5–2 years old) revealed 3.4–14.4% prevalence. Adult cats showed a high infection rate of approximately 28–30%. Male cats constituted 71.1% of all sero-positive animals. The infection rate in 716 cats with a history of fighting wounds was 34.9% while that in cats without any history was 15.0%. Infection rates in cats with or without any clinical signs were 28.9% and 12.6%, respectively. Proviral DNA was detected in 404 cases of the 411 sero-positive cats. In addition, nineteen sero-negative cats were positive by PCR analysis. The concordance between SNAP and PCR tests was 98.5%. DNA fragments of the FIV *env* V3-V5 region isolated from 347 cases proceeded to phylogenetic analysis. This analysis revealed that four subtypes (A, B, C and D) of FIV were still distributed in Japan. Regional characteristics were also observed: Subtype A in Hokkaido, Chugoku, Shikoku and southern Kyushu; Subtype B in the Tohoku and Kanto areas, Subtype C in mid-Japan; and Subtype D in the area Kinki and northern Kyushu. In addition, subtype D viruses were possibly divided into two groups, classic D and varied D. The varied type virus was mainly distributed in the Hokuriku area. Limited information about subtype C virus in Japan has previously been available; however, a number of subtype C virus cases were detected in this study.

We identified that FIV is epidemic in Japanese cats, especially in outdoor accessing cats. Even though an FIV vaccine was introduced in Japan, close attention still has to be paid to epidemic and genotypic trends of FIV.

ABSTRACT #309

EVALUATION OF CD4+ AND CD8+ T LYMPHOCYTES IN CATS NATURALLY INFECTED WITH FELINE LEUKEMIA VIRUS

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Feline leukemia virus (FeLV) is known as a cause of immune dysfunction and opportunistic infections in naturally and experimentally infected cats. Humoral and cell-mediated immune response abnormalities have been reported by many investigators, implicating in particular T-cell alterations. Cats exposed to FeLV may either become persistently viremic or recover from infection. Viremic cats showed little evidence of an immune response to the virus, and are at very high risk of developing a fatal FeLV-related disease within 2 to 4 years. The pathogenesis of the immune deficit caused by FeLV, however, remains incompletely understood. Changes in T-cell subpopulations in FIV-infected cats, especially decreased CD4+/CD8+ ratios have been reported to be similar to those occurring in HIV infection.

As FeLV infection is known to induce lymphopenia and there is little information on changes in lymphocyte subpopulations, T-cells subpopulations of FeLV infected cats were phenotyped and compared to FeLV-non infected cats in this particular study.

Eleven adult cats (7 male and 4 female, mean age –2.7 yrs) were identified as persistently infected with FeLV (IFA test for FeLV antigen). All cats with exception of one had presented chronic upper respiratory disease, and two of them, also had an associated severe gingivitis. Sixteen healthy adult cats (7 male and 9 female; mean age – 4.7 yrs) sharing the same household were negative for FeLV test. Neither one was infected with feline immunodeficiency virus (FIV). Blood from those cats was collected for CBC and lymphocytes phenotyping in a tube with EDTA–K3 (Vacutainer®). An additional twenty five young adult cats were included as FeLV-non infected controls. Absolute leukocyte counting was done on an authomatized blood analyser (ABC-Vet, ABX Diagnostics, Fr) and lymphocytes subsets were analysed by flow cytometry (FACScan; Becton Dickinson; Mountain View, CA). Mouse anti feline CD4: FITC mAb (Serotec MCA 1346F) and mouse anti-feline CD8+ mAb (Serotec MCA 1347PE) were used for lymphocytes phenotyping. The non parametric Mann-Whitney test was performed to compare both groups.

Decreased lymphocyte cells (2556.01±1697.76 and 6249.93±3666.70 lymphocytes/μL; $p=0.03$), T-CD4+ lymphocytes (585.73±543.46 and 1648.78±11. 86/μL; $p<0.001$), T-CD8+ lymphocytes (396.33±690.78 and 690.78±461.93/μL, $p=0.039$) and CD4+/CD8+ ratio (2.31 and 3.03; $p=0.018$) were observed among FeLV-infected cats compared to non-infected cats. The resulting immunodeficiency might be responsible for chronic upper respiratory infection found in almost all of FeLV-infected cats. It is concluded that a decreased CD4+/CD8+ ratio occurs in FeLV infection and evaluation of CD4+ T lymphocytes might be an useful tool to assess the prognosis of the infection.

ABSTRACT #310

CUTANEOUS MYCOFLORA AND CD4+:CD8+ RATIO OF CATS INFECTED WITH FELINE IMMUNODEFICIENCY VIRUS

A Reche Junior, AGT Daniel, TCPL Strauss, CP Taborda, SAV Marques, K Haipek, LJ Oliveira, JM Monteiro, JR Kfoury Junior. School of Veterinary Medicine – University of São Paulo, Brazil.

Opportunistic mycoses occur mainly when immunocompromising conditions are present. FIV-infected cats show a progressive depletion of CD4+ lymphocytes and other immunologic abnormalities. Considering that FIV-infected cats may have a higher isolation rate of fungal flora than FIV-uninfected cats.

The purpose of the present study was to compare cutaneous mycoflora isolation and CD4+:CD8+ ratio in FIV-infected cats with that in FIV-uninfected cats.

Sixty client-owned cats were examined. Twenty-five were FIV-infected cats and 35 were FIV-uninfected cats. All the 60 cats were FeLV-negative. They were matched as closely as possible for age, sex, breed and lifestyle. None of the 60 cats had dermatologic problems at the moment of specimens collection.

Animals were tested for the retroviruses with a commercially available ELISA test kit. Cutaneous specimens were obtained from the cat coat by rubbing the whole hair with a sterile piece of carpet. Fungi were isolated from specimens using Sabouraud dextrose agar incubated at 27 °C. Immunophenotyping of peripheral CD4+ and CD8+ T lymphocytes was done by flow cytometry.

Statistical analyses were done by one-way ANOVA and Tukey-Kramer multiple comparisons test. A P value <0.05 was considered significant. Results are presented as means±standard deviation (SD) where appropriate.

Fungal colonies were isolated from at least one specimen from 22 of 25 (88%) FIV-infected cats. Among the FIV-uninfected cats fungal colonies were recovered from 13 of 35 (37%) specimens. Dermatophytes were recovered from 2 of 25(8%) FIV-infected cats, from one coat specimen was isolated *Microsporum canis* and from the other *Microsporum gypseum*. Among the FIV-uninfected cats the only dermatophyte isolated was *Microsporum gypseum* in 3 out of 35 (8.5%) cats. *Malassezia* spp was the most commonly isolated organism overall (85.7%). *Malassezia* spp was more commonly isolated from FIV-infected cats, compared with FIV-uninfected cats (68.8% vs 28.6%).

The CD4+ to CD8+ lymphocyte ratio for FIV-infected cats was significantly less than the CD4+ to CD8+ ratio in the FIV-uninfected cats (0.328±0.222 and 1.250±0.507, respectively; $P<0.01$). The CD4+ to CD8+ lymphocyte ratio for FIV-infected cats with cutaneous overall fungal isolation was significantly less than the CD4+ to CD8+ lymphocyte ratio in the FIV-infected cats but without cutaneous fungal isolation (0.187±0.044 and 0.683±0.085, respectively; $P<0.01$). We can conclude that immunologic depletion due to retroviral infection might represent a risk factor to cutaneous fungal infection in cats.

ABSTRACT #311
PREVALENCE OF FELINE CALICIVIRUS AND HERPESVIRUS TYPE 1 IN MAINE COON CATS WITH CHRONIC GINGIVITIS. A Reche Júnior, AGT Daniel, CA Geraldo Júnior, MVC Albino, MN Arena, A Pellegrino, BM Teixeira, PE Brandão. School of Veterinary Medicine – University of São Paulo, Brazil.

Inflammatory diseases of feline oral cavity are extremely common in daily clinical practice. Factors that may contribute to the development of gingivitis and stomatitis are: diet, oral conformation, specific breed characteristics (juvenile gingivitis of Maine Coons), immune-mediated diseases and infectious diseases, like FIV, FeLV, feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1). The Maine Coon breed seems to be predisposed to develop chronic gingivitis. The aim of this study was to investigate the prevalence and correlation of the FCV and FHV-1 in Maine Coons with chronic gingivitis.

Seventy-two client-owned cats were placed in one of four groups: group I (n=22) – Maine Coons with chronic gingivitis; group II (n=23) – Maine Coons without oral disease; group III (n=10) – Other breeds [domestic shorthair, Bengal, Norwegian forest cat, Ragdoll] with chronic gingivitis and group IV (n=17) other breeds without oral disease. These animals were matched as closely as possible for age, sex and lifestyle. All cats of all groups were negative for FIV and FeLV. Oral samples were collected with a sterile swab and RT-PCR was performed to search for FCV and PCR for FHV-1. Fisher's Exact Test was used to statistic analyze. A P value <0.05 was considered significant. Results are presented as percentage of presence of virus.

Seventeen cats (77.2%) of group I, two cats (8%) of group II, none cat in group III and one cat (6%) of group IV were positive for FCV. Only two cats of group IV were positive for FHV-1. Statistically significant difference was obtained ($P<0.0001$) between group I and all other groups, for the presence of FCV. No statistically significant difference was obtained for FHV-1. Maine Coon cats of group I showed significant prevalence of FCV in gingival tissue, suggesting that this virus might have some influence on the chronic gingivitis in this breed. Although other researches reinforce the presence of the FCV in the pathogenesis of gingivitis, in this study the virus was found predominantly in Maine Coon cats with gingivitis. Considering that most of the Maine Coons used in the present study were born in catteries and FCV infection is usually a common problem among these cats, the results of the present study may reflect only differences in the environment rather than a Maine Coon predisposition to FCV infection. Although many of the other cats, including the Maine Coons without gingivitis were also born in catteries and did not show the same prevalence for FCV infection. Further studies are necessary to investigate the role of FCV on pathogenesis of chronic gingivitis in Maine Coon cats.

ABSTRACT #312
DISEASE PREVALENCE AND CAUSES OF DEATH IN AMERICAN KENNEL CLUB REGISTERED GREYHOUNDS. S Zaldivar, LM Marin, H Hamilton, CG Couto. The Ohio State University College of Veterinary Medicine, Columbus, OH.

Recently, we reported the prevalence of diseases and causes of death in retired racing Greyhounds (RRGs) using a web-based survey. Some of the diseases we see in RRGs appear to be uncommon in American Kennel Club (AKC) Greyhounds, and viceversa. The objective of the current study was to obtain comparable information for AKC Greyhounds, and compare the most relevant pathologies in both groups.

The data were collected through a survey posted online. The AKC announced the study and the owners/breeders completed the survey voluntarily; 214 responses were obtained.

Only 211 questionnaires were analyzed; 3 dogs were excluded because they were RRGs. Forty dogs were dead (19%) at the time of the study. The main cause of death was cancer (7 dogs, 17.5%). The most commonly reported group of diseases were dental (42, 20.2%), digestive (32, 15.5%), and skin (22, 10.5%). On the other hand, the most frequent disorders in RRGs were skeletal (33%), skin (28%), and digestive (18%).

Comparing these results with the data previously reported, we can conclude that in general the distribution of diseases in AKC Greyhounds is different than in retired racing Greyhounds. Cancer was the most common cause of death in both groups, and osteosarcoma (OSA) was the main tumor type found exclusively in retired racers (no AKC Greyhounds had OSA). Behavioral disorders were less frequent in the AKC group. The different results obtained could be explained by a progression of this breed towards two different genetic lines or changes due to environmental factors.

ABSTRACT #313
THROMBOELASTOGRAPHIC CHANGES AFTER GONADECTOMY IN RETIRED RACING GREYHOUNDS. P Vilar, N Westendorf, MC Iazbik, L Marín, MA McLoughlin, and C. G Couto. The Ohio State University, Veterinary Clinical Sciences, Columbus, OH.

Postoperative bleeding occurs in 26% of retired racing Greyhounds (RRG) that are spayed or neutered (Lara A et al. 2008). Identifying the patients at risk for prevention of this complication will be extremely valuable. Thromboelastography (TEG®) allows for a global analysis of the hemostatic system providing information about the primary and secondary hemostasis, and the fibrinolytic system. The objective of this study was to evaluate perioperative hemostatic features in RRGs using TEG®, and determine whether we could predict bleeding in Greyhounds that undergo surgical procedures. We evaluated 21 healthy Greyhounds (11 females and 10 males); 8 were classified as "Bleeders" and 13 as "Non-Bleeders" based on a bleeding scoring system we recently validated. Blood samples obtained via jugular venipuncture were collected into one 2.7 ml Vacutainer 1:9 with 3.2% buffered sodium citrate collection tube, and TEG® test were done using citrated native technique as previously described. There were no statistical differences ($p>0.05$) preoperatively between "Bleeders" and "Non-Bleeders" for TEG® parameters, fibrinogen concentration ($p=0.7465$), platelet count ($p=0.673$), or hemoglobin concentration ($p=0.1853$). In the "Non-Bleeders", the "MA" ($p=0.0017$) and "G" ($p=0.0047$) increased significantly after surgery. In contrast, in the "Bleeders" the "R" ($p=0.0315$) and angle ($p=0.0444$) were significantly prolonged and decreased, respectively after surgery. In addition, there were no significant changes in "MA" ($p=0.0873$) or "G" ($p=0.0959$) from the presurgery values. "Non-Bleeder" TEG® values after surgery suggest the formation a stronger clot and a decrease in fibrinolysis, an expected response to surgery also described in humans (Okamura K et al; 2007). "Bleeder" TEG® values after surgery supports the theory of slower clot formation and reduced clot strength, thus failing to reach a reactive hypercoagulable postsurgical state and contributing to the bleeding observed in the breed. Preoperative TEG® failed to predict bleeding in RRG that undergo surgical procedures.

ABSTRACT #314
IMMUNOLOGICAL RESPONSES IN PERSISTENTLY BOVINE VIRAL DIARRHEA VIRUS (BVDV) INFECTED ALPACAS. D Bedenice[a], W Davis[b], MJ Hamilton[b], A Grimm[b], E Dubovi[c], L Costa[a]. [a]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA; [b]Washington State University, Pullman, WA; [c]Cornell Diagnostic Laboratory, Ithaca, NY.

The objective of this study was to characterize the effect of chronic and persistent BVDV infection in alpacas on immunological function.

Baseline flow cytometry and serum protein electrophoresis were performed on 9 persistently infected (PI), 16 age-matched control (BVDV PCR negative) and 2 chronically BVD infected alpacas. Chronic infection (CI) was defined by a positive BVD PCR for >3 weeks in the face of rising serum neutralizing BVDV antibodies. To further assess the functional activity of peripheral blood mononuclear cells [PBMC] in four PI, two CI and four control animals, cultured PBMC were separately stimulated with concanavalin A (ConA), phorbol myristate acetate (PMA), phytohemagglutinin (PHA) and pokeweed mitogen (PWM) for 3 days. Cells were subsequently collected and labeled for flow cytometry. Analyses were repeated at two separate time points for all animals. Overall results were reported as mean (±standard deviation) and analyzed via independent samples T-test ($P\leq0.05$).

Significant anemia and hypoproteinemia (reduced albumin but increased $\alpha2$ globulin fraction), a reduction in T-lymphocytes (CD

4) and relative monocytosis were evident in PI compared to healthy control animals. Furthermore, monocyte, B lymphocyte and γΔ T cell proliferation were observed in mitogen stimulated cells. Superior stimulation of B cells was achieved using PWM (71–144%). PI alpacas consistently showed greater lymphocyte proliferation than control animals when stimulated with ConA, PHA and PWM.

Despite absolute reduction in peripheral T cells, PBMC from persistently BVDV infected alpacas demonstrated comparable, if not superior proliferation in response to in vitro mitogen stimulation.

ABSTRACT #315
HUMORAL RESPONSE TO BLUETONGUE VACCINATION IN ALPACAS. CE Whitehead[1], CA Batten[2], J Brownlie[1], B Jackson[1]. [1]The Royal Veterinary College, London, UK. [2]Institute for Animal Health, Pirbright, UK.

Bluetongue virus (serotype 8) was first documented in the UK in September 2007. This study evaluates a new vaccine (Bovilis® BTV8, Intervet) in alpacas. Three groups of alpacas were enrolled in a field trial: Group 1 (10 alpacas) received one dose of vaccine only; Group 2 (10 alpacas) received two doses, 3 weeks apart; Group 3 (6 alpacas, control group) received no vaccine. All alpacas were healthy, adult male and non-pregnant female alpacas kept at pasture. Blood samples were collected at Days 0, 21 (prior to vaccination), and 42. All samples underwent routine hematology and biochemistry, and ELISA and PCR testing for presence of antibody and virus respectively. Seropositive samples were tested for presence of neutralizing antibodies by serum neutralisation (SNT). Soft tissue reactions to the vaccine were common, and one alpaca exhibited transient lameness. All alpacas were seronegative at the start of the study and were negative for virus throughout the period of study. Control alpacas were negative for antibody and virus throughout. After one dose of vaccine (day 21), only 7/20 alpacas were seropositive. At day 42, Group 2 alpacas were all seropositive, had significantly greater percentage inhibition (PI) on ELISA compared with Group 1 alpacas ($p<0.0001$), and significantly higher serum neutralization titres ($p=0.002$). This study suggests that the vaccine appears to be safe in healthy alpacas and that it can stimulate an antibody response in alpacas. Further, this study suggests that two doses given 3 weeks apart is an acceptable vaccination strategy in alpacas.

ABSTRACT #316
INCIDENCE OF *CLOSTRIDIUM DIFFICILE* IN FECES OF MALE HOLSTEIN VEAL CALVES. MC Costa, LG Arroyo, DL Pearl, JS Weese, H Staempfli. Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Neonatal calf diarrhea (NCD) is a multifactorial disease complex causing loss to the bovine industry. Recently, *Clostridium difficile* has been identified as potential additional cause for NCD in dairy calves. *Clostridium difficile* has been found in retail beef samples and was associated with sudden death in a group of veal calves. Very little information is available about *C. difficile* colonization in young calves. The objective of this study was to monitor longitudinally *C. difficile* colonization in a group of Holstein male calves reared on a milk replacer diet for meat on one farm.

For this study, 163 male Holstein-Friesen calves originating from several different farms were housed either individually ($n=70$) or in group pens ($n=93$). Calves were medicated with oral oxytetracycline for five days after arrival and were fed a complete milk replacer diet. Fecal samples were obtained by rectal swab within 48 hours post arrival and again six days after the first sampling. Several swabs were taken from the environment at the moment of the calves' arrival. Selective culture for *C. difficile* was performed, and isolates were characterized using standard methods.

C. difficile was isolated initially from 22/70 (34%) individually housed and in 42/69 (61%) 6 days later ($P<0.001$). Similarly, *C. difficile* was isolated from 31/93 (33%) group housed calves initially and in 46/93 (49%) 6 days later ($P=0.037$). Overall, *C. difficile* was isolated from 53/163 (33%) calves initially and from 88/162 (54%) at the second sampling. There was no difference in the prevalence of colonization between management types at either sampling point ($P=0.87$ and 0.16 respectively). 14 (45%) of group housed calves and 7 (32%) of individually housed calves that were initially positive were negative at the 2nd sampling. No *C. difficile* was grown from any of the 7 samples taken from the environment at the first sampling.

In conclusion the colonization rate of calves in this study was high, even at admission from different source farms and increased significantly shortly after arrival. Comingling, stress and antimicrobial therapy could have contributed to the high incidence. Virtually all calves had some degree of diarrhea at the time of the second sampling. Recently, toxigenic strains of *C. difficile* have been positively associated with diarrhea in dairy calves. Despite the significant increase in the prevalence of the bacterium concomitant with the increase in diarrhea, molecular analysis would be necessary to establish a clear association. Evaluation of typing data will provide additional insight into the epidemiology of *C. difficile* in calves reared in high-density environments and its' role in NCD.

ABSTRACT #317
PRAIRIE RATTLESNAKE ENVENOMATION IN 25 NEW WORLD CAMELIDS. JM Sonis, ES Hackett, RJ Callan, TB Hackett. Colorado State University Department of Clinical Sciences, Fort Collins, CO.

Morbidity and mortality from rattlesnake envenomation is regionally specific due to variability in relative toxicity of the species of snake encountered. A previous report of rattlesnake envenomation in New World Camelids from the western coastal United States documented high mortality rates and guarded prognosis for survival. The purpose of this study was to describe clinical findings, treatments, and outcome of New World Camelids following envenomation in the Rocky Mountain region of the United States by Prairie rattlesnakes (*Crotalus viridis viridis*). We hypothesized that Prairie rattlesnake envenomation of New World Camelids would result in a higher survival rate than that reported for rattlesnakes in other regions, and that the survival rate would more closely approximate that for Prairie rattlesnake envenomation in other mammalian species.

Records of New World Camelids evaluated for rattlesnake envenomation from 1992 to 2007 were reviewed retrospectively. Signalment, area of bite, clinical signs, clinicopathologic data, treatment, and outcome were recorded. Continuous data were reported as mean and 95% confidence interval. Fisher's exact test was used to evaluate for an association between platelet count, and treatments of steroids, NSAIDS, and commercial antivenin on outcome. Logistic regression was used to evaluate for an association between age, days of hospitalization, and outcome. For all comparisons, significance was set at a value of $p<0.05$.

Twenty-five New World Camelids experienced envenomation in this time period, consisting of 24 Llamas and 1 Alpaca. Llamas were overrepresented compared to hospital caseload. Mean age of envenomation was 3.8 years [95%CI: 2.8–4.9 years]. Presenting clinical signs included respiratory distress (7), fever (12), tachycardia (10), and tachypnea (12). The face was the most common site of envenomation (20 of 21). Most camelids were treated with intravenous fluids, antibiotic therapy, and NSAIDS. Eight animals required tracheotomy to maintain airway and 8 were treated with supplemental oxygen. Mean hospitalization was 2.4 days [95%CI: 1.4–3.4 days]. Overall survival rate was 72%. Age did not significantly impact outcome. Of the treatments evaluated, only commercial antivenin administration was protective for survival.

In conclusion, mortality rate for prairie rattlesnake envenomation in New World Camelids was lower than that reported in other regions of the United States and similar to that reported for prairie rattlesnake envenomation in horses. Commercial antivenin treatment should be considered for prairie rattlesnake envenomation in this species and may favorably impact outcome.

ABSTRACT #318
CONCENTRATIONS OF CARDIAC TROPONIN I MEASURED WITH AN I-STAT®1 ANALYZER IN NORMAL HORSES UNDERGOING A STANDARD TREADMILL PERFORMANCE EXAMINATION. MS Kraus, TG Divers, DV Nydam, SA Jesty, AR Gelzer, ND Ducharme. Cornell University, Ithaca, NY.

Elevated serum concentration of cardiac troponin I (cTnI) is a biomarker for myocardial damage in horses. Treadmill exercise is a

common method for evaluation of horses with poor performance, but detection of occult heart disease is difficult. Treadmill exercise may induce increased cTnI concentrations, thereby unmasking myocardial disease. We hypothesize that in healthy horses, treadmill exercise does not induce cTnI concentrations above the normal range. Our objective was to determine cTnI concentrations in normal horses undergoing a standardized treadmill performance examination.

Eleven healthy horses (8 Thoroughbreds, 2 Standardbreds and 1 Warmblood) were exercised using a stepwise incremental treadmill protocol. Blood samples for cTnI were taken prior to exercise and at 5, 60, 180 minutes, and 24 post exercise and then daily for 5 days post exercise. Heparinized plasma samples were frozen at −20°C and analyzed for cTnI concentration utilizing the (i-STAT®1, Heska Corporation). Within each horse the change in cTnI over time was assessed using linear regression. The regression coefficients were then compared to baseline levels of cTnI using a 1 sample Wilcoxon rank test. The p-value was 0.61, indicating that there was no significant change in cTnI concentration over time in these 11 horses. Clinical significance of this study is that cTnI concentrations are not increased in clinically healthy horses undergoing a standardized treadmill exam. Thus, cTnI concentrations above the reference range measured in a horse at any time point following similar treadmill exercise should be considered abnormal.

ABSTRACT #319
THE EFFECTS OF DEHYDRATION ON CENTRAL VENOUS PRESSURE IN ADULT HORSES. RD Nolen-Walston, JL Norton, R Boston, C Underwood, J Slack, BL Dallap. New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.

Central venous pressure (CVP) is used in many species to monitor right-sided intravascular volume status especially in critical care medicine. Our hypothesis was that dehydration in adult horses is associated with a proportional reduction in CVP. Ten healthy adult horses underwent central venous catheter placement via the right jugular vein, and CVP readings were obtained using water manometry. The horses were then deprived of water and administered furosemide (1 mg/kg IV QID) for up to 36 hours. Weight, CVP, vital signs, PCV, total solids, and serum lactate were monitored at baseline and every 6 hours until a target of 5% dehydration was achieved. Linear regression analysis was used to assess the association of CVP and other clinical parameters with degree of dehydration over time. A value of $P<0.05$ was used to separate statistical differences from those due to the causes explored in this investigation. A significant association was found between CVP and percent dehydration ($P<0.001$), with a decrease in CVP of 2.2 cmH_2O for every percentage point increase in dehydration. Other significant associations between dehydration and parameters measured included increased total protein ($P=0.007$) and increased serum lactate concentration ($P=0.048$). There was no significant association between CVP and heart rate, respiratory rate, rectal temperature, or PCV. In conclusion, there is a strong association between central venous pressure and the degree of dehydration caused by diuretic use and water deprivation. These findings suggest that CVP monitoring may be a useful addition to the clinical evaluation of hydration status in adult horses.

ABSTRACT #320
COMPARISON OF WATER MANOMETRY TO TWO ELECTRONIC PRESSURE MONITORS FOR CENTRAL VENOUS PRESSURE MEASUREMENT IN HORSES. JL Norton, C Underwood, J Slack, BL Dallap, RD Nolen-Walston. New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.

Central venous pressure (CVP) has customarily been measured in veterinary patients using water manometry. However, many institutions are now using bedside/stallside electronic monitors in both anesthesia and intensive care units for many aspects of patient monitoring. The aim of this study was to evaluate the agreement of CVP values obtained using water manometry and two commercially available bedside monitoring devices (Datascope Passport LT™ and Medtronic Lifepak 12™). Central venous catheters were placed routinely in 10 healthy adult horses. Measurement sets were taken every 12 hours over the course of 3 days. At each time point, three measurements were obtained with each of the three methods. Data were analyzed using Bland-Altman limits of agreement, with all devices compared pairwise. Compared to water manometry, agreement (bias) of the Passport was −1.94 cmH_2O (limits of agreement, −8.54 to 4.66 cmH_2O) and of the Medtronic was −1.83 cmH_2O (limits of agreement, −8.60 to 4.94 cmH_2O). When compared to the Passport, agreement of the data obtained with the Medtronic was 0.27 cmH_2O (limits of agreement, −4.39 to 4.93 cmH_2O). These data show that both electronic monitors systematically provide measurements that are approximately 2 cmH_2O lower than water manometry; however, differences between the two electronic devices are small enough (less than 0.5 cmH_2O) to be considered clinically insignificant. This discrepancy should be taken into account when interpreting data obtained with these monitoring devices. Although water manometry has typically been considered the gold standard technique, this study does not identify which of these methods is more accurate.

ABSTRACT #321
REPEATABILITY AND EFFECT OF HEAD POSITION ON CENTRAL VENOUS PRESSURE MEASUREMENT IN STANDING ADULT HORSES. JL Norton, R Boston, C Underwood, J Slack, BL Dallap, RD Nolen-Walston. New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.

Central venous pressure (CVP) measurements are typically obtained from equine patients using water manometry, but the reliability of these measurements and the effect of changes in head elevation have not been established. In humans, variation in CVP is seen when the patient moves from supine to standing position. We hypothesized that, in the horse, alteration in head position relative to the heart would significantly alter CVP. Jugular CVP catheters were placed in 10 healthy adult horses and measurements were obtained every twelve hours for three days. Readings were taken in triplicate ($n=498$) at three different head positions: neutral (muzzle level with point of shoulder), elevated (muzzle at the level of the withers) and lowered (muzzle on the ground). Data were analyzed using mixed effect modeling, with significance set at $P<0.05$. Mean CVP in the neutral head position was 9.4±3.6 cmH_2O. In regards to repeatability, variation in the "neutral" measurements obtained at each time point was minimal (2%), as compared to variation as a function of day and subject. No significant difference ($P>0.4$) was demonstrated between repetitions at any time point. Head height had a significant and directional effect on CVP. The elevated head position decreased CVP by a mean of −2.0±6.5 cmH_2O ($P<0.001$) while the lowered head position increased CVP by 3.7±5.5 cmH_2O ($P<0.001$). In conclusion, CVP values obtained using water manometry are highly repeatable in adult horses, provided head position is standardized to prevent inconsistent readings.

ABSTRACT #322
CARDIAC TROPONIN I CONCENTRATIONS IN HORSES REFERRED FOR COLIC. OS Diaz, MM Durando, EK Birks, VB Reef. University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA.

Cardiac dysrhythmias are observed in horses hospitalized with colic. Endotoxemia, hypovolemia, electrolyte and acid-base disturbances commonly associated with colic may predispose these horses to myocardial damage and rhythm disturbances. This study was performed to determine if a relationship exists between a measure of myocardial damage, plasma cardiac troponin I (cTnI), and outcome (survival/non-survival), treatment (medical/surgical), or the presence of dysrhythmias.

111 horses presented to the University of Pennsylvania Large Animal Hospital with a complaint of colic were included. Blood for cTnI was drawn at admission, and 12 and 24 hours post admission or surgery, for medical or surgical colics, respectively. A 24-hour ambulatory ECG was placed the morning after admission (medical colics) or surgery to record cardiac rhythm. Medical records were reviewed to obtain clinical and clinico-pathologic data and outcome. Data at admission were evaluated by ANOVA, chi-square

and linear regression where appropriate. For all tests, $P < 0.05$ was considered significant.

38/110 horses had an abnormal cTnI at admission. Higher cTnI concentrations were significantly associated with the occurrence of ventricular arrhythmias (VA) ($p=0.01$) and outcome ($p<0.001$), but not with treatment (medical/surgical). An abnormal cTnI concentration was significantly associated with increased HR ($p=0.03$), PCV ($p=0.012$) and lactate concentration ($p=0.002$), and VA ($p=0.012$), surgery ($p=0.032$) and mortality ($p=0.012$).

These data suggest that horses presenting for colic with elevated cTnI concentrations have a less-favorable prognosis for successful recovery from colic. Also, the association between higher cTnI concentrations and VA in horses presenting for colic suggests concurrent myocardial injury in these horses.

ABSTRACT #323

ASSOCIATION OF GROWTH HORMONE AND GHRELIN WITH THE ENERGY METABOLISM AND MORTALITY IN SEPTIC FOALS

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Sepsis is a major cause of mortality in newborn foals and has been shown to cause dysregulation of hormones involved in the hypothalamic-pituitary axis and energy metabolism in affected foals. Even though growth hormone is a central factor in energy regulation, information on growth hormone in neonatal foals (healthy or diseased) is limited. Ghrelin is produced by P/D1cells in the gastric mucosa to stimulate short-term hunger and the secretion of growth hormone from the pituitary gland. Both hormones play important roles in energy homeostasis.

The goal of this study was to determine the association between growth hormone and ghrelin concentrations with sepsis, mortality of sepsis, severity of disease and serum glucose and triglyceride concentrations in critically ill foals.

We measured the plasma concentrations of ghrelin and growth hormone in 44 septic, 62 sick non-septic (hospitalized for other diseases) and 19 healthy foals. Sepsis was defined as a sepsis score >11.

Ghrelin concentrations were higher in septic foals than in sick non-septic and healthy foals ($p<0.001$). Growth hormone concentrations were higher in septic and sick non-septic foals than in healthy foals ($p<0.001$). Septic foals had lower serum glucose and higher triglyceride concentrations than healthy foals ($p<0.01$). Among the septic foals, non-survivors had lower glucose and higher triglyceride values than survivors ($p<0.05$). Ghrelin and growth hormone concentrations were positively correlated with sepsis scores as well as triglyceride concentrations, but negatively correlated with glucose concentrations ($p<0.05$). No difference was found in ghrelin and growth hormone concentrations between septic foals that died or survived.

The results of this study suggest that ghrelin and growth hormone concentrations increase in response to systemic inflammation in septic foals, which probably is an attempt to overcome the negative energy balance by stimulating hunger and lipolysis. As septic foals are often anorexic, the neuronal response to ghrelin is likely to be impaired during sepsis leading to a lack of energy intake and a catabolic state in sick foals.

ABSTRACT #324

SEASONAL CHANGES IN PLASMA ALPHA-MELANOCYTE-STIMULATING HORMONE AND ADRENOCORTICOTROPIC HORMONE IN RESPONSE TO THYROTROPIN-RELEASING HORMONE ADMINISTRATION IN NORMAL AGED HORSES

RA Funk, AJ Stewart, AA Wooldridge, RJ Kemppainen, EN Behrend, AK Johnson. Auburn University College of Veterinary Medicine, Auburn, AL.

Diagnostic tests for equine pituitary pars intermedia dysfunction (PPID) including endogenous adrenocorticotropic hormone (ACTH) and the overnight dexamethasone suppression test (DST) have been demonstrated to be affected by season in the Northeastern United States. Adrenocorticotropic hormone response to thyrotropin-releasing hormone (TRH) is a new and potentially more sensitive diagnostic test for equine PPID, but has not been evaluated for seasonality. The purpose of this study was to evaluate seasonal changes in plasma ACTH and alpha melanocyte-stimulating hormone (αMSH) responses to TRH administration in the southeastern United States.

Ten healthy aged horses with normal DST (indicating absence of PPID) were administered synthetic TRH (1 mg) intravenously. Adrenocorticotropic hormone and αMSH concentrations were measured at 0, 5, 10, 15, 20, 25, 30, 45, 60, and 180 minutes. Testing was performed in February, July, August, September, October, and November. Mean ACTH and αMSH concentrations at each time point were compared across months using 2-way analysis of variance. Significance was set at $p<0.05$. Concentrations of ACTH (post-TRH administration) were significantly higher in August, September and October compared to February. Concentrations from July and November were not significantly different than concentrations in any other month. Concentrations of αMSH (post-TRH administration) were significantly higher in August compared to February. Concentrations in July were not significantly different than concentrations in February or August.

Adrenocorticotropic hormone and αMSH responses to TRH administration experience seasonal variation, with increased TRH-stimulated ACTH and αMSH concentrations in the fall. These results support previous evidence of increased activity of the equine adrenocortical axis in the fall months.

ABSTRACT #325

EFFECT OF INSULIN INFUSION INTO THE DIGITAL ARTERY ON GLUCOSE CONCENTRATIONS IN THE DIGITAL VEIN IN HEALTHY HORSES

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Laminitis is often associated with insulin resistance and carbohydrate metabolism. The objective of this research was to determine whether insulin infused into the digital artery can affect the arterial-venous (A/V) glucose differential in healthy horses as an indication that glucose uptake by the foot is regulated by insulin.

Seven healthy horses were used. Both digital arteries and the lateral digital vein from one foot were catheterized with 18G X 3.2 cm Teflon catheters. Infusion was through the lateral artery by infusion pump. Blood samples were collected concurrently from the medial artery and lateral vein at 1 or 5 min intervals during untreated-control, saline-infusion, and insulin infusion at 0.1, 0.5, 1.0, 5.0 units/min (0.50 ml/min infusion speed). Each infusion period lasted 20 min.Glucose concentrations were determined by a validated glucometer.

Glucose A/V concentration differential were: Mean±SD, (lowest-highest value), number of samples, % samples with arterial glucose concentrations greater than venous. No treatment-control period**:** 9.4±8.6 (1–39), n=32, 91.4%. Saline-infusion period**:** 9.8±7.6 (1–33), n=19, 70.3%. Insulin infusion period **(**all dosages combined): 9.0±5.2 (1–23), n=78, 98.7%; compared to controls there was no difference ($P>0.05$). A/V differential was not affected by saline infusion, insulin or hypoglycemia (34–40 mg/dL level). The glucose A/V differential (9–10 mg/dL) demonstrated utilization of glucose by the digit. However, insulin infusion into the digital artery did not increase glucose A/V differential. This suggests that the foot is not affected by insulin.

ABSTRACT #326

VALIDATION OF A SPECIES SPECIFIC ENZYME-LINKED IMMUNOSORBENT ASSAY FOR MEASUREMENT OF SERUM INSULIN IN HORSES

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Equine serum insulin has previously been analyzed with methods developed for human serum insulin. The performance and results of these human RIA and ELISA methods vary considerably because of different cross-reactivity of the anti-human antibodies and matrix problems. A species optimized, quantitative method (Mercodia Equine Insulin ELISA) for measurement of equine serum insulin has currently been produced by Mercodia, Uppsala, Sweden. It is a

direct sandwich ELISA in which two monoclonal antibodies are directed against two separate antigenic determinants on the equine insulin molecule. The detection limit is 0.01 μg/L and the detection range is 0.02–1.50 μg/L.

Our study evaluated the precision and linearity of the Mercodia Equine Insulin ELISA and compared it with two methods for human insulin (Mercodia Human Insulin ELISA and Coat-A-Count Insulin RIA kit from DPC, Siemens Diagnostics, Los Angeles, CA, US). Biological relevance of the assay was evaluated by measuring insulin before and after feeding.

Forty healthy horses were sampled before and 90 minutes after feeding. These 80 serum samples were analyzed with all three methods. Pearsońs correlation values were calculated. Precision for the equine ELISA was evaluated by determining the intra- and inter-assay coefficient of variation (CV). Sera from one horse with a mean concentration of 0.116 μg/L was analyzed in duplicate on fifteen different assay runs. Another sample (mean 0.017 μg/L) was analyzed in duplicate four times on one assay the same day. One sample with high insulin value (0.998 μg/L) was diluted with physiologic saline in five steps. The results were compared with mathematically predicted values and recovery was determined.

Results from the equine ELISA had high correlation both with the human ELISA and the RIA, $r^2=0.97$ and 0.97 respectively. Inter-assay CV was 10.7% and intra-assay CV was 4.6%. Recovery was 92–122%. Insulin concentration was significantly greater after feeding compared with the pre-feeding sample in 40 horses (Sign test $p<0.0001$). The median insulin level using the equine ELISA before feeding was 0.07 μg/L compared with 0.29 μg/L after feeding. In comparison, the insulin results from human RIA and ELISA increased from median 4.8 and 7.8 mU/L to 21.1 and 26.1 mU/L respectively.

The Mercodia Equine Insulin ELISA had good precision and linearity, and showed high correlation with both the human ELISA and the Coat-A-Count RIA. This commercial assay's advantages are that it is developed for equine samples and results are quantitative (μg/L) instead of using activity units based on human insulin.

ABSTRACT #327
HYPERINSULINEMIA IN HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION AND EFFECTS OF HOUSING ON INSULIN RESPONSE DURING A GLUCOSE TOLERANCE TEST. HC Schott II[1], L Groppi[1], S Wismer[1], S Beyerlein[2], LJ McCutcheon[1], P Schenck[2], RJ Geor[1]. [1]Department of Large Animal Clinical Sciences, [2]Diagnostic Center for Population and Animal Health, Michigan State University College of Veterinary Medicine, East Lansing, MI.

Insulin resistance (IR) can be a comorbid condition in horses with pituitary pars intermedia dysfunction (PPID) and presence of hyperinsulinemia, supportive of IR, is considered a negative prognostic indicator for long-term survival. Although the prevalence of PPID in older equids likely approaches 20%, the prevalence of concurrent hyperinsulinemia has not been documented. Thus, basal serum insulin concentration was determined (double antibody RIA, Diagnostic Systems Laboratories, Webster, TX) in nine horses with PPID diagnosed by presence of hirsutism and supported by an overnight dexamethasone suppression test. Blood samples were collected between 9–10 am after hay (but no concentrate) had been fed at 8 am. In addition, the insulin response to glucose administration (100 mg/kg, IV bolus) was determined in the nine PPID horses under three conditions: 1) at winter pasture (15–30°F); 2) after an overnight stay in the hospital environment; or 3) after 5 or 6 days in the hospital environment (60–65°F). Diet for this subgroup was mixed grass-alfalfa hay with no concentrate feed or other supplements throughout the study period (December, 2008). While in the hospital environment, horses remained confined in individual box stalls with no forced exercise.

Basal insulin concentration was 133±32 (SE) pmol/L with a range of 14–911 pmol/L. Only one of the nine horses had a basal insulin concentration >300 pmol/L. The insulin response to glucose administration was not different when the subgroup of horses was tested at pasture or after 1 night in the hospital (Table) but insulin concentration at baseline and 5 and 30 min after glucose administration was greater ($p<0.01$) after 5–6 days of hospitalization. These data demonstrate that ambient conditions and perhaps amount of daily exercise can have a substantial impact on testing for IR.

Housing	Pre-glucose administration	5 min	30 min
Pasture	106±28[a]	402±79[a]	189±54[a]
Hospital 1 day	90±24[a]	495±117[a,b]	325±119[a,b]
Hospital 5–6 days	204±89[b]	677±152[b]	504±194[b]

[a]different letters indicate significant difference ($p<0.01$) within each column.

ABSTRACT #328
CHARACTERIZATION OF THE CDNA SEQUENCE ENCODING FOR THE PLATELET INTEGRIN ALPHAIIB AND BETA3 IN A HORSE WITH GLANZMANN THROMBASTENIA. S Macieira, J Lussier, C Bédard. Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.

Glanzmann thrombastenia (TG) is characterized by a defect of platelet aggregation. It is an autosomal recessive genetic disorder caused by an abnormality of the platelet receptor for fibrinogen. The integrin receptor located on the plasma membrane is formed by a complex of alphaIIb and beta3 subunits, encoded by two distinct genes. Recently, we identified a horse with clinical and pathological features of TG. Flow cytometry studies revealed a deficiency of the alphaIIb subunit, suggesting the presence of one or several mutations in the gene encoding for the protein.

The aim of this study was to describe this case of TG at the molecular level by characterizing the cDNAs encoding for the beta3 and alphaIIb.

Total RNA was extracted from platelets and converted into cDNA by reverse transcription and poly-dT. Genomic DNA was extracted from white blood cells. Specific primers for alphaIIb and beta3 were used to amplify by PCR the corresponding cDNA or genomic regions that were further characterized by sequencing and compared by BLAST analysis (GenBank). A point mutation from G to C was identified in exon 2 of alphaIIb causing the substitution of amino acid Arg72, normally found to Pro72. This amino acid change may result into abnormal structural conformations that yield an inactive alphaIIb subunit. The analysis of genomic DNA showed that this horse was homozygous for the missense mutation Arg to Pro. The genomic DNA sequences encoding exon 2 of the dam and the sire were heterozygous for this nucleic acid change and were clinically normal.

ABSTRACT #329
COMPARISON OF HEMATOLOGIC AND BIOCHEMICAL VALUES OBTAINED VIA INTRAVENOUS CATHETER VERSUS VENIPUNCTURE IN HOSPITALIZED HORSES. ML May, ME Utter, RD Nolen-Walston. New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.

During hospitalization, horses typically undergo frequent blood sampling for diagnostic testing and monitoring. The nature of veterinary patients and the need for numerous samples makes acquisition from an intravenous catheter both convenient and less stressful to the patient. We hypothesized that there would be no significant difference in the plasma chemistry and hemoconcentration parameters from venous blood samples obtained from a jugular catheter versus direct jugular venipuncture. Paired samples were obtained simultaneously from 23 adult hospitalized horses, of which 14 were receiving intravenous crystalloids, and 9 were receiving no fluid therapy. Where applicable, fluids were stopped for 2 minutes, and a pre-sample of 15 ml withdrawn from the catheter and discarded. Using identical syringes and needles, 10 ml samples were obtained from both the catheter and the contralateral jugular vein. Samples were submitted for blinded analysis including complete blood count, standard equine plasma chemistry analysis, serum lactate, PCV, and total protein concentration. Data obtained were analyzed using a mixed-model ANOVA and Bonferroni adjustment

for multiple comparisons. There was no significant difference associated with sampling method (venipuncture versus catheter) irrespective of fluid administration status in any of the 24 analytes measured. In addition, the absolute differences between sampling methods were subjectively judged to be to be clinically insignificant. In conclusion, blood samples obtained via intravenous catheter have clinically equivalent values to those taken using venipuncture in commonly performed analyses. Further investigation is warranted to establish if this technique is associated with increased complications such as phlebitis or bacteremia.

ABSTRACT #330
EFFECTS OF LIPOPOLYSACCHARIDE, LIPOTEICHOIC ACID, AND PEPTIDOGLYCAN ON TUMOR NECROSIS FACTOR PRODUCTION IN EQUINE WHOLE BLOOD CULTURE. JL Day, PJ Johnson, AR Honaker, J Amorim, AE DeClue. University of Missouri College of Veterinary Medicine, Columbia, MO.

Whole blood culture (C_{wb}), an *ex vivo* method for studying inflammatory mediator response to stimuli, maintains interactions between populations of blood cells, and plasma and can be used to evaluate anti-inflammatory therapies. Although C_{wb} has been used in horses, dose response curves and Gram-positive microbial products have not been evaluated. The purpose of this study was to determine the concentration at which TNF is maximally stimulated by several microbial products using C_{wb}.

Blood, collected from 4 healthy adult mares, diluted 1:2 with RPMI/penicillin/streptomycin was cultured on 12 well plates. The blood was stimulated with various concentrations of lipopolysaccharide (LPS) (5–1000 ng/ml), lipoteichoic acid (LTA) (100–2500 ng/ml), peptidoglycan (PG) (100–2500 ng/ml), or control solution (PBS) and incubated at 37 °C for 24 h. A cell killing bioassay was used to measure TNF in the C_{wb} supernatant. Data were analyzed using ANOVA and *post-hoc* Fisher LSD method with $p < 0.05$ considered significant.

Multiple concentrations of LPS, LTA and PG significantly stimulated TNF production from the cultured blood. The lowest concentrations that stimulated maximal mean±SD TNF bioactivity for each microbial product compared to control (1.6±0.7 ng/ml) were 50 ng/ml for LPS (10.7±3.4 ng/ml; $p=0.007$), 500 ng/ml for LTA (17.7±8.8 ng/ml; $p=0.039$) and 500 ng/ml for PG (26.1±4.9 ng/ml; $p=0.006$).

In conclusion, C_{wb} is an efficient method of evaluating TNF production in response to LPS, LTA and PG in horses. These data provide optimum stimulant concentrations for use with C_{wb} in future studies investigating anti-inflammatory interventions for both Gram negative and positive infections in this species.

ABSTRACT #331
LAMINAR INFLAMMATORY GENE EXPRESSION IN THE CARBOHYDRATE OVERLOAD MODEL OF EQUINE LAMINITIS. BS Leise[1], PJ Johnson[2], RR Faleiros[1,3], SJ Black[4], JK Belknap[1]. [1]The Ohio State University College of Veterinary Medicine, Columbus, OH. [2]University of Missouri College of Veterinary Medicine, Columbia, MO. [3]Universidade Federal de Minas Gerais, Brazil. [4]University of Massachusetts, Amherst, MA.

Similar to sepsis-related organ injury in the human patient, laminar injury in the black walnut extract (BWE) model is associated with marked increases in laminar inflammatory indices, including increases in laminar mRNA concentrations of COX-2, IL-1β and IL-6. However, no reports have detailed the inflammatory response in the carbohydrate overload (CHO) laminitis model. The CHO model more closely mimics other clinical causes of acute laminitis (i.e. grain overload, enterocolitis, strangulating intestinal lesions) due to the presence of endotoxemia, the extended time frame of systemic signs, and the higher frequency of structural failure of the laminae. The purpose of this study was to determine if a similar pattern of laminar inflammation characterized by proinflammatory cytokine expression occurs in the CHO model as has been previously reported for the BWE model. Twelve horses were administered 17.6 g of starch (85% corn starch/15% wood flour)/kg body weight via nasogastric tube; these horses were anesthetized either after a two degree increase in rectal temperature (DEV group, n=6) or at the onset of Obel grade 1 lameness (OG1 group, n=6). Control horses (CON group, n=7) were anesthetized 24 hours after NG administration of 6 liters of deionized water. Serial physical examinations including gait analysis and complete blood counts were performed. Laminar tissue was collected from horses while under anesthesia, followed by humane euthanasia. Complementary DNA was made from laminar mRNA isolated from 3 different segments of the dorsal laminae of each horse. Real-time PCR for IL-1β, IL-6, TNFα, IFNγ, COX-1 and COX-2 was performed. Increased mRNA concentrations ($P<0.05$) for IL-1β IL-6 and COX-2 were present in laminae from horses with OG1 lameness when compared to the CON and DEV horses. No differences between the groups were found for TNFα, IFNγ, or COX-1 at either the DEV or OG1 time points. In contrast to the BWE model, no increases in IL-1β, IL-6 or COX-2 mRNA concentrations were present in the DEV group. Furthermore, the CHO model also differs from results reported in the oligofructose model where no increase in IL-1β was reported, but an increase in IFNγ was present at the onset of lameness. In conclusion, although the onset of cytokine expression is delayed in the CHO model compared to the BWE model and is slightly different from the oligofructose model, a similar prominent laminar inflammation occurs at onset of lameness, further supporting the need to address inflammatory signaling in the acute clinical case of laminitis.

ABSTRACT #332
IL-10 DOWNREGULATES PRODUCTION OF PEROXYNITRITE IN EQUINE MONOCYTE-DERIVED MACROPHAGES: IMPLICATIONS FOR RHODOCOCCAL INFECTION. BA Sponseller, SK Clark, MM de Macedo, DM Wong, DE Jones. College of Veterinary Medicine, Iowa State University, Ames, IA.

Rhodococcus equi is an important cause of pneumonia, the leading cause of morbidity and mortality among foals aged one to six months, and a cause of significant economic loss to the equine industry. Infection of foals by *R. equi* is believed to occur within the first few weeks of life. Why some foals are susceptible to infection while adults are not is incompletely understood.

In previous *in vitro* studies we determined that activated neonatal equine macrophages express higher levels of IL-10 cytokine mRNA than adult controls (Sponseller et al., 2008). Similarly, Giguère et al. found that foals infected at approximately 3 weeks of age with virulent *R. equi* 103^{+} had increased levels of IL-10 in lung tissue at 3 and 14 days post-inoculation compared to foals infected with the avirulent, plasmid-cured *R. equi* 103^{-} and uninfected controls (Giguère et al., 1999). IL-10 down-regulates many effector functions of macrophages, including the release of reactive nitrogen and oxygen intermediates. In addition, studies in mice have convincingly demonstrated that peroxynitrite, a very reactive product of superoxide and nitric oxide, is required for killing of virulent *R. equi* in murine macrophages (Darrah et al., 2000). In fact, neither superoxide nor nitric oxide alone is effective in killing *R. equi*. Interestingly, vap-A and -G genes, contained on the *R. equi*-virulence plasmid, undergo increased transcription when *R. equi* is exposed to oxidative stress, indicating an adaptive bacterial response to such cellular defenses. Thus, we speculate that equine macrophage oxidative responses, and peroxynitrite in particular, are curtailed in the presence of increased expression of IL-10.

The purpose of this study was to determine whether or not IL-10 modulates production of peroxynitrite in equine monocyte-derived macrophages. Replicates of adult monocyte derived macrophage cultures in a 96 well-format were exposed to 0.2, 0.6, and 1.0 μg of anti-IL-10 antibody alone (R&D Systems), or with recombinant equine IFN-γ (R&D Systems), or with IFN-γ+LPS (Sigma-Aldrich). Intracellular peroxynitrite-mediated oxidation of dihydrorhodamine-123 (Invitrogen, Carlsbad, CA) was measured as rhodamine fluorescence with the FluoStar Omega fluorimeter. In all culture conditions, increasing concentrations of anti-IL-10 antibody resulted in increased levels of peroxynitrite, suggesting that a cytokine milieu rich in IL-10 would result in decreased macrophage production of peroxynitrite. Since activated foal macrophages express high levels of IL-10, our results indicate that neonatal foals infected with *R. equi* may have compromised macrophage oxidative responses and an inherent inability to clear infection.

ABSTRACT #333

EFFECT OF INTRAVENOUS LIDOCAINE ADMINISTRATION ON LEUKOCYTE EMIGRATION IN THE BLACK WALNUT EXTRACT MODEL OF LAMINITIS. Williams JM[1], Ravis W[2], Loftus J[3], Peroni J[4], Hubbell J[1], Faleiros R[1], Black SJ[3], Belknap JK[1]. [1]Department of Clinical Sciences, The Ohio State University, College of Veterinary Medicine, Columbus, OH; [2]Dept. of Pharmacal Sciences, Auburn University, Auburn, AL; [3]Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA; [4]Dept. of Large Animal Medicine, University of Georgia, Athens, GA.

Leukocyte emigration, the extravasation and migration of leukocytes into target tissue, is a central event in cellular injury resulting in organ failure in human sepsis. Recent research in the black walnut extract (BWE) laminitis model suggests that these animals exhibit a similar systemic inflammation as seen in sepsis, including leukocyte emigration into the laminar tissue that is likely to play a major role in laminar injury in laminitis. In models of human sepsis, intravenous lidocaine has been reported to decrease leukocyte emigration via the inhibition of leukocyte activation, endothelial activation, and expression of proinflammatory cytokines and chemokines. Anti-inflammatory properties of lidocaine in sepsis have also been proposed to be valuable in the treatment of ileus in the equine patient. The purpose of this study was to assess the efficacy of intravenous lidocaine therapy in the inhibition of leukocyte emigration patterns documented to occur in the BWE model of laminitis. Due to the fact that laminar tissue is an integumentary structure, investigators have used skin biopsies in two studies of the BWE model to demonstrate similar leukocyte extravasation into the skin as occurs in the laminae. Therefore, in this study, immunohistochemistry for a myeloid leukocyte marker, calprotectin, was used on serial skin biopsies to assess the effect of intravenous lidocaine on leukocyte emigration. Twelve horses were administered BWE, and were immediately treated with a constant rate infusion (CRI) of either lidocaine (0.05 mg/kg/min, LD group, n=6) or saline (CON group, n=6) for 10 hours (H). Skin samples (6 mm diameter) were obtained from 3 areas of the neck (approx. 10 cm.apart) for the following time points after BWE administration: 0 H, 3 H, and 10 H. Compared to the 0 H time point, increased integumentary leukocyte (neutrophil and monocyte/macrophage) numbers were present at the 10 H time point ($P<0.05$), with a trend for increased leukocyte numbers at the 3 H time point in both the LD and CON groups. No significant differences were present between LD and CON for any time point. These data are consistent with previously presented data from our laboratory showing no effect of lidocaine CRI on laminar cytokine or endothelial adhesion molecule expression or laminar leukocyte concentrations in the same horses. In conclusion, these data indicate that intravenous lidocaine does not effectively inhibit events leading to leukocyte emigration into tissues in disease states characterized by systemic inflammation.

Previously presented at IVECCS, September 2008.

ABSTRACT #334

THE IMMUNE RESPONSE OF FOALS TO NATURAL INFECTION WITH EQUID HERPESVIRUS-2 AND ITS ASSOCIATION WITH FEBRILE ILLNESS. SA Bell, MT Blanchard, JL Stott, N Pusterla, SM Mapes, W Vernau, KD DeJong, NJ MacLachlan. University of California School of Veterinary Medicine, Davis, CA.

Equid herpesvirus-2 (EHV-2) infection of horses is ubiquitous. EHV-2 infection anecdotally has been associated with a variety of disease syndromes in horses, but its true pathogenic significance remains uncertain. Other gammaherpesviruses such as Epstein Barr virus (EBV) have been shown to cause febrile illness in humans related to viral immunopathologic effects. Thus, the purpose of this study was to describe the ontogeny of the immune response of a cohort of 9 foals to natural infection with EHV-2 by evaluating serial complete blood counts, lymphocyte morphology, cytokine gene expression in peripheral blood mononuclear cells (PBMC), viral load in nasal swabs and peripheral blood, and antigen-specific cellular immune responses of PBMC, in conjunction with clinical evaluation of the foals. The occurrence of fever in foals was not related to lymphocytosis or lymphocyte morphology, cytokine gene expression, or viral load, but was significantly associated with increased levels of antigen-specific PBMC in peripheral blood of the foals. These data suggest that cellular immune responses to EHV-2 may lead to an immunologically mediated disease of foals that is perhaps analogous to infectious mononucleosis caused by EBV infection in humans.

Previously presented at The Second Havemeyer EHV-1 Workshop, Steamboat Springs, CO, September 2008.

ABSTRACT #335

USE OF QUANTITATIVE REAL-TIME PCR FOR THE DETECTION OF *SALMONELLA* SP. IN FECAL SAMPLES OF HORSES ADMITTED TO A VETERINARY MEDICAL TEACHING HOSPITAL. Emir Hodzic[1], Barbara A. Byrne[2], Samantha Mapes[3], Spencer S. Jang[4], K. Gary Magdesian[3], N. Pusterla[3]. [1]The Lucy Whittier Molecular and Diagnostic Core Facility, [2]The Department of Medicine and Epidemiology, [3]The Department of Pathology, Microbiology and Immunology and [4]The William R. Pritchard Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California, Davis, CA.

The purpose of this study was to compare quantitative real-time PCR with conventional culture in the detection of *Salmonella* sp. from feces of horses admitted to our veterinary hospital.

A quantitative real-time PCR assay was developed for the detection of *Salmonella* sp. in the feces of 911 equids admitted to a veterinary hospital. Fresh feces and feces following a 24 hour enrichment step in selenite broth were tested by conventional culture and real-time PCR targeting the *invA* gene of *Salmonella* sp. The detection limit for the real-time PCR assay was 3 and 10 *Salmonella* sp. organisms, when spiked samples were purified from selenite broth and feces, respectively. The analytical specificity was 100% based on the detection of a panel of 40 *Salmonella* serotypes from 5 serogroups and the lack of cross-reactivity with non-related microorganisms.

Salmonella sp. was not cultured from any of the fresh feces; however, *Salmonella* was cultured from the enrichment broth of 6 out of 911 fecal submissions (0.6%). *Salmonella* was detected by real-time PCR in 3 (0.3%) and 22 (2.4%) fecal and enrichment broth samples, respectively. Six broth samples were dually positive by culture and real-time PCR, while 16 additional broth samples tested positive only by real-time PCR. The *Salmonella* load in enrichment broth samples varied from 3 to 861,037 *Salmonella invA* gene copies/μl of DNA. Broth samples testing positive by both culture and real-time PCR had significantly higher pathogen load than broth samples testing only positive by real-time PCR. The PCR assay used in this study achieved an overall relative accuracy of 98%, a relative sensitivity of 100% and a relative specificity of 98% when compared to conventional culture.

The use of real-time PCR has the potential to reduce turnaround-time, with results being available within 22–28 hours from the time of sample collection. Further, the use of absolute quantitation may aid in the assessment of the infectious nature of hospitalized animals and may be an excellent alternative to conventional culture methods for surveillance and research studies.

ABSTRACT #336

COMPARİSON OF PCR TESTİNG FOR MANAGEMENT OF AN OUTBREAK OF EHVI AT A RACETRACK. AF Roy[1], SC Eades[2], RS McConnico[2], FM Andrews[2]. Louisiana Animal Disease Diagnostic Laboratory and[2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

An outbreak of Equine Herpes Virus I (EHV-I) involving racing Thoroughbred horses at a Louisiana racetrack afforded the opportunity to evaluate diagnostic testing techniques used to manage the outbreak situation. EHVI virus was determined to be the wild-type and not the neuropathogenic strain of the virus even though the index horse showed abnormal neurologic signs. A total of 74 exposed horses and 3 additional febrile and/or neurologic horses were sampled. Nasal swabs were passed at least 6 inches (15 cm) into the horse's nasal passages via the ventral meatus and swirled or held in place for at least 30–60 seconds to absorb respiratory secretions. Jugular venipuncture blood samples were taken for serology and molecular testing of the buffy coat. A single-step real-time PCR was performed on blood and nasal swabs using TaqMan reporter probes

for discrimination between neuropathogenic and non-neuropathogenic strains of EHV-l. The realtime PCR was performed using an Applied Biosystems 7500 real-time PCR system. A 96 well format was used and the turnaround time from extraction to results is less than 12 hours. This single-step real-time PCR was compared with a nested PCR performed at another laboratory. The single-step real-time PCR identified more horses with EHV-1 viral DNA in nasal swabs than did the nested PCR and appears to be more sensitive. All PCR positive samples were identified as the non-neuropathogenic strain of EHV-1 Serological testing was performed using paired, acute and convalescent sera with an Indirect Fluorescent Antibody procedure.

ABSTRACT #337
SALMONELLA ORANIENBURG IN A LARGE ANIMAL VETERINARY TEACHING HOSPITAL. KJ Mitchell, KJ Cummings, LD Rodriguez Rivera, M Wiedmann, P McDonough, DM Ainsworth, TJ Divers, L Cavaney, GA Perkins, LD Warnick. Cornell University, Ithaca, NY.

To describe an outbreak of multidrug-resistant *Salmonella* Oranienburg within the Cornell University Equine Farm Animal Hospital (CU-EFAH) and discuss control and prevention measures.

Medical records of patients that were fecal positive for *S.* Oranienburg in the CU-EFAH from 2006–2008 were evaluated. Pulsed-field gel electrophoresis (PFGE) was performed on isolates.

Salmonella Oranienburg was identified in 28 animals: 3 cows, 5 alpacas, and 20 horses. The index case was admitted 8/15/2006; an additional 26 cases occurred in 2007, with a cluster between 09–10/2007. One case occurred in 01/2008, with no further cases for 12 months. Six animals negative during previous hospitalization were positive on re-admission, with 3/6 returning due to clinical signs of infection. Salmonellosis developed in 18/28 cases, five animals were euthanized. Antimicrobial sensitivity patterns revealed resistance to multiple antimicrobials. The minimum inhibitory concentration for enrofloxacin was $\geq$1 (intermediate sensitivity) for 18/28 cases, with one isolate showing *in vitro* resistance. The PFGE patterns from 23/26 animals were identical.

Environmental disinfection and culturing increased during the cluster of Salmonella cases in the fall of 2007. *S.* Oranienburg was isolated from the environment four times (from 611 cultures) during 2007 and once in 02/2008. Additional hospital-wide biosecurity measures were implemented, including twice weekly fecal cultures of all patients.

Salmonella Oranienburg is of concern given the multidrug resistance and evidence of high morbidity associated with infection. The PFGE data support hospital-acquired infection in the majority of cases. Improving biosecurity has led to elimination of animal infection and environmental positives for over a year.

ABSTRACT #338
PARADOXICAL ACIDURIA IN HORSES: 37 CASES (2000–2008). HC Schott II[1], JR Rossetto[2]. [1]Michigan State University College of Veterinary Medicine, East Lansing, MI. [2]The Ohio State University College of Veterinary Medicine, Columbus, OH.

Paradoxical aciduria (PA) is well recognized in ruminants with gastrointestinal disorders accompanied by hypochloremic metabolic alkalosis, hypokalemia, and decreased circulating volume. The proposed renal mechanisms that contribute to these clinicopathological abnormalities include increased bicarbonate reabsorption (consequent to hypochloremia) and maximal sodium reabsorption, partly via exchange with hydrogen ions (into the tubular lumen). In urine samples collected from dehydrated horses within the first few hours of hospitalization, the authors have also observed PA. Thus, we retrospectively examined 37 case records of horses that were $>$1 year of age, had a urine sample collected within the initial 6 hours of hospitalization that was acidic (pH $<$ 7), and had a venous blood pH $>$7.35 on admission blood gas analysis. Horses with primary renal disease were excluded. Urine pH ranged from 4.0–6.5 (reagent strip analysis), venous blood pH ranged from 7.36–7.49, and specific gravity ranged from 1.004–1.037 (no correlations found). Primary medical disorders included enterocolitis (16), colic (8), liver disease (2), neurological disorders (2), metritis (2), myopathy (1), inflammatory bowel disease (1), cellulitis (1), heart failure (1), pleuropneumonia (1), red maple leaf toxicosis (1), and fever of unknown origin (1). A common feature of all horses was partial to complete anorexia ranging from 1–14 days. Serum biochemical abnormalities included hypoproteinemia (20), azotemia (18), chloride (Cl^-) $<$100 mmol/L (19), total calcium $<$10 mg/dL (17), sodium (Na^+) $<$130 mmol/L (12), and potassium (K^+) $<$3.0 (9). The only significant correlations detected were between Na^+ and Cl^- (0.80, $p<0.01$), Cl^- and creatinine (-0.43, $p<0.01$), and Na^+ and creatinine (-0.36, $p<0.02$). Electrolyte and creatinine concentrations were measured in 18 urine samples and Na^+, Cl^-, and K^+ ranged from 10–148, 15–162, and 13–112 mmol/L, respectively, resulting in fractional clearances of 0.05–7.1%, 0.14–8.4%, and 6.3–69% for Na^+, Cl^-, and K^+, respectively. The only urine measure significantly correlated to urine pH was fractional Na^+ clearance (0.51, $p<0.04$), providing support that PA in horses may also be a consequence of increased renal Na^+ reabsorption.

ABSTRACT #339
DIAGNOSTIC UTILITY OF COMPUTED TOMOGRAPHY OF THE HEAD IN HORSES AFFECTED BY NEUROLOGIC DISORDERS. C Sogaro-Robinson[1], VA Lacombe[2], SM Reed[3]. [1]Presque Isle, ME. [2]The Ohio State University, Columbus, OH. [3]Rood and Riddle, Lexington, KY.

The use of computed tomography (CT) and contrast-enhanced CT to image the head is common in horses presented for a wide variety of neurologic conditions. However, the validity of CT as a diagnostic indicator of neurological diseases in horses is unknown.

The validity of CT was estimated by comparing clinical, clinicopathologic, and histopathologic findings to CT findings in 11 horses presented for neurologic clinical signs, for which pre- and post-contrast CT images of the head were reviewed.

All horses with abnormal head CT had abnormal neurologic examinations on presentation and 90% (10/11) of horses exhibited abnormal mentation during hospitalization. Ten horses had abnormal postmortem examinations of the head, and CT imaging identified histopathologic lesions in 6 of these cases, which included: skull fractures, temporohyoid osteoarthropathy, large intracranial masses (oligodendroglioma, nasal adenocarcinoma, choleosteatomas) and acute vascular event. In 17% (1/6) of these CT studies, abnormal findings (meningeal congestion) were only detected after intravenous iodinated contrast injection. In 4 cases, pre- and post-contrast CT imaging failed to identify histopathologic lesions, such as encephalitis, meningitis, brainstem hemorrhage and local gliosis. CT sensitivity was 60% (95% confidence interval [CI]: 27–86), which was not greater than the sensitivity of CSF analysis (78%, 95% CI: 40–96). Postmortem examination of the head was unremarkable in 1 horse, for which CT imaging was normal (specificity, 100%).

Although it showed an excellent specificity, CT had a limited sensitivity, in detecting inflammatory disorders and small soft tissue lesions in the equine brain, which was not increased by contrast enhancement.

ABSTRACT #340
THE EFFECT OF METFORMIN ON INSULIN SENSITIVITY IN HORSES. AM Firshman, Hustace JL, Peterson K, Mata J. College of Veterinary Medicine, Oregon State University, Corvallis, OR.

Metformin is an oral antihyperglycemic drug used to enhance peripheral tissue sensitivity to insulin in humans. Metformin has been insufficiently studied in horses and conflicting results regarding efficacy have been reported. Equine Metabolic Syndrome (EMS) is a common condition that results in abnormal adipose tissue distribution, elevated plasma lipids, laminitis, and infertility. Horses with EMS are commonly glucose intolerant and insulin resistant. Treatment for horses with EMS includes feeding a low glycemic index diet and increasing exercise. Treatment with metformin in horses with EMS is appealing due to its potentially beneficial method of action and low cost. We hypothesized that insulin-stimulated glucose uptake could be increased by administering metformin to horses using dosages obtained from a preliminary pharmacokinetic study. The specific aim was to determine if metformin increases insulin sensitivity compared to placebo.

Eight normal adult horses, fed grass hay, were used in a 2$\times$2 switch back design using 15 mg/kg metformin PO q8 hrs for 15d or saline

placebo. Horses were allowed 6–8 hrs of paddock turnout daily with a 2-week washout between treatments. At the end of each treatment period, a 3 hr hyperinsulinemic euglycemic clamp was performed.

No effect of treatment was found on the rate of glucose infusion necessary to maintain euglycemia during the clamp compared to placebo ($P=0.223$). The results of this study indicate that 15 days of metformin treatment did not increase insulin sensitivity in normal horses. This lack of response may be related to the poor bioavailability of metformin in horses, however further study of the effect of metformin in insulin resistant individuals is warranted.

ABSTRACT #341

THE POSITIVE INTERACTION OF PROPIONATE, GLUCOSE, INSULIN AND CORTISOL IN THE HORSE. DT Lewis, ML Katz. Department of Veterinary and Animal Science, University of Massachusetts, Amherst, MA.

Propionate is the gluconeogenic substance derived through fermentation in the colon of the horse. Propionate has been demonstrated to increase plasma insulin in the pre-ruminal and ruminal sheep, goat and cow and plasma cortisol in the cow. Previously, propionate has been shown to not increase plasma insulin in the horse. During gluconeogenesis, propionate might stimulate hormonal changes similar to glucose. Therefore, this investigation examined the effects of increased plasma propionate on the concentrations of glucose, insulin and cortisol in the horse.

Four miniature horses were fed only low soluble carbohydrate first cutting grass hay and a vitamin mineral supplement for at least one year. Thus, we attempted to maximize gluconeogenesis as the source of glucose in these horses. We increased the plasma level of propionate about two fold in four miniature horses, based on the data of Argenzio & Hintz (1971) who reported a propionate turnover half-time of 8 minutes and an average basal plasma level of 4 mmol/l. Assuming an extracellular fluid volume of 20%, a priming dose of Na propionate in phosphate-buffered saline was administered as an intravenous bolus to increase the plasma propionate concentration to about 8 mmol/l and then a constant rate infusion pump was used to maintain this propionate concentration in the horse for 4 hours. Blood samples were taken every twenty minutes beginning one hour before and during the 4 hour infusion period. The horses had hay available to them during the experiments. Glucose was measured using the glucose-oxidase method and insulin and cortisol were measured by RIA (Coat-A-Count) methods.

Plasma glucose concentration steadily increased after the start of infusion so that plasma glucose was 10–20 mg/dl higher in all horses by 180 minutes after the beginning of propionate infusion. Each horse exhibited a marked rise (four to five fold) in insulin levels immediately upon the initial bolus injection of propionate, which was followed by a return towards baseline by the time of the 20 minute sample. Insulin levels then gradually increased to about two fold in all horses after 180 minutes of propionate infusion. During the propionate infusion plasma cortisol concentrations in all horses rose by 20–200% by 20 minutes and increased 200–500% by 180 minutes.

This study reveals a transient immediate insulin peak and a steady increase in glucose, insulin and cortisol concentrations in the plasma of the horse throughout propionate infusion, when fed a diet requiring gluconeogenesis to meet glucose needs. This data seems to reveal a direct temporal relationship between propionate and insulin.

ABSTRACT #342

ACUTE HYPERINSULINEMIA MODULATES EXPRESSION OF GENES INVOLVED IN GLUCOSE AND FAT METABOLISM IN SUBCUTANEOUS ADIPOSE TISSUE OF HORSES. JK Suagee[1], RJ Geor[2], LJ McCutcheon[2], BA Corl[1], MW Hulver[1]. [1]Virginia Tech, Blacksburg, VA. [2]College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Low insulin sensitivity and/or high insulin concentrations have been associated with laminitic predisposition in ponies and prolonged experimental hyperinsulinemia (>1000 mU/L) while maintaining euglycemia has induced laminitis in healthy ponies. This study examined the effects of elevated plasma insulin concentrations on expression of genes associated with glucose and fat metabolism in subcutaneous adipose tissue of healthy, non-obese horses.

Ten mares (BCS 6.3±0.5) received a 6 hr infusion of insulin (6 mIU/kg/min) and a saline infusion, 13 d apart, in a balanced cross-over design. Euglycemia was maintained during insulin infusion by variable rate dextrose infusion. Blood samples for measurement of insulin concentrations were collected every 15 min.Following the clamp procedure, biopsy samples of subcutaneous adipose tissue (neck crest) were collected and RNA extracted to determine, by quantitative RT-PCR, the expression of CD36, FATP, GLUT-4, GLUT-1, and the insulin receptor (IR). Statistical analyses were performed using the mixed models procedure (SAS software system), with significance taken at $P<0.05$.

Mean plasma insulin concentrations during the insulin and saline infusions were 933.5±22.0 and 15.9±21.7 mIU/mL, respectively. Expression of CD36 ($P=0.002$), FATP ($P=0.012$), and IR ($P=0.006$) were decreased in adipose tissue following insulin infusion. The mRNA of GLUT-1 and GLUT-4 were not affected by treatment.

This study demonstrated that a 6-h period of supraphysiological hypersinulinemia decreased expression of genes involved in fatty acid transport (CD36, FATP) and insulin signaling (IR) in the subcutaneous adipose tissue of horses.

ABSTRACT #343

INSULIN SENSITIVITY IN PONIES IS INFLUENCED BY SEASON AND PASTURE. KH Treiber[1], RJ Geor[1], PA Harris[2]. [1]Virginia Tech MARE Center, Middleburg, VA. [2]Waltham Centre for Pet Nutrition, Melton Mowbry, UK.

Insulin sensitivity is a principle factor in glucose and energy regulation. Reduced insulin sensitivity (i.e. insulin resistance) has been implicated in equine pasture associated laminitis, which develops predominantly in the spring season. Increased risk of laminitis in the spring has been attributed to changes in pasture carbohydrates, however underlying metabolic predisposition may be influenced by other seasonal environmental factors. This study performed the Minimal Model of Glucose and Insulin Dynamics on 12 pasture-kept ponies in northern Virginia in January, March, May, July and September of 2007. Ponies were maintained together on the same pasture for at least 1 week prior to testing each month. On the morning of the test ponies were brought into stalls, jugular catheters were placed and 4 hour insulin-modified (20 mIU/kg) frequently sampled intravenous glucose (200 mg/kg) tolerance tests (FSIGTs) were performed. Ponies were then released into a drylot and fed grass hay. After 1 week on drylot the FSIGT was repeated. Glucose and insulin curves from the FSIGTs were modeled using MinMod Millenium and WinSAAM software to determine insulin sensitivity (SI, $mIU/L^{-1} \cdot min^{-1} \cdot 10^4$).

Table 1. Insulin sensitivity (SI) of ponies reported as median (IQR) and compared by Wilcoxon's matched-pairs signrank test.

	January	March	May	July	September
Drylot	0.17[c]	0.26[a,b]	0.45[a]	0.19[b,c]	0.22[b,c]
	(0.27)	(0.98)	(1.11)	(0.34)	(0.56)
Pasture	0.69[a]	0.24[b]	0.16[b]	0.48[b]	0.27[b]
	(3.06)	(1.38)	(0.60)	(0.44)	(0.55)
Enclosure[1]	0.003	0.69	0.019	0.026	0.69
	(0/11)	(6/6)	(11/1)	(1/10)	(4/8)

[a,b,c]Values with differing superscripts are different between months in the same row.

[1]Differences between pasture and drylot reported as P-value (negative/positive differences).

Drylot resulted in low SI values, possibly in response to energy or exercise restriction. In May, however, drylot SI was higher than other months, suggesting a spring increase in SI which would promote utilization of carbohydrates available in the pasture, facilitate activity during the breeding season, and potentially protect against laminitis. Conversely, SI on May pasture was numerically lowest, corresponding with clinical cases of laminitis which were

successfully treated by restriction to drylot. Reduced SI on May pasture may result from the glycemic or digestive response to exaggerated carbohydrate fractions accumulating in improved pastures in the spring. Pasture SI values in March resembled drylot values, not surprisingly as very little grass was available and ponies were subsisting primarily on supplemented hay. Low SI values on September pasture may reflect a return to a carbohydrate and energy conserving metabolic state in preparation for the winter season. Understanding seasonal adaptations in metabolism and responses to nutritional environment will improve the management of equids, especially ponies, to avoid the development of pasture associated laminitis.

ABSTRACT #344
MONONUCLEAR LEUKOCYTES IN THE LAMINAE OF NORMAL HORSES AND THOSE WITH BLACK WALNUT EXTRACT-INDUCED LAMINITIS. RR Faleiros[1,2], GJ Nuovo[1], AD Flechtner[1], JK Belknap[1]. [1]Ohio State University, Columbus, Ohio. [2]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

The laminar interstitium is reported to have no resident leukocyte populations, but undergo a marked neutrophil infiltration in early stages of laminitis. In addition to neutrophils, both resident lymphocytes and monocyte/macrophages have recently been reported to play important roles in human sepsis-related organ injury. The purpose of this study was to assess monocyte/macrophage and lymphocyte populations in laminae of normal horses and those with black walnut extract (BWE)-induced laminitis. Immunohistochemistry was performed on laminar samples from 20 horses divided equally in 4 groups: control animals (CON), and 3 different time points after BWE administration: 1.5 hours (ETP group), onset of leucopenia (DTP group), and onset of lameness (LAM group). Antibodies against CD3, CD20 and CD163 were used to recognize lymphocytes (T and B), and macrophages (CD163+ indicates resident/"alternatively activated" macrophage), respectively. CON laminae contained $CD3^+$ and $CD20^+$ lymphocytes situated around deep dermal vessels, and $CD163^+$ resident macrophages in both the deep dermis (perivascular orientation) and in dermal laminae (primary and secondary). BWE treatment resulted in no changes in lymphocyte counts, but a marked increase (P=.0016) in $CD163^+$ macrophages in the secondary dermal laminae (SDL) in the ETP and DTP groups, returning to CON values in the LAM group. In conclusion, resident leukocytes in the form of lymphocytes and macrophages are present in the laminae of clinically normal horses, and BWE administration induces increases in the numbers of $CD163^+$ macrophages in SDL. Thus, mononuclear cells are likely to play both homeostatic and pathologic roles in laminitis.

ABSTRACT #345
ABSTRACT WITHDRAWN

ABSTRACT #346
EVALUATION OF VARIOUS MATRIX METALLOPROTEINASE INHIBITORS (MMPIs) IN THE HORSE. LA Fugler, SC Eades, CE Koch. Louisiana State University School of Veterinary Medicine, Baton Rouge, LA.

The purpose of this study was to establish a non-terminal model of MMP induction in the horse, and to use this model for evaluating various MMPIs that may have potential therapeutic use in the treatment of equine laminitis. Lipopolysaccharide (LPS) was administered intravenously to normal horses, and digital venous plasma samples were collected hourly for 24 hours. Plasma MMP-2 and MMP-9 activities were assessed using zymography. The administration of LPS resulted in significant increases in MMP-2 and MMP-9 concentrations in the digital circulation. This model of MMP induction was then used to evaluate the effects of doxycycline, oxytetracycline, flunixin meglumine, and pentoxifylline on equine MMP inhibition. Horses were treated with the inhibitors every 12 hours beginning 12 hours prior to LPS administration, and digital venous plasma samples were evaluated for MMP-2 and MMP-9 activities using zymography. Pentoxifylline and oxytetracycline appeared to be potent MMP-9 and modest MMP-2 inhibitors in the horse. Flunixin meglumine and doxycycline were potent inhibitors of equine MMP-2, but only weak inhibitors of equine MMP-9. Recent studies suggest that MMP-9 may play an important role in the development of equine laminitis; therefore, these findings warrant the evaluation of pentoxifylline and oxytetracycline as MMPIs in the prevention/treatment of equine laminitis.

ABSTRACT #347
ULTRASONOGRAPHIC MEASUREMENT OF SPLEEN VOLUME IN HORSES. C Navas de Solis, JH Foreman, CR Byron, RA Carpenter. University of Illinois, Urbana, IL.

Ante-mortem measurement of spleen size might be helpful in clinical determination of splenomegaly. Our hypothesis was that spleen volume can be calculated from percutaneous ultrasonographic measurements in live standing horses.

Eight donated horses free of splenic disease were studied after obtaining IACUC approval. Ultrasonographic spleen volume and Evans blue dye-dilution blood volume (BV) were measured before (baseline) and after intravenous administration of detomidine and epinephrine (separately). Spleen volume was calculated using a standard ellipsoid formula ($VOL_{standard}$) and with the formula modified for the unique shape of the equine spleen ($VOL_{modified}$). Horses were euthanized using pentobarbital sodium, spleens were removed immediately, and spleen measurements were repeated. Descriptive statistics, correlations, and regression analysis were performed. The level of significance was set at $P < 0.05$.

Mean $VOL_{standard}$ was 31.4±9.8 L at baseline, 34.0±10.5 L after detomidine, and 5.4±3.1 L after epinephrine. $VOL_{modified}$ was 25.1±9.1 L at baseline, 27.4±9.6 L after detomidine, and 4.2±2.4 L after epinephrine. Post-mortem spleen volume was 14.1±4.6 L and was correlated with $VOL_{standard}$ (r=0.86, P=0.006) and $VOL_{modified}$ (r=0.87, P=0.004). BV was correlated with $VOL_{standard}$ (r=0.81, P=0.014) and $VOL_{modified}$ (r=0.78, P=0.02). PCV after epinephrine was correlated with $VOL_{standard}$ (r=0.95, P=0.0002) and $VOL_{modified}$ (r=0.96, P=0.0001). When compared to baseline, spleen volume was smaller after epinephrine (P=0.009) but not larger after detomidine (P=0.12). BV after detomidine was larger than after epinephrine (P=0.009).

The study demonstrated that splenic size, under various pharmacological influences, can be calculated from ultrasonographic measurements in the live standing horse. PCV after epinephrine was the best indicator of spleen volume.

ABSTRACT #348
EFFECTS OF FLUNIXIN MEGLUMINE ON LEUKOCYTE ACTIVATION IN A LOW-DOSE ENDOTOXEMIA MODEL IN HORSES. G Forbes, SR Bailey, S Church, CJ Savage. University of Melbourne, Victoria, Australia.

Endotoxemia remains a major cause of equine morbidity and mortality. Tissue damage and organ dysfunction resulting from endotoxemia are partly brought about by leukocyte activation and their subsequent attachment and migration through the vascular endothelium and into tissues. Cytokines, matrix metalloproteinase enzymes and reactive oxygen species are released. Flunixin meglumine, in addition to its cyclo-oxygenase inhibitory actions as a non-steroidal anti-inflammatory drug, has been postulated to have further anti-endotoxic effects on leukocytes by inhibiting the transcription factor, NFkB. The purpose of this study was to determine the effects of flunixin on clinical signs and leukocyte counts in an established model of equine endotoxemia.

Endotoxin (lipopolysaccharide isolated from *E. Coli* 055:B5 bacteria) was administered intravenously at a dose of 1 ng/kg/min, over 30 min (total dose 30 ng/kg; dissolved in 500 ml sterile saline), into six adult female standardbred horses. Horses were pre-treated with either flunixin meglumine (1.1 mg/kg, intravenously) or saline. Each horse underwent both treatments (in randomised order), with a washout period of at least 14 days between each experiment. Clinical signs (including rectal temperature, heart rate, respiratory rate and mentation) were monitored at frequent intervals (initially every 15 min for 2 hours then every 30 min from 2 hours until 6 hours). Jugular venous blood samples were taken at the same time points. Leukocyte and platelet counts were performed using a Coulter Counter (model Z1; Coulter Electronics Inc.).

The endotoxin caused a small but significant increase in rectal temperature, reaching a peak of 39.18±0.15 °C at 210 minutes after the start of the LPS infusion. Other clinical signs were mild, and included transient muscle fasciculations. Blood leukocyte counts decreased from $9.15\pm1.36\times10^9$/L at time 0 to a nadir of 3.70±0.77 at 105 mins, before recovering back to baseline values by 5 hrs. Flunixin meglumine significantly reduced the peak temperature (maximum 38.48±0.14 °C at 120 mins). However, the blood leukocyte counts were not significantly affected by flunixin, reaching a nadir of $3.25\pm0.80\times10^9$/L and slowly recovering to $5.25\pm1.30\times10^9$/L at 5 hours.

These data suggest that while flunixin may effectively reduce some of the signs of endotoxemia, including pyrexia, it may not affect leukocyte activation in terms of endothelial adherence and subsequent tissue damage. Even at the recommended anti-inflammatory dose of 1.1 mg/kg, leukocyte margination was unaffected. Further studies are required to examine the cytokines and signalling pathways involved, affected by flunixin in this model; however, this study indicates that other therapeutic agents may be required to effectively inhibit leukocyte activation in endotoxemia.

ABSTRACT #349

THE PHARMACOKINETICS OF INTRAVENOUS AND INTRAMUSCULAR BUPRENORPHINE IN THE HORSE. KM Messenger, JL Davis, DH LaFevers, BM Barlow, LP Posner. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Pain management options in equine medicine are limited due to a lack of available pharmacokinetic and safety data, expense of treatment, and a high risk of adverse effects with some classes of drugs. Opioid analgesics, particularly butorphanol and morphine are frequently used for moderate to severe pain in horses. However, these drugs have a short duration of action, require frequent dosing, and are associated with adverse effects such as gastrointestinal stasis and behavioral changes. Buprenorphine is a highly potent, longer acting mu opioid agonist analgesic frequently used in human and small animal medicine. In humans, therapeutic effects are achieved at concentrations greater than 0.5–1 ng/mL. Its analgesic effects have been studied in the horse, however its pharmacokinetics in this species are unknown. The purpose of this study was to determine the pharmacokinetics of buprenorphine after intravenous (IV) and intramuscular (IM) administration in healthy horses.

Six healthy adult horses were used in this study. Each horse received either IV or IM buprenorphine at a dose of 0.005 mg/kg in a randomized, crossover design, with a minimum 1 week washout period between experiments. Plasma samples were collected via jugular catheter immediately prior to drug administration, and then at 10, 20, 30, 45, and 60 minutes, and 1.5, 2, 4, 6, 8, 12, and 24 hours after drug administration. Horses were monitored for adverse reactions throughout the study. Plasma buprenorphine concentrations were measured using ultra performance liquid chromatography with electrospray ionization mass spectrometry.

Following IV administration, buprenorphine had a clearance of 7.88±4.47 mL/kg/min, and a plasma half-life ($t_{1/2}$) of 7.14±4.97 hr. The volume of distribution at steady state was 3.39±1.46 L/kg. Following IM administration, the average maximum plasma concentration was 1.74±0.09 ng/mL, which was significantly lower than the highest measured concentration (4.34±1.22 ng/mL) 10 minutes after IV administration ($P<0.001$). The time to maximum plasma concentration was 0.9±0.69 hr. The plasma $t_{1/2}$ was 7.34±5.06 hr. Bioavailability was highly variable, ranging from 41–93%. After administration of buprenorphine via either route, several of the horses showed signs of mild to moderate excitement. Gut sounds were decreased for 10±2.19 hours in the IV group and 8.67±1.63 hours in the IM group. The time to first defecation was approximately 5 hours after administration in both groups.

Buprenorphine has a long half-life in the horse and was detected in the plasma at concentrations expected to be therapeutic after a dose of 0.005 mg/kg after IV and IM administration. Signs of excitement and gastrointestinal stasis may be noted in horses.

ABSTRACT #350

BIOAVAILABILITY OF DETOMIDINE ADMINISTERED TO HORSES AS AN OROMUCOSAL (SUBLINGUAL) GEL AND COMPARISON OF ABSORPTION OF DETOMIDINE BY THE SUBLINGUAL AND INTRAMUSCULAR ROUTES. H Kaukinen[1], J Aspegrén[1], S Hyyppä[2], JS Salonen PhD[1], L Tamm[1]. [1]Orion Corporation, Turku, Finland. [2]Agrifood Research Finland, Ypäjä, Finland.

Detomidine is a potent and specific alpha-2 adrenoceptor agonist that is widely used as sedative for horses. Injectable detomidine has also been shown to produce effective sedation when given to horses sublingually.

The objective of this open randomised study was to determine the bioavailability of detomidine administered to horses as an oromucosal gel, and to compare the absorption and the sedative effect of detomidine by sublingual and intramuscular routes.

Nine healthy institute-owned horses received approximately 40 µg/kg detomidine either intravenously, intramuscularly (Domosedan® 10 mg/mL solution for injection, Orion Pharma, Espoo, Finland), or as an oromucosal gel (Domosedan Gel® 7.6 mg/mL oromucosal gel, Orion Pharma, Turku, Finland) administered under the tongue with a 7-day withdrawal period between treatments. All horses received three treatments with a three sequences crossover design.

Blood was collected from an indwelling catheter before drug administration and at 10, 20, 30, 40, 60, 120, 180, 240, 300, 360 and 480 minutes post-dosing for the determination of detomidine level in serum. The effect of routes of administration of detomidine on heart rate and rhythm was evaluated, and sedation was assessed by measuring head droop and scoring ataxia at the time points of blood collection. The mean bioavailability of detomidine was 22% (±5.3%) as the oromucosal gel and 38.2% (±7.9%) injected intramuscularly. The sedative effects correlated with drug concentrations in serum with all routes of administration.

Sublingual bioavailability of detomidine is lower than after intramuscular injection, as part of the gel is swallowed and does not reach the systemic circulation. Despite the moderate bioavailability, oromucosal detomidine gel at a dose of about 40 mcg/kg produces safe sedation in horses. The bioavailability is lower than after intramuscular injection, as part of the gel is swallowed and does not reach the systemic circulation. The slower absorption seems to have the advantage of less and milder adverse effects.

ABSTRACT #351

PHARMACOKINETICS OF ORALLY ADMINISTERED TERBINAFINE IN HORSES. Butch KuKanich, Megan Montgomery, and Elizabeth Davis. Kansas State University, Department of Anatomy and Physiology, Manhattan, KS.

The purpose of the study was to assess the pharmacokinetics of the antifungal drug terbinafine administered orally to horses. Terbinafine has a broad spectrum of antifungal effects and with the introduction of generic formulations is no longer cost prohibitive for use in veterinary medicine.

The study was approved by the Animal Care and Use Committee and Kansas State University. Six healthy horses weighing between 446 and 625 kg were administered 20 mg/kg of commercially available terbinafine tablets (250 mg per tablet) per os crushed and mixed with 60 mL light corn syrup. Blood samples were obtained at predetermined intervals for the determination of plasma drug concentrations with liquid chromatography and mass spectrometry. The pharmacokinetic parameters were estimated with noncompartmental analyses.

The plasma profile consisted of an absorption phase followed by a biphasic decline in plasma concentrations. The geometric mean (range) of the half-life (T 1/2), 8.1 (3.9–11.6) hours, clearance per fraction of the drug absorbed (Cl/F), 187 (132–282) mL/min/kg, and volume of distribution per fraction of the drug absorbed (Vd/F) 131 (50–266) L/kg were estimated for terbinafine. The maximum plasma concentration 0.31 (0.21–0.61) mcg/mL occurred at 1.7 (0.75–4) hours. The area under the curve extrapolated to infinity was 1.79 (1.18–2.51) hr*mcg/mL. One of the horses during the pilot study (data not presented) pawed at the ground, acted anxious and began circling in her stall 17 minutes after drug administration which resolved spontaneously within 30 minues. One of the horses during

the pharmacokinetic study pawed the ground 10 minutes after drug administration, curled its lips and shook its head 10 minutes after drug administration which resolved spontaneously within 30 minute. No other adverse effects were noted.

In conclusion, further studies are indicated to assess the pharmacokinetics and safety of higher and multiple doses of terbinafine in horses. Further studies are also indicated to assess the efficacy of terbinafine for the treatment of fungal diseases in horses.

ABSTRACT #352
ASSESSMENT OF CARDIAC FUNCTION, SKELETAL MUSCLE MORPHOLOGY, AND MUSCLE ENZYMES IN HORSES ON LONG-TERM CLENBUTEROL THERAPY. JA Thompson, SC Eades, AM Chapman, SA Barker, DB Paulsen, RS McConnico. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Potential for drug abuse exists in the horse industry, due to the perception that clenbuterol, a beta-adrenergic agonist approved for veterinary use as a bronchodilator in horses with reactive airway disease, increases performance and lean muscle mass. Recent studies into the effects of clenbuterol on exercising horses suggest that clenbuterol doses within therapeutic ranges negatively impact aerobic capacity and cardiac function. Studies in murine models show that clenbuterol causes skeletal muscle cell death at high doses, and clinical cases of equine rhabdomyolysis have been documented with clenbuterol overdose. This study examined the effects of oral clenbuterol, given at a dose up to 3.2ug/kg for 14 days, on skeletal and cardiac muscle in clinically healthy horses undergoing treadmill exercise, as compared to a control group.

Twelve clinically healthy Thoroughbred-type horses between the ages of 2 and 10 years old were evaluated. The study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Horses were randomly assigned to either the control group (n=6) or the clenbuterol group (n=6). Animals in the control group received saline by mouth twice daily for 14 days. Horses in the clenbuterol group received clenbuterol as Ventipulmin® syrup (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) by mouth twice daily for 14 days. The dose was incrementally increased during the treatment period to minimize adverse side effects, according to the following schedule: 0.8ug/kg twice daily for 3 days, then 1.6ug/kg twice daily for 3 days, then 2.4ug/kg twice daily for 3 days, then 3.2ug/kg twice daily for 5 days. Horses were subjected to daily submaximal treadmill exercise during the treatment period. Venous blood samples were collected from the jugular vein(s) every 3 days during treatment. Echocardiography was repeated 7 days after beginning treatment. Muscle biopsies were collected before and after treatment for determination of percent myocyte necrosis and apoptosis. Echocardiographic measurements, serum levels of creatinine kinase, aspartate aminotransferase, and cardiac troponin I, and serum clenbuterol levels were measured before, during, and after treatment.

Response variables were compared between treatment groups and across time periods using a Mann-Whitney U test and Friedman's test for repeated nonparametric data, respectively. An adjusted level of significance at $p<0.01$ was used to reduce type I error. Response variables were summarized as median and range.

No significant effect of clenbuterol or exercise on response variables was found between treatment and control groups at any time point, nor within groups over time. This study did not show any adverse effects of clenbuterol treatment on equine cardiac or skeletal muscle at the approved dose level for treatment in horses.

ABSTRACT #353
EFFECTS OF CLOPIDOGREL ON PLATELET FUNCTION IN THE HORSE. Dorothy D. Whelchel, Benjamin M. Brainard, Barbara Fortes, James N. Moore. College of Veterinary Medicine, University of Georgia, Athens, Georgia.

Clopidogrel (Plavix®), an antagonist at the platelet P2Y12 ADP receptor, significantly reduces platelet aggregation in many species. This study provided preliminary data regarding the pharmacodynamics of clopidogrel on platelet aggregation in the horse. Specifically, a compounded paste formulation of Clopidogrel was administered (2 mg/kg) orally once a day in the morning to 3 healthy horses for 3 days. Blood was collected before and at specified time intervals after the first oral dosing (t=0, 3, 6, 24, 48, 72, and 96 hours). A complete blood count and serum chemistry was performed at the start and conclusion of the dosing interval (t=0 and t=72). Serum serotonin (5-HT) concentrations were measured at each time point using a commercial ELISA. Mean platelet volume increased at 72 hr (9.1 ± 0.8 fl, $p<0.012$) from baseline values (8.0 ± 0.6 fl). ADP-induced platelet aggregation decreased from baseline values (mean±SD; $72.7\pm23\%$) at 72 h ($23.7\pm4\%$; $p<0.002$) and 96 h ($28.0\pm3\%$; $p<0.003$). Collagen-induced platelet aggregation decreased from baseline values ($86.3\pm10\%$) at 3 h ($19.7\pm4\%$, $p<0.0001$), 6 h ($20.7\pm5\%$ $p<0.0001$), 24 h ($18.3\pm5\%$; $p<0.0001$) and 72 h ($33.3\pm13.9\%$; $p<0.0001$). Serum 5-HT concentrations decreased from baseline (1580.9 ± 164.6 ng/mL) at 48 h (126.8 ± 44.3 ng/mL, $p<0.05$). Clopidogrel given at 2 mg/kg PO q 24 h appears to decrease both platelet aggregation and secretion of 5-HT after oral administration in healthy horses.

ABSTRACT #354
UPPER AND LOWER AIRWAY ENDOSCOPIC FINDINGS AND BRONCHOALVEOLAR LAVAGE-INDUCED LOWER AIRWAY COLLAPSE IN EQUINE RESPIRATORY INFLAMMATORY DISEASES. R Léguillette[1,2], K Koblinger[2], AJ Wasko[1]. [1]University of Calgary Faculty of Veterinary Medicine, Calgary, AB. [2]Moore&Co Veterinary Services, Calgary, AB.

In this study, we tested the hypotheses 1) that the lung inflammation in equine inflammatory airway disease (IAD) and recurrent airway obstruction (RAO) is correlated to the endoscopic inflammatory and mucus scores of upper and lower airway and 2) that horses with RAO have bronchial collapse during bronchoalveolar lavage (BAL) aspiration.

We used a random population of 138 horses around Calgary of age ranging 1–28 years. Both healthy horses and horses with respiratory symptoms not compatible with a respiratory infection were included in the study. One BAL was performed and recorded on each of the 138 subjects using a video endoscope, followed by a BAL cytological analysis. Each BAL video was viewed and scored on-site by one veterinarian, then *a posteriori* by two veterinarians. Pharyngitis was scored I to IV, pharyngeal mucus accumulation was scored 0 to 5, tracheal and lower airway mucus accumulation were scored 1 to 5, using previously published scales. We made a new scale to grade lower airway inflammation and collapse upon aspiration as 0 (none), 1(moderate) or 2 (severe). RAO was defined as a BAL $\geq$15% neutrophils and$\leq$2% mast cells and$\leq$1% eosinophils. IAD was defined as a BAL $>2\%$ mast cells or $>1\%$ eosinophils or 5–15% neutrophils.

Inter-observer correlations for upper airway pharyngitis and mucus was significant ($R^2=0.54$ and $R^2=0.24$ respectively). Inter-observer correlations for tracheal and lower bronchial mucus was significant ($R^2=0.51$ and $R^2=0.32$ respectively). There was no significant inter-observer correlation for neither lower airway inflammation nor airway collapse ($R^2=0.05$ and $R^2=0.15$ respectively).

Neutophil correlation: When collapse was scored on-site during the BAL procedure it was significantly correlated with neutrophil percentage in the BAL fluid ($R^2=0.29$). Horses with severe collapse were also 3.8 times more likely to have RAO than to be normal or have IAD.

When scores were averaged between observers, tracheal and bronchial mucus accumulation scores were significantly correlated with neutophils ($R^2=0.29$ and $R^2=0.37$ respectively), but it was not the case for the lower airways inflammation score ($R^2=0.10$).

Pharyngitis and pharynx mucus accumulation are not correlated to lung inflammation. Lower bronchial mucus accumulation is better correlated to the BAL neutrophil percentage than tracheal mucus score, possibly because it is more distal thus closer from the sampling site. Our lower airway inflammation score is too subjective. BAL-induced lower airway collapse is difficult to evaluate *a posteriori* but not on-site. Airways collapse during the BAL procedure is correlated to RAO and neutrophilia possibly due to airway remodeling.