American College of Veterinary Internal Medicine

Research Abstract Program of the 24th Annual ACVIM Forum
Louisville, KY, May 31 – June 3, 2006

Index of Abstracts

ORAL PRESENTATIONS – Wednesday, May 31

Time	#	Presenting Author	Abstract Title
SMALL ANIMAL – INFECTIOUS DISEASE			
2:00 pm	1	A. Litster	Isolation and Identification of *Staphylococcus Felis* and its Role as a Feline Urinary Tract Pathogen
2:15 pm	2	M. Lappin	*Bartonella* spp. Antigen Recognition Patterns in Cats with and without Fever
2:30 pm	3	J. Huebner	Is it Possible to Get a Protective Immunity against Feline Leukemia Virus Infection by Immunization with Its Transmembrane Envelope Protein?
2:45 pm	**4**	**M. Haber**	***Cytauxzoon felis* in Feral Cats from North Carolina and Florida**
3:00 pm	5	J. Levy	Impact of Vaccination on Parvovirus Testing in Kittens
3:15 pm	6	J. Levy	Serological Responses of Feral Cats to Vaccination in Trap-Neuter-Return Programs
3:30 pm	7	C. Crawford	Crossreactivity of Canine and Equine Influenza Antibodies
		BREAK	
4:15 pm	8	D. Spector	Antigen Testing for the Diagnosis of Blastomycosis
4:30 pm	9	J. Huebner	Mycoplasma Infection in Anaemic and Non Anaemic Dogs in Germany
4:45 pm	10	M. Lappin	*Wolbachia* spp. DNA in Blood of Dogs with *Dirofilaria immitis* Infection
5:00 pm	11	P. Diniz	*Anaplasma spp, Babesia canis, Bartonella henselae, Bartonella vinsonii subsp. berkhoffii, Borrelia burgdorferi, Dirofilaria immitis,* and *Ehrlichia canis* seroprevalences in Brazilian Dogs
5:15 pm	12	R. Goldstein	Evaluation of Serology and Circulating Immune Complexes in Dogs Naturally Infected with *Borrelia burgdorferi*
5:30 pm	13	R. Goldstein	Serology and Circulating Immune Complexes in Dogs Naturally Infected with *Borrelia burgdorferi* Before and After Doxycycline Therapy
5:45 pm	14	E. Breitschwerdt	*Borrelia burgdorferi* and *Anaplasma phagocytophilum*: Potenial Implications of Co-infection on Clinical Presentation in the Dog
SMALL ANIMAL – ONCOLOGY/HEMATOLOGY/IMMUNOLOGY			
2:00 pm	15	F. Rizzo	Thrombocytosis in Cats: A Retrospective Study of 51 Cases (2000-2005)
2:15 pm	16	L. Jessen	Dalteparin Causes Significant Dose Related Changes in Tissue Factor and Kaolin Activated Whole Blood Thromboelastography (TEG) Parameters
2:30 pm	17	R. Lobetti	Serum Urea: Creatinine Ratio in Hemolytic Disease

2:45 pm	18	M. Whelan	Use of the Canine Hemolytic Anemia Objective Score (CHAOS) to Predict Survival in Dogs with Immune-mediated Hemolytic Anemia
3:00 pm	19	E. Spangler	Retrospective Evaluation of D-dimer as an Indicator of Coagulation Status in 323 Dogs Admitted to a Veterinary Teaching Hospital
3:15 pm	20	S. Lavergne	Association of Drug-serum Protein Adducts and Anti-drug Antibodies in Dogs with Sulfonamide Hypersensitivity
3:30 pm	21	S. Lavergne	Anti-Platelet and Anti-Neutrophil Antibodies in Dogs with Sulfonamide-Associated Blood Dyscrasias
		BREAK	
4:15 pm	22	M. Isotani	PCR Analysis of C-kit Juxtamembrane Domain Mutation in Eight Cases of Canine Mast Cell Tumor Treated with Imatinib Mesylate (Gleevec)
4:30 pm	23	Y. Kato	Plasma Vascular Endothelial Growth Factor and Angiopoietin-2 Levels in Dogs with Hepatocellular Carcinoma, Hemangiosarcoma and Mammary Gland Tumor
4:45 pm	24	N. Mason	Development of an Artificial Antigen-presenting Cell to Support Ex-vivo Expansion of Canine T Cells for Adoptive Immunotherapy
5:00 pm	25	S. Winkler	Identification of Tumour Markers in a Newly Established Cell-line Allowing Development of Therapeutic Strategies for Prostate Cancer
5:15 pm	26	K. Kutara	Levovist-enhanced Contrast Harmonic Imaging of Canine Abdominal Masses
5:30 pm	27	S. Kim	Adjuvant Epirubicin in the Treatment of Splenic Hemangiosarcoma in Dogs
5:45 pm	**28**	**P. Woods**	**A Retrospective Analysis of Coarse Fractionation (0-7-21) External Beam Radiation Therapy of Canine Oral Melanoma (88 Cases)**

EQUINE**

2:00 pm	**29**	**T. Muirhead**	**Effect of Age on Systemic Antibody Response Following Rabies and Influenza Vaccinations in Healthy Horses**
2:15 pm	**30**	**M. Saulez**	**Altitude May Affect the Incidence and Severity of Exercise-Induced Pulmonary Hemorrhage in Thoroughbred Racehorses**
2:30 pm	**31**	**M. Miskovic**	**Evaluation of Lung Function and Airway Cytology in Horses with Recurrent Airway Obstruction in Long-term Remission**
2:45 pm	**32**	**J. Lawler**	**Evaluation of Di-tri-octahedral Smectite Neutralization of Alpha, Beta and Beta-2 Toxins of *Clostridium perfringens***
3:00 pm	33	M. Riihimäki	Clinical Alterations in Horses with Bronchoalveolar Lavage Eosinophilia
3:15 pm	**34**	**P. Jackson**	**Domperidone Causes an Increase in Plasma Endogenous ACTH in Horses with Pars Intermedia Tumors**
3:30 pm	**35**	**K. Chaney**	**PCR Results for EHV-1 Following Vaccination with Modified Live Virus**
		BREAK	
4:15 pm	36	**S. Bell**	**Temporal Detection of Equine Herpesvirus Infections of a Cohort of Mares and Their Foals**
4:30 pm	**37**	**J. Gold**	**Cytokine Profiles of Peripheral Blood Mononuclear Cells Isolated from Septicemic and Healthy Neonatal Foals**
4:45 pm	**38**	**J. Gold**	**ACTH, Cortisol and Vasopressin Levels of Septic (Survivors and Nonsurvivors) in Comparison to Normal Foals**
5:00 pm	**39**	**K. Chaney**	**The Effect of Uterine Torsion on Mare and Foal Survival: A Retrospective Study 1985-2005**
5:15 pm	40	P. Franzén	Long Term Follow Up of Experimentally Induced Infection with *Anaplasma Phagocytophilum* in Horses: Clinical and PCR Findings
5:30 pm	41	A. Hollis	The Effects of Norepinephrine and a Combined Norepinephrine and Dobutamine Infusion on Systemic Haemodynamics and Indices of Renal Function in the Normotensive Neonatal Thoroughbred Foal

Also see Equine sessions 113 – 126 (Thursday, June 1: 8:00 am – 12:30 pm) and Equine sessions 162 – 168 (Saturday, June 3: 8:00 am – 9:45 am)

5:45 pm	42	L. Armengou	Coagualtion Profile and Plasma D-dimer Concentration in Septic Newborn Foals

FOOD ANIMAL

2:00 pm	**43**	**M. Chigerwe**	**Comparison of Four Methods To Assess Colostral IgG Concentration**
2:15 pm	**44**	**J. Johnson**	**The Effect of Feeding Heat-Treated Colostrum on Serum Immunoglobulin Concentrations in Dairy Calves**
2:30 pm	**45**	**A. Rodriguez-Palacios**	**Oral Inoculation of Neonatal Calves with a Toxigenic Strain of Clostridium Difficile**
2:45 pm	**46**	**D. Foster**	**Evaluation of Two Colostrum Replacers Products in Dairy Calves**
3:00 pm	**47**	**S. Buczinski**	**Ultrasonographic Assessment of Bovine Fetal Well Being During Late Pregnancy in Normal, Compromised and Cloned Pregnancies**
3:15 pm	**48**	**G. Hallowell**	**Ultrasonographic Anatomy of the Bovine Eye**
3:30 pm	49	J. Nagy	Ultrasonographic Comparison of the Mammary Gland in Cows with Experimentally Induced Versus Naturally Occuring *E. coli* Mastitis
		BREAK	
4:15 pm	50	R. Nolen-Walston	Eastern Equine Encephalitis (EEE) in South American Camelids: 9 Cases
4:30 pm	**51**	**L. Waitt**	**Comparison of Insulin and Non-insulin Treated Camelids with Hypertriglyceridemia: 33 Cases**
4:45 pm	52	A. Gruntman	Albendazole Toxicity in Nine Alpaca Crias
5:00 pm	53	M. Miesner	Serum and Cerebrospinal Fluid Concentrations of Fenbendazole and Oxfendazole in Alpacas after Five Daily Oral Doses of 50mg/kg Fenbendazole 10% Suspension
5:15 pm	54	D. Nagy	Enhanced Periparturient Transmission of Bovine Leukosis Virus in Colostrum-deprived Calves
5:30 pm	55	M. Meylan	mRNA Levels and Binding Sites for Alpha2-adrenoceptor Subtypes in Muscle Layers of the Ileum and Spiral Colon of Dairy Cows
5:45 pm	56	M. Meylan	mRNA Levels and Densities of 5-hydroxytryptamine-4 receptors in the Gastrointestinal Smooth Muscles of Healthy Dairy Cows

ORAL PRESENTATIONS – Thursday, June 1

SMALL ANIMAL – ENDOCRINOLOGY

8:00 am	**57**	**R. Berg**	**Serum Insulin-like Growth Factor-I Concentration in Diabetic and Acromegalic Cats**
8:15 am	58	F. Tschuor	Intravenous Arginine Stimulation Test in Cats with Transient and Non-transient Diabetes Mellitus
8:30 am	59	R. Singh	Switching to an Ultra-low Carbohydrate Diet Has a Similar Effect on Postprandial Blood Glucose Concentrations to Administering Acarbose to Healthy Cats Fed a High Carbohydrate Diet
8:45 am	60	J. Wakeling	Does Subclinical Hyperthyroidism Exist in Cats?
9:00 am	61	M. Coradini	Delayed Gastric Emptying May Contribute to Prolonged Postprandial Hyperglycemia in Meal-fed Cats
9:15 am	62	L. Davison	Use of the Medtronic MiniMed Continuous Glucose Monitoring System for Assessment of Diabetes Mellitus in 40 Dogs
9:30 am	63	K. Verkest	Compensation for Obesity-Induced Insulin Resistance in Dogs: Causal Web Analysis of the Associations of Leptin and GLP-1
		BREAK	
10:45 am	64	S. Brennan	Clinical Efficacy and Safety of Leventa™ for Treatment of Hypothyroid Dogs
11:00 am	65	M. Mazaki-Tovi	Serum Leptin and Insulin Concentrations in Dogs with Hypothyroidism
11:15 am	66	M. Rick	Liquid Thyroxyl® is an Alternative to Soloxine® for Treating Canine Hypothyroidism

11:30 am	67	A. Milgrom	In Normal Dogs, ACTH Stimulation Test Results are Altered by Time in Cage, ACTH Dose, Previous Stimulation Tests and Steroid Therapy
11:45 am	68	E. Lennon	Use of Basal Plasma Cortisol Concentrations to Rule Out a Diagnosis of Hypoadrenocorticism
12:00 pm	**69**	**A. Thompson**	**Characterization of Glucocorticoid Deficient Hypoadrenocorticism in Dogs: A Retrospective Study**
12:15 pm	**70**	**C. Broemel**	**Serum Inhibin Immunoreactivity in Neutered Dogs with Adrenal Dysfunction**

SMALL ANIMAL – CARDIOLOGY**

8:00 am	71	S. Gordon	Atrial Septal Defects in an Extended Family of Standard Poodles
8:15 am	72	D. Hogan	Use of a Peripheral Vascular Occlusion Device for Correction of Patent Ductus Arteriosus in Dogs
8:30 am	**73**	**T. Nguyenba**	**Patent Ductus Arteriosus Occlusion with an Investigational Amplatzer Canine Ductal Occluder**
8:45 am	74	M. Oyama	Effect of Carvedilol in Dogs with Dilated Cardiomyopathy: Results from a Prospective Placebo-Controlled Randomized Clinical Trial
9:00 am	**75**	**R. Roland**	**Acute Cardiovascular Effects of Pimobendan in Dogs with Stable Congestive Heart Failure Due to Chronic Degenerative Atrioventricular Valve Disease**
9:15 am	76	S. Rosenthal	Association of Pimobendan with Ventricular Arrhythmias in Dogs with Congestive Heart Failure
9:30 am	77	H. Green	Dual-Chamber Cardiac Pacing in 25 Dogs
		BREAK	
10:45 am	78	S. Disatian	Effects of Age and Heart Rate on Right Ventricular Myocardial Velocities Assessed by Tissue Doppler Imaging in Healthy Nonsedated Cats
11:00 am	79	V. Gouni	Tissue Doppler Imaging Detects Radial and Longitudinal Myocardial Dysfunction in a Cat Model of Hypertrophic Cardiomyopathy
11:15 am	80	J. Abbott	Evaluation of Relationships between Doppler-Derived Aortic Ejection Velocity and Ventricular Performance in Healthy Anesthetized Dogs
11:30 am	81	G. Wess	Strain and Strain Rate Imaging - New Ultrasound-based Parameters for Quantification of Regional Myocardial Function
11:45 am	**82**	**S. Cole**	**Use of Real Time Three Dimensional Echocardiography in the Characterization of Congenital and Acquired Cardiac Disease**
12:00 pm	83	M. Oyama	Predictive Value of Natriuretic Peptide and Cardiac Troponin-I Testing in Dogs with Moderate to Severe Congenital Subaortic Stenosis
12:15 pm	**84**	**R. Baumwart**	**Elevated Serum Cardiac Troponin I Levels In Boxer Dogs with Arrhythmogenic Right Ventricular Cardiomyopathy**

SMALL ANIMAL – NEUROLOGY

8:00 am	85	D. O'Brien	Standard Poodle Neonatal Encephalopathy Locus Maps to Canine Chromosome 36
8:15 am	**86**	**R. Pettigrew**	**CNS Hypomyelination in a Litter of Rat Terriers with Goitrous Cretinism and a Mutation in the Thyroid Peroxidase Gene**
8:30 am	**87**	**R. Packer**	**Characterization and Mode of Inheritance of an Episodic Dyskinesia in the Chinook Dog**
8:45 am	88	D. Geiger	Inherited Encephalomyelopathy and Polyneuropathy in Two Boxer Littermates
9:00 am	**89**	**M. Campbell**	**NF-2 gene Expression in Canine Meningiomas**
9:15 am	90	J. Burkitt	Retrospective Evaluation of Tetanus in Dogs: 38 Cases
9:30 am	**91**	**D. Westworth**	**Clinicopathological Features of Choroid Plexus Tumors in Dogs: 44 Cases**
		BREAK	
10:45 am	**92**	**S. Petersen**	**Spinal Cord Disease in Dogs with Neoplasia Arising from the Central Nervous System**

Also see Cardiology sessions 132 – 140 (Friday, June 2: 9:15 am – 12:30 pm)

11:00 am	93	S. Platt	Cerebrospinal Fluid Glutamate Levels in Dogs with Intracranial Neoplasia
11:15 am	94	T. Flegel	Computer-assisted MRI Analysis of the Cerebellum in American Staffordshire Terriers with Cerebellar Cortical Degeneration
11:30 am	**95**	**S. Cerda-Gonzalez**	**Morphology of the Caudal Fossa in Cavalier King Charles Spaniels**
11:45 am	**96**	**S. Cerda-Gonzalez**	**Characteristics of Cerebrospinal Fluid Flow in Cavalier King Charles Spaniels**
12:00 pm	**97**	**R. da Costa**	**One-year Magnetic Resonance Imaging Follow-up of Dogs with Cervical Spondylomyelopathy**
12:15 pm	**98**	**R. da Costa**	**Cervical Spondylomyelopath in Dogs: Comparison of Conservative and Surgical Treatments – 104 Cases**

SMALL ANIMAL – NEPHROLOGY/UROLOGY

8:00 am	**99**	**A. Defarges**	**Use of Electrohydraulic Shock-wave Lithotripsy for the Fragmentation of Bladder Calculi: A Pilot Study in Dogs**
8:15 am	100	P. Lazaretti	Qualitative Changes in Proteinuria Correlate with Changes in Kidney Structure and Function as X-Linked Hereditary Nephropathy (XLHN) Progresses in Affected Male Dogs
8:30 am	**101**	**A. Berent**	**Palliative Stenting for Malignant Urethral Obstructions in 13 Dogs**
8:45 am	102	J. Barsanti	Accuracy of Urinalysis in Predicting the Type of Infecting Bacteria in Urinary Tract Infection (UTI)
9:00 am	103	H. Xu	Effect of Dietary Sodium on Urine Characteristics in Healthy Adult Cats
9:15 am	104	G. Lees	Identification of the Gene Mutation that Causes Autosomal Recessive Hereditary Nephropathy in English Cocker Spaniels
9:30 am	105	B. Egner	Prenatal Programming and Arterial Hypertension in Marmoset Monkeys
		BREAK	
10:45 am	106	B. Gerber	Complement Protein C3, Circulating Immune Complexes and Antibodies Against *Borrelia burgdorferi* in Bernese Mountain Dogs
11:00 am	107	A. Craig	Refining the Reference Interval for Plasma Creatinine in Dogs: Effect of Age, Gender, Body Weight and Breed
11:15 am	108	R. Jepson	Evaluation of Cystatin C as a Marker of GFR in Hyperthyroid Cats
11:30 am	109	M. Robson	Intrinsic Acute Renal Failure (ARF) Associated with Non-Steroidal Anti-Inflammatory Drug (NSAID) Use in Juvenile Cats Undergoing Routine Desexing-16 Cases 1998-2005
11:45 am	110	C. Lapointe	N-acetyl-ß-D-glucosaminidase Index as an Early Biomarker for Chronic Renal Insufficiency in Cats with Hyperthyroidism
12:00 pm	111	A. Hezel	Influence of Hydrochlorothiazide on Urinary Calcium Oxalate Relative Supersaturation in Healthy Adult Cats
12:15 pm	112	S. Schellenberg	The Effect of Hydrocortisone on Urinary Protein Excretion in Dogs

EQUINE

8:00 am	113	L. Fielding	Estimation of Acute Fluid Shifts in Horses Using Bioelectrical Impedance Analysis
8:15 am	114	L. Pantaleon	Cardiopulmonary Effects of Small Volume Resuscitation in Anesthetized Endotoxemic Horses
8:30 am	115	M. Bowen	The Effects of Aging on the Structure and Function of the Equine Aortic Valve
8:45 am	116	M. Durando	Echocardiographic Estimation of Pulmonary Arterial Pressures in Horses with Cardiac Disease
9:00 am	117	T. Holbrook	Biochemical Evidence of Cardiac Injury in Horses with Cantharidin Toxicosis
9:15 am	118	N. Frank	Effects of Long-Term Levothyroxine Administration on Adipose and Skeletal Muscle Tissue Glucose Transporter Gene Expression in Mares
9:30 am	119	M. McCue	The Prevalence of Polysaccharide Storage Myopathy in the Quarter Horse Population
		BREAK	

10:45 am	120	A. Firshman	Hyperinsulinemic Euglycemic Clamping and Insulin Sensitivity in Belgian Draft Horses with Polysaccharide Storage Myopathy
11:00 am	**121**	**L. Lecoq**	**Genomic and Non genomic Effects of Dexamethasone on Equine Peripheral Blood Neutrophils**
11:15 am	122	C. Sanchez	Effect of Fentanyl on Somatic and Visceral Nociception in Conscious Horses
11:30 am	123	R. MacKay	Use of a Quantitative Intradermal Terbutaline Test for Measuring Sweat Production in Normal and Anhidrotic Horses
11:45 am	124	T. Meredith	Equine Adipose Tissue is a Source of Multi-potent Stem Cells
12:00 pm	125	D. Thamm	Single-Dose Pharmacokinetics of Oral Piroxicam in Horses
12:15 pm	126	D. Thamm	Cyclooxygenase-2 Expression in Equine Tumors

ORAL PRESENTATIONS – Friday, June 2

SMALL ANIMAL – RESPIRATORY/CARDIOLOGY

8:00 am	127	L. Taylor	Bordetellosis in Dogs: 2000-2005
8:15 am	**128**	**E. Schooley**	**Effects of Cyproheptadine and Cetirizine on Airway Eosinophilia in Experimental Feline Asthma**
8:30 am	129	L. Johnson	Flexible Bronchoscopy and Bronchoalveolar Lavage in the Cat: Procedure and Outcome (2001-2006)
8:45 am	130	B. Schulz	Clinical and Laboratory Findings in 17 Cats with Chronic Bronchial Disease
9:00 am	131	J. Willesen	Hematological Changes in 42 Dogs Naturally Infected with *Angiostrongylus Vasorum*
9:15 am	132	C. Schwarzwald	Atrial, SA Nodal, and AV Nodal Electrophysiology in Standing Horses: Reference Values and Electrophysiologic Effects of Quinidine and Diltiazem
9:30 am	133	C. Schwarzwald	Echocardiographic Detection of Atrial Mechanical Dysfunction in Horses after Conversion of Atrial Fibrillation to Sinus Rhythm
		BREAK	
10:45 am	134	M. Oyama	Arrhythmogenic Right Ventricular Cardiomyopathy in Boxer Dogs is Associated with Calstabin2 (FKBP12.6) Deficiency
11:00 am	135	R. Prošek	Comparision of Sotalol and Mexiletine Versus Standalone Sotalol in Treatment of Boxer Dogs with Ventricular Arrhythmias
11:15 am	136	L. Olsen	Physiological Flow Murmurs in Cavalier King Charles Spaniels
11:30 am	137	L. Pedersen	The Transcription of Endothelin-1 and Receptors in Mitral Valves Exposed to Static Stretch
11:45 am	138	D. Hogan	Clopidogrel (Plavix®) and Collateral Vessel Development in Experimental Feline Aortic Thrombosis
12:00 pm	**139**	**C. Vargo**	**Determination of the Effect of Low Molecular Weight Heparin Administration on Coagulation Parameters in Healthy Cats**
12:15 pm	140	M. Small	Effects of Clinically Recommended Doses of IV Adenosine and Vagal Maneuvers on Heart Rate and Blood Pressure in Conscious Dogs

SMALL ANIMAL – GASTROENTEROLOGY/HEPATOLOGY

8:00 am	**141**	**K. Mix**	**Evaluation of the Normal Feline Pancreas With Computed Tomography**
8:15 am	142	A. Schweighauser	Evaluation of Endosonography as a New Diagnostic Tool in Feline Pancreatitis
8:30 am	143	J. Sinclair	Continuing Pancreatic Inflammation or Reduced Exocrine Function are Common in Dogs after Acute Pancreatitis
8:45 am	144	P. Xenoulis	Association between Serum Triglyceride and Canine Pancreatic Lipase Immunoreactivity Concentrations in Miniature Schnauzers
9:00 am	145	J. Steiner	False Positive Results of Measurement of Fecal Elastase Concentration for the Diagnosis of Exocrine Pancreatic Insufficiency in Dogs

9:15 am	146	N. Luckschander	Activation of Nuclear Factor-Kappa B in Dogs with Chronic Enteropathies
9:30 am	147	P. Xenoulis	Detection of the Gene Encoding Clostridium Perfringens Enterotoxin in the Small Intestine of Healthy Dogs and Dogs with Diarrhea
		BREAK	
10:45 am	**148**	**D. Dereszynski**	**Clinical Features of Foodborne Aflatoxin Hepatotoxicity in 23 Dogs**
11:00 am	149	G. Hoffmann	Double-blind, Placebo-controlled Treatment with D-penicillamine Against Hepatic Copper Accumulation in Labrador retrievers
11:15 am	**150**	**L. Sepesy**	**Vacuolar Hepatopathy in Dogs: 336 Cases (1993-2005)**
11:30 am	151	C. Webster	Chronic Inflammatory Hepatic Disease in Labrador Retrievers: Clinical Presentation and Prognostic Factors
11:45 am	152	C. Weisse	Percutaneous Transvenous Coil Embolization (PTCE) of Canine Intrahepatic Portosystemic Shunts: Experience in 33 Dogs
12:00 pm	153	S. Torisu	Sustained Severe Hypoglycemia During Surgery as a Genesis of Global Brain Damage in Post Ligation Seizure of Congenital Portosystemic Shunts Dogs
12:15 pm	154	G. Hoffmann	Copper-Associated Chronic Hepatitis in Labrador Retrievers: 15 Clinical Patients and Their Family – ***ECVIM Award Winning Abstract***

ORAL PRESENTATIONS – Saturday, June 3

SMALL ANIMAL – PHARMACOLOGY/NUTRITION/METABOLISM

8:00 am	155	S. Thomasy	Pharmacokinetics of Penciclovir Following Oral Administration of Famciclovir to Cats
8:15 am	156	M. Papich	Cetirizine (Zyrtec®) Pharmacokinetics In Healthy Cats
8:30 am	157	J. Hall	Dose Not Ratio of (n-6) and (n-3) Fatty Acids Is Responsible for Changes in the Plasma Fatty Acid Profile of Normal Dogs
8:45 am	158	J. Bauer	Docosapentaenoic Acid (22:5n-3) is not a Substrate for Lecithin:Cholesterol Acyl Transferase in n-3 Fatty Acid Fed Dogs
9:00 am	159	D. Chan	Relationship between Plasma Amino Acids, C-reactive Protein, Illness Severity and Outcome in Critically Ill Dogs
9:15 am	160	C. Frondoza	Inhibition of Pro-Inflammatory Cytokine and COX-2 Expression in Chondrocytes and Monocytes by Avocado Soybean Unsaponifiables (ASU)
9:30 am	161	C. Kirk	Canine Dietary Iron Recommendations are Insufficient to Correct Iron Deficiency in Growing Dogs

EQUINE

8:00 am	162	T. Goetz	Arterial Hypoxemia in Exercising Thoroughbred Horses is not Affected by Pre-exercise Bronchodilator Administration
8:15 am	163	C. Swiderski	The Ratio of Urine Deoxypyridinoline to Pyridinoline Identifies Horses with Hyperelastosis Cutis (a.k.a. Hereditary Equine Regional Dermal Asthenia or HERDA)
8:30 am	164	P. Johnson	Stimulated Secretion of Matrix Metalloproteinases from Equine Keratinocytes by Different Strains of *Streptococcus bovis*
8:45 am	165	B. Darien	Tahitian Noni® Equine Essentials™: A Novel Anti-inflammatory and a COX-2 Inhibitor which Regulates LPS-induced Inflammatory Mediator Expression in Equine Neonatal Monocytes
9:00 am	166	M. Lendau	Clinical Evaluation of a Serum IgE (Fc Epsilon Receptor) Test for Equine Insect Hypersensitivity
9:15 am	167	J. Slack	Non-invasive Estimation of Pulmonary Arterial Pressures in Horses with Recurrent Airway Obstruction
9:30 am	168	F. Andrews	Effects of Intravenously Administrated Omeprazole on Gastric Juice pH and Gastric Ulcer Scores in Adult Horses

POSTER PRESENTATIONS

On Display: Wednesday, May 31, 2:00 pm – 7:00 pm; Thursday & Friday, June 1 & 2, 7:00 am – 10:00 pm; Saturday, June 3, 7:00 am – 4:30 pm

Attended by Author: Thursday, June 1, 6:00 pm – 7:00 pm & Friday, June 2, 9:50 am – 10:45 am

#	Presenting Author	Abstract Title
SMALL ANIMAL – INFECTIOUS DISEASE		
175	**A. DeClue**	**Effects of Ketamine Infusion on Hemodynamic and Immunologic Variables in a Canine Model of Endotoxemia**
176	N. Drazenovich	Use of Conventional And Real-Time PCR to Determine the Epidemiology of Hemoplasmosis in Anemic and Non-Anemic Dogs
177	N. Drazenovich	Use of Conventional And Real-Time PCR to Determine the Epidemiology of Hemoplasmosis in Anemic and Non-Anemic Cats
178	**M. Haber**	**Prevalence of *Cytauxzoon felis* DNA in Blood of Cats with Suspected Hemoplasmosis**
179	S. Tanaka	Inhibitory Effect of R-9-(2-phosphonomethoxypropyl) Adenine on the Replication of Feline Immunodeficiency Virus In Vitro and Possible Use of Its Oral Medication Derivative
180	A. Reche, Jr.	CD4+ T-lymphocytes Count and CD4:CD8 Ratio in a Colony of Cats with Chronic Gingivitis and Naturally-infected with Feline Immunodeficiency Virus
181	J. Levy	The Effect of Anesthesia and Surgery on Serological Responses to Vaccination in Kittens
182	M. Lappin	Prevalence of *Rickettsia felis* in the Blood of Cats and their Fleas in the United States
183	**D. Bayliss**	**Prevalence of *Rickettsia felis* Infections in Cats with and without Fever**
184	**A. Ishak**	**Marbofloxacin for the Treatment of Experimentally-Induced *Mycoplasma haemofelis* Infection in Cats**
185	K. Dowers	Detection of Anti-Erythrocyte Antibodies Using Direct Coomb's Testing in Cats Experimentally Infected with *Mycoplasma haemofelis*
186	**C. Gurnee**	**Molecular Characterization of Multiple-drug Resistant *E. coli* at a Veterinary Teaching Hospital**
187	D. Boothe	*Escherichia coli* Antimicrobial Resistance in Small Animals: The Scope of the Problem
188	**B. Hanselman**	**Methicillin-resistant *Staphylococcus aureus* Colonization in Veterinary Professionals**
189	M. Vengust	Methicillin-resistant Staphylococci in Healthy Dogs and Horses in Slovenia
190	M. Littman	Seroprevalence of Borrelia Burgdorferi Antibodies in Dogs at a Veterinary Teaching Hospital in a Lyme Endemic Area
191	K. Song	Seroprevalence of *Ehrlichia canis* and *Borrelia burgdoferi* Infection in Dogs, South Korea
192	**J. Diroff**	***Bartonella* Seroprevalence in Diseased Dogs and Healthy Blood Donor Dogs in the Northeastern United States**
193	P. Diniz	Molecular Prevalence of *Bartonella* spp. in Brazilian Dogs
194	A. Alleman	Experimental Inoculation of Dogs with a Human Isolate (NY18) of *Anaplasma Phagocytophilum* and Demonstration of Persistent Infection following Doxycycline Therapy
195	H. Wakabayashi	Possible Treatment Strategy and Clinical Estimation Factors for Onset, Relapse and Prognosis of *Bebesia gibsoni* Infection
196	K. Kasper	Development of a Real-Time PCR Assay for the Detection of Pathogenic Leptospires in Canine Urine
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ABSTRACT #1

ISOLATION AND IDENTIFICATION OF *STAPHYLOCOCCUS FELIS* AND ITS ROLE AS A FELINE URINARY TRACT PATHOGEN.

Annette Litster[1], Susan Moss[1], Robert Rees[2] and Darren Trott[1]. School of Veterinary Science, The University of Queensland[1] and Bayer Animal Health Ltd[2], Australia.

The objective of this study was to investigate the incidence of *Staphylococcus felis* as a pathogen in infections of the urinary tract in Australian cats. *S. felis* is an infrequently recognized coagulase-negative staphylococcal species, first identified in 1989 from feline clinical specimens. It has similar phenotypic characteristics to *Staphylococcus simulans*, except for its sensitivity to bacitracin (2 IU/mL) and alkaline phosphatase activity. It also can be definitively differentiated from *S. simulans* by partial 16 s rRNA gene sequencing.

Urine was collected by cystocentesis from cats exhibiting clinical signs of urinary tract disease, and submitted to The University of Queensland School of Veterinary Science diagnostic laboratory over a 2-year period (2004–2005; n = 97). All specimens were obtained as part of a clinical trial and subjected to complete urinalysis as well as culture and sensitivity. The identity of each isolate was confirmed by standard veterinary diagnostic microbiological methods. In addition, all *Staphylococcus* spp. were fully identified using standard biochemical protocols and partial 16 s rRNA gene sequencing, and antimicrobial susceptibility patterns were determined using Clinical and Lap;oratory Standards Institute (CLSI) performance standards.

The 97 urinary specimens yielded 117 isolates, which were identified as follows – *E. coli* (n = 46; 39.3%), *Enterococcus faecalis* (n = 30; 25.6%), *S. felis* (n = 21; 17.9%), *Proteus sp.* (n = 7; 6.0%), *Enterobacter/Klebsiella spp.* (n = 4; 3.4%), *Staphylococcus aureus* (n = 3; 2.6%), *Pseudomonas aeruginosa* (n = 2; 1.7%), *Staphylococcus intermedius* (n = 2; 1.7%), *Enterococcus faecium* (n = 1; 0.9%), and *Streptococcus bovis* (n = 1; 0.9%). On urinalysis, specific gravity (SG) was higher in urine positive for *S. felis* (n = 21; median SG = 1.034) than in urine that was culture positive for other bacterial species (n = 74 median SG = 1.021 p = 0.024). Erythrocyte count (EC) tended to be higher in urine positive for *S. felis* (n = 21; median EC = 1000) than in urine that was culture positive for other bacterial species (n = 74 median EC = 100 p = 0.101), but this difference did not reach statistical significance. Nineteen of the 21 *S. felis* isolates were sensitive to the full range of antimicrobials tested (n = 12), and the remaining two isolates were resistant only to penicillin and ampicillin. All *S. felis* were also sensitive to bacitracin.

Staph. felis is a feline urinary tract pathogen that may easily be overlooked because it is difficult to differentiate from other coagulase-negative *Staphylococcus* species. It is more likely to act as a primary pathogen in cats with urine that has a relatively high SG.

ABSTRACT #2

BARTONELLA SPP. ANTIGEN RECOGNITION PATTERNS IN CATS WITH AND WITHOUT FEVER.

MR Lappin,[1] JR Hawley,[1] EB Breitschwerdt.[2] From Colorado State University,[1] Fort Collins, CO and North Carolina State University, [2] Raleigh, NC.

Bartonella spp. infections are common in cats. Some cats with *Bartonella* spp. DNA in blood or antibodies in serum are clinically ill and have fever leading to the hypothesis that the organisms may be associated with fever of unknown origin. In addition, it has been hypothesized that western blot immunoassay (WB) results can be used to predict *Bartonella* spp. bacteremia. The objectives of this study was to use WB to determine whether *Bartonella* spp. antigen recognition patterns exist that correlate to the presence of fever or presence of *Bartonella* spp. DNA in blood.

An IgG heavy chain-specific, WB using blood agar grown *B. henselae* as the antigen source was optimized using sera collected over time from experimentally-inoculated cats; 9 immunodominant antigens were selected by analysis of the pre-infection and post-infection results. Matched serum and blood samples from client-owned cats with (n = 20) or without fever (n = 19) that resided in high flea prevalence states were selected for analysis using the optimized WB and a previously published conventional PCR assay. Results of the serum WB were considered positive if any 2 or more of the 9 antigens were recognized and were greater than a predetermined appropriate density. Results of the PCR assay on blood were considered positive if an amplicon of the appropriate size was detected. Fisher's exact test was used to compare between groups for some parameters; significance was defined as $p < 0.05$.

WB results were positive in 10 of 20 cats (50%) with fever and 6 of 19 cats (31.6%) without fever; differences between groups were not significant (p = 0.1998). PCR assay results were positive in 5 of 20 cats (25%) with fever and 1 of 19 cats (5.3%) of cats without fever; differences between groups were not significant (p = 0.1022). Of 16 WB positive cats, 3 were PCR positive (percentage concordance = 59%). All 3 PCR positive but WB negative samples were from acutely ill, febrile cats. Three or more antigens were recognized by 5 of 10 (50%) WB positive cats with fever and 5 of 6 (83.3%) WB positive cats without fever. Antigens with the apparent molecular masses of 48, 57, 62, 69, and 82 kD were each recognized by at least 4 cats but there was no antigen recognition pattern that was specifically detected in cats with or without fever.

We conclude that determination of *Bartonella* spp. antigen recognition patterns in this sample set could not be used to predict which cats had fever associated with *Bartonella* spp. infection. In addition, presence of antibodies detected by WB did not reliably correlate with the presence or absence of *Bartonella* spp. DNA in blood and so should not be used to predict the infection status of individual cats. Lastly, in peracute infections, *Bartonella* spp. serum antibody test results can be falsely negative.

ABSTRACT #3

IS IT POSSIBLE TO GET A PROTECTIVE IMMUNITY AGAINST FELINE LEUKEMIA VIRUS INFECTION BY IMMUNIZATION WITH ITS TRANSMEMBRANE ENVELOPE PROTEIN?

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The importance of neutralizing antibodies specific for retrovirus infections like Feline Leukaemia Virus (FeLV) became recently a focus in feline medicine. Since retroviruses integrate their genome in the genome of the infected cell, where they may persist undetected from cellular immunity, the induction of neutralizing antibodies is therefore of great advantage in preventing infection of cells early after exposure of an individual to the virus. Owing to its conserved domains, the transmembrane (TM) envelope protein is a suitable target. We showed recently induction of neutralizing antibodies specific for FeLV using their TM protein p15E. The feline leukaemia virus (FeLV) vaccines that are currently in wide use are generally poor inducers of virus-neutralizing antibodies, although such antibodies appear after recovering from challenge. However, the presence of neutralizing antibodies in cats recovering from natural FeLV infection clearly correlates with resistance to subsequent infection and passive transfer of antibodies can protect other animals. Cats immunized with the transmembrane envelope protein p15E of FeLV developed high titres of neutralizing antibodies specific for FeLV. The ability of p15E to induce neutralizing antibodies in cats leads to the suggestion that it might be reasonable to be included in the next generation of vaccines.

To study whether vaccinating with the TM protein could protect cats against FeLV infection, cats were immunized with the TM protein p15E of FeLV-A, with the commercial vaccine Leucogen® comprising the unglycosylated surface envelope protein p45 of FeLV-A, or with a combination of both and were subsequently challenged oronasally with 4 doses of 1 × 106 ffu/ml FeLV-A Glasgow-1 strain.

All of the cats in the present experiment became provirus positive by real time PCR after FeLV challenge, indicating that neither the commercial vaccine, Leucogen®, p15E nor the combination of both protected from provirus acquisition and minimal viral replication. But animals immunized with Leucogen® or the combination of Leucogen® and p15E had the lowest provirus load and were always p27 antigen negative using a commercial ELISA test.

So in contrast to non-immunized cats, characterized by provirus load and p27 antigenaemia, protection was achieved in all animals immunized with Leucogen® and with the combination. Interestingly 3 of 6 animals immunized with p15E alone showed a successful immunisation regarding proviral load and p27 antigenaemia. This is the first report showing protection from retrovirus infection in vivo by immunization with a TM protein. However, in none of the animals immunized with p15E or the commercial vaccine or both was sterilizing immunity observed. These data may have an impact on the generation of vaccines against other retroviruses, including HIV.

ABSTRACT #4

CYTAUXZOON FELIS IN FERAL CATS FROM NORTH CAROLINA AND FLORIDA.

MD Haber[1], MD Tucker[1], HS Marr[1], JK Levy[2], MR Lappin[3], AJ Birkenheuer[1]. [1]North Carolina State University, Raleigh, NC. [2]University of Florida, Gainesville, FL. [3]Colorado State University, Fort Collins, CO.

Cytauxzoon felis causes fatal disease in domestic cats. Reports of survival of some domestic cats have been documented. Persistent parasitemia in these cats without clinical illness has led to our speculation that domestic cats may serve as an additional reservoir host for *C. felis*. To our knowledge there are no prevalence studies of *C. felis* in domestic cats. Therefore, the purpose of this study was to determine the prevalence of *C. felis* infected domestic cats

presenting to trap-neuter-release programs in Northern Florida and Central North Carolina.

Blood samples from 886 domestic cats (494 from Northern Florida; 392 from Central North Carolina) were tested using a *C. felis*-specific PCR assay. Amplification of feline DNA was performed to evaluate for PCR inhibitors on all samples that tested negative for *C.felis*. Positive and negative controls consisted of *C. felis* infected samples and water (no DNA) respectively.

Two Florida samples tested positive for the presence of *C. felis* DNA. These samples were sequenced and confirmed to be *C. felis*. Six samples were excluded due to PCR inhibitors.

This is the first report of *C. felis* in prospectively sampled domestic cats. Based on these results, the prevalence of *C. felis* infection in this population is less than 0.1% (99% confidence level). This is much lower than in the previously described Oklahoma bobcat population (~50%). Domestic cats are therefore less likely to be a major reservoir host. Further epidemiologic investigations should consider the role of chronically infected cats especially in areas in which cytauxzoonosis appears hyperendemic.

ABSTRACT #5

IMPACT OF VACCINATION ON PARVOVIRUS TESTING IN KITTENS. JK Levy, EV Patterson, MJ Reese SJ Tucker. University of Florida College of Veterinary Medicine, Gainesville, FL.

Animal shelters are increasingly reporting large-scale outbreaks of the parvovirus, feline panleukopenia virus (FPV). Primary immunization against FPV results in detectable antibodies within 5 to 7 days, but confers protection against infection even earlier. For this reason, guidelines for shelters suggest vaccination immediately upon admission. Definitive diagnosis of FPV can be made by identification of parvovirus in the feces of affected cats. In dogs, it is believed that parvovirus vaccines result in transient fecal shedding of vaccine virus that is indistinguishable from natural infection by the diagnostic test kits. If this is true for cats, FPV vaccination may interfere with diagnosis in cats with clinical signs suggestive of FPV. The purpose of this study is to test the most commonly used FPV vaccines for fecal viral shedding that would interfere with interpretation of diagnostic test results for FPV.

A total of 64 8 to 10 week-old SPF kittens were randomized into 8 treatment groups to receive one of 8 vaccines against FPV, FCV, and FHV (6 modified live, 2 inactivated; 7 SC, 1 IN) vaccines. Blood was collected for FPV antibody titer prior to vaccination and at the end of the 2-week test period. Feces were tested daily for a total of 15 days using 3 different tests designed for detection of canine parvovirus (IDEXX, Synbiotics, Agen).

All 3 tests reacted strongly positive when feces from a FPV-infected kitten was tested. All kittens had negative fecal parvovirus tests prior to vaccination. Several kittens had positive tests (IDEXX n = 1, Synbiotics n = 13, Agen n = 4) for 1 to 8 days. Of the 36 individual positive tests, only 2 (from the same kitten) were considered to be ''strong-positives.'' Positive results occurred in each vaccine group except that receiving the IN product. None of the kittens had protective FPV titers prior to vaccination. At 2 weeks post-vaccination, 25–38% of kittens receiving inactivated vaccines had protective titers, whereas 75–100% of kittens receiving modified live vaccines had protective titers.

Tests used for detection of parvovirus may have positive test results (usually weak) when used with feces from recently vaccinated kittens, and the frequency of this occurrence varies among different brands of tests. MLV vaccines produced protective titers by 2 weeks more frequently than inactivated vaccines.

ABSTRACT #6

SEROLOGICAL RESPONSES OF FERAL CATS TO VACCINATION IN TRAP-NEUTER-RETURN PROGRAMS. JK Levy, SM Fisher, CM Quest, SJ Tucker. University of Florida College of Veterinary Medicine, Gainesville, FL.

Trap-neuter-return (TNR) programs are an increasingly popular method of controlling unowned feral cat populations. In these programs, cats are humanely trapped, undergo sterilization surgery, then returned to their colonies. In order for TNR programs to be successful, they must maximize the number of cats sterilized with the limited resources available. In the largest programs, a herd health approach is taken, and many treatments routinely offered to pet cats are considered to be too impractical or expensive for feral cats. Controversy exists over whether a single vaccine administered during the stressful events of capture, anesthesia, and surgery offers sufficient protection to warrant routine vaccination of feral cats. The purpose of this study was to determine the response rate of feral cats to modified live virus (MLV) and inactivated (IA) vaccines against panleukopenia virus (FPV), calicivirus (FCV), and herpes virus (FHV) and IA rabies vaccines.

Feral cats were trapped by their caretakers and transported to the TNR clinic. Cats were anesthetized with a combination of tiletamine-zolazepam-ketamine-xylazine (TKX) IM. Blood was collected, microchip identification was implanted, and then cats were spayed or castrated. Vaccines were administered SC (MLV n = 29, IA n = 32) before anesthesia was reversed with yohimbine IV. Cats were discharged to their caretakers the same day for return to their colonies with instructions for recapture in 8 to 12 weeks for repeat blood collection. Antibody titers for FPV, FCV, FHV, and rabies virus were determined for the paired serum samples.

Percent protected	FPV	FCV	FHV	Rabies
Pre-vaccination	33%	64%	21%	10%
Post-vaccination	90%	93%	56%	98%

A total of 61 cats weighing 3.0 ± 0.7 kg comprised of 57% females and 92% adults were included. Total anesthesia time was 44 ± 25 min and body temperature was 97.6 ± 2.0 F at the time of reversal. Cats were recaptured 10.1 ± 2.7 weeks after vaccination for repeat testing. The proportions of cats with protective titers at the first and second testing are shown in the table. There was no difference in proportions of cats with protective titers relative to vaccine type, but median titers were significantly higher for FPV in the MLV group and for FHV in the IA group.

A proportion of feral cats have protective titers at the time of surgery due to previous exposure or vaccination. A majority of cats respond well to a single vaccination given at the time of surgery.

ABSTRACT #7

CROSSREACTIVITY OF CANINE AND EQUINE INFLUENZA ANTIBODIES. PC Crawford,[1] JM Katz,[2] J Pompey,[2] TC Anderson,[1] RO Donis.[2] University of Florida,[1] Gainesville, FL and Centers for Disease Control and Prevention,[2] Atlanta, GA.

Canine influenza virus (CIV) is a newly emerging respiratory infection in dogs. Molecular and serological analyses indicate that the entire genome of an equine influenza virus (EIV) has been transferred to dogs with subsequent dog-to-dog transmission. Currently, there is no vaccine for CIV. There is much debate on whether hemagglutination inhibition (HI) assays employed for diagnosis of EIV are also suitable for diagnosis of CIV, and whether commercially available vaccines for EIV protect against CIV infection of dogs. The purpose of this study was 1) to determine the crossreactivity of antibodies from CIV-infected dogs with various strains of EIV, and 2) to determine the crossreactivity of antibodies in dogs vaccinated with an EIV vaccine to CIV.

Serum from 41 dogs naturally infected with CIV were tested for antibody crossreactivity with equine/KY/91, equine/KY/93, equine/NY/99, and equine/OH/03 influenza virus strains using an HI assay, and the antibody titers were compared to those for the canine/FL/04 strain of CIV. Six dogs were vaccinated subcutaneously with a commercial EIV vaccine containing inactivated equine/KY/93 virus and boosted 4 weeks later. Serum samples were collected from the dogs weekly from 1 to 5 weeks, and at 8, 12, and 16 weeks postvaccination. Antibody titers to the equine/KY/93 vaccine strain, equine/OH/03 strain, and canine/FL/04 strain were determined in the HI assay.

Antibody titers to canine/FL/04 in the 41 infected dogs ranged from 32 to 2048. Titers to equine/OH/03 were 2-to 4-fold lower than corresponding titers to canine/FL/04 and misdiagnosed one dog as uninfected. Titers to equine/NY/99 were 4-to 32-fold lower than corresponding titers to canine/FL/04 and misdiagnosed 12 (29%) of the dogs as uninfected. Titers were negative in all samples when equine/KY/93 and equine/KY/91 were used. Three of the 6 vaccinated dogs developed antibody titers to the equine/KY/93 vaccine strain by 1 week following the booster injection. The titers ranged from 32 to 128 and quickly declined to undetectable levels over the next 12 weeks. These vaccine-induced antibodies did not inhibit hemagglutination by the canine/FL/04 or equine/OH/03 viruses.

Accurate serological diagnosis of CIV infection in dogs requires use of the CIV in the HI assay. The current EIV vaccines are unlikely to protect dogs against CIV.

ABSTRACT #8

ANTIGEN TESTING FOR THE DIAGNOSIS OF BLASTOMYCOSIS. Deborah Spector[1], Joseph Wheat[4], David Bemis[3], Bart Rohrbach[3], Joseph

Taboada[2] and Alfred M. Legendre[1]. [1]Small Animal Clinical Sciences, [2]Louisiana State University, Baton Rouge, LA. [3]Department of Comparative Medicine, University of Tennessee, Knoxville, TN. [4]MiraVista Diagnostics, Indianapolis, IN.

Up to 30% of dogs with blastomycosis lack measurable serum antibodies at the time of initial evaluation. When the diagnosis cannot be made by identification of *Blastomyces dermatitidis* organisms by cytology, culture or histopathology of suspected lesions, making a diagnosis can be difficult. Antigen must be present before the development of an antibody response. Antigen detection would be especially valuable in immunosuppressed dogs that cannot produce an antibody response to infection and in dogs prior to antibody development. Early diagnosis and treatment with an antifungal drug provides the best outcome to treatment.

Paired serum and urine samples were obtained pre treatment from 46 dogs with blastomycosis confirmed by cytology or histopathology. There were 15 follow up urine samples and 13 follow up serum samples obtained at 30, 60 and 90 days after starting itraconazole treatment. Paired urine and serum samples to be used as negative controls were obtained from 44 dogs seen at the University of Tennessee for a variety of non-fungal medical and surgical problems and used as negative controls. An antigen assay was done on all samples.

The antigen assay is an enzyme immunoassay which uses rabbit antibodies to the formalin-killed, mold form of *B. dermatitidis*. Wells are coated with the immunoglobulin G anti-*B.dermatitidis* antibodies. Antigen bound to the antibody is detected with a substrate bound biotinylated antibody that initiates a color change. Optical density is determined at 450 nm. The optical density is then divided by a cutoff value to obtain enzyme immunoassay units (EU). The cut off value is determined by evaluating the mean value of negative controls × 1.5.

Most urine and serum samples from dogs infected with blastomycosis had antigen titers well above the range for negative specimens. Only 1 of 44 control urine samples and none of the 44 control serum samples had values considered to be positive. The sensitivity and specificity for the assay on urine was 93% and 98% respectively while the serum assays had a sensitivity of 87% and a specificity of 100%. There was a dramatic decline in serum antigen concentrations 30 days after the initiation of treatment with itraconazole. Urine antigen concentrations were maintained at 30 days but declined after 60 days of treatment. Urine antigen concentrations declined at a slower rate than the serum antigen concentrations.

Preliminary findings support the use of the antigen assay in the diagnosis of blastomycosis in dogs. There were fewer false negative results from the urine antigen assay than from the serum antigen assay. False positive results appear to be rare making the assay valuable to rule in the diagnosis of blastomycosis. Further studies are needed to determine if there are cross reactions in the assay between *Blastomyces sp*. and other fungal organisms. Further studies are needed to determine if antigen concentrations can be used to determine duration of antifungal treatment needed for cure of blastomycosis.

ABSTRACT #9

MYCOPLASMA INFECTION IN ANAEMIC AND NON ANAEMIC DOGS IN GERMANY. J Huebner[1], TW Vahlenkamp[2], E Müller[1], I Langbein-Detsch[1] [1]LABOKLIN, Bad Kissingen, Germany. [2]Federal Research Institute for Animal Health Greifswald-Isle of Riems, Germany.

Mycoplasma haemocanis infections of dogs are well documented with a world wide distribution. First thought this red blood cell bacterium causes only disease in immunosuppressed or splenectomized dogs, it is now known to be one of the main infectious agents in canine immunhaemolytic anaemia (IMHA). Most of these animals are presented with severe haemolytic anaemia with a PCV often below 10%. Performing a Coombs' test 80 to 85% of theses cases are positive for anti-erythrocyte-antibodies. Some dogs deteriorate quickly and the infection can be fatal. To differentiate between the variety of parasites and bacteria that can be responsible for the clinical picture of IMHA like *Babesia canis, Ehrlichia canis* and *Anaplasma phagozytophilum* PCR test should be performed. Especially under the aspect of treatment a distinct diagnosis is essential.

To get an overview and reveal the prevalence of *Mycoplasma* in Germany we performed a study in the year 2005 in our laboratory with randomised samples of 976 dogs using real-time PCR. A highly conserved region of the 16 smRNA gene specific for *Mycoplasma haemocanis* and *Candidatus Mycoplasma haemominutum* was used. Results showed that 167 dogs (17%) were positive for *Mycoplasma*. None of these dogs showed noticeable signs of anaemia or changes in their differential white blood profile.

Further investigations of 116 blood samples from dogs with anaemia were added to this study. These samples were send in for an anaemia screening, including a blood profile, reticulocyte count, total protein, iron and PCR tests for *Babesia canis, Ehrlichia canis* and *Mycoplasma*. Results show that 20 (18%) of these dogs were positive for *Mycoplasma*, whereas only 2 (2%) for *Babesia canis* and 8 (7%) for *Ehrlichia*.

All of these dogs were from Germany and had never left the country. This shows the wide distribution of these infectious agents in Middle Europe and the importance to test for *Mycoplasma* in the anaemic dog. Furthermore it shows that nearly as much asymptomatic dog carry *Mycoplasma*. These are important facts in kennel management or blood donation.

ABSTRACT #10

WOLBACHIA SPP. DNA IN BLOOD OF DOGS WITH *DIROFILARIA IMMITIS* INFECTION. MR Lappin,[1] JR Hawley,[1] JK Levy,[2] PC Crawford,[2] SJ Tucker.[2] Colorado State University,[1] Fort Collins, CO and University of Florida,[2] Gainesville, FL.

Wolbachia bacteria are endosymbionts of arthropods. The organisms have been demonstrated in *Dirofilaria immitis* and appear to contribute to the pathogenesis of disease. Some cats and dogs with dirofilariasis develop humoral responses against *Wolbachia*. The purpose of this study is to report the prevalence of *Wolbachia* DNA in the blood of dogs with and without dirofilariasis

In May 2004, blood samples were collected from 95 dogs on Isla Isabela, Galapagos while completing a project to control the dog population via sterilization. The ages of the dogs were estimated to range from 6 months to adult and both sexes were included. DNA was extracted from blood (200 µl) by use of a commercially available kit (QIAamp DNA Blood Mini Kit, Qiagen, Chatsworth, CA) and assayed with a conventional PCR assay that amplifies a region of the 16S–23S intergenic spacer gene (5′ CTG GGG ACT ACG GTC GCA AGA C 3′-forward; 5′ CTC CAG TTT ATC ACT GGA AGT T 3′- reverse) common to *Ehrlichia* spp., *Anaplasma* spp., *Neorickettsia risticii*, and *Wolbachia*. For samples with an amplicon of the appropriate size, sequencing was performed by a commercially available service and resultant sequences analyzed by comparison to sequences in GenBank using the BLAST program on the National Institutes of Health (NCBI) website. Sera were tested for *D. immitis* antigen, *Ehrlichia canis* antibodies, and *Borrelia burgdorferi* antibodies by use of a commercially available kit (Canine SNAP® 3Dx® Test, IDEXX Laboratories, Portland, ME).

Of the 95 dogs, 32 (34.0%) were positive for *D. immitis* antigen in serum. DNA consistent with *Wolbachia* bacteria (accession number Z492961.1) was amplified from the blood of 21 of the *D. immitis* antigen positive dogs (65.6%) but none of the *D. immitis* negative dogs. For 20 *Wolbachia* DNA positive samples, homologies with Z402061.1 were 97–100%; one sample had 90% homology. DNA consistent with *A. platys* (97% homology with accession # AF536828) was amplified from the blood of one *D. immitis* seronegative dog. Antibodies against *E. canis* or *B. burgdorferi* were not detected in any dog.

These results document that DNA of *Wolbachia* bacteria is commonly present in blood of dogs with *D. immitis* infection. In addition, the results suggest that infection with *Wolbachia* bacteria or *D. immitis* do not result in falsely positive *E. canis* or *B. burgdorferi* antibody test results.

ABSTRACT #11

ANAPLASMA SPP, BABESIA CANIS, BARTONELLA HENSELAE, BARTONELLA VINSONII SUBSP. BERKHOFFII, BORRELIA BURGDORFERI, DIROFILARIA IMMITIS, AND *EHRLICHIA CANIS* SEROPREVALENCES IN BRAZILIAN DOGS. PPVP Diniz[1], DS Schwartz[1], RZ Machado[2], JM Bradley[3], BC Hegarty[3], EB Breitschwerdt[3]. [1]School of Veterinary Medicine and Animal Science (FMVZ-UNESP), São Paulo State University, Botucatu, SP, Brazil. [2]School of Agronomical and Veterinary Sciences (FCAV-UNESP), Jaboticabal, SP, Brazil. [3]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

Canine ehrlichiosis is one of the most prevalent infectious diseases in Brazil; however, the prevalence of other vector-borne pathogens remains unclear. *Anaplasma spp*, *Babesia canis, Bartonella henselae, Bartonella vinsonii* subsp. *berkhoffii*, *Borrelia burgdorferi, Dirofilaria immitis*, and *Ehrlichia canis* seroprevalences were determined for 197 sick dogs examined because of clinical signs consistent with a tick-borne disease at Botucatu Veterinary Teaching Hospital, located in southeastern Brazil. Inclusion criteria included at least three of the following: presence of ticks, fever, bleeding, neurological signs, inflammatory ocular disorders, anemia, leukopenia, thrombocytopenia and/or hyperproteinemia. *Anaplasma spp*, *B. burgdorferi*, *E. canis* antibodies and *D. immitis* antigens were evaluated with an in-clinic ELISA (SNAP® 4Dx®, IDEXX Laboratories). *B. vinsonii*

(berkhoffii), B. henselae and *B. canis* antibodies were detected using an indirect immunofluorescence assay with reciprocal titers of 64, 64 and 80 respectively representing seroreactivity. Seroreactivity to *E. canis* peptides was detected in 145 dogs (73.6%), to *B. canis* antigens in 112 dogs (56.6%) and to *Anaplasma* spp. peptides in 81 dogs (41.1%). Four and 3 dogs (2.0%, 1.5%) were seroreactive to *B. henselae* and *B. vinsonii (berkhoffii)* antigens respectively. No dog was *B. burgdorferi* C6 peptide or *D. immitis* antigen seroreactive. Twenty seven samples (13.7%) were negative for all test organisms. Forty seven dogs (23.9%) were seroreactive to a single organism, whereas 123 dogs (62.4%) had antibodies to multiple organisms as summarized below.

Organism(s) Seroreactive	N	%	Organism(s) Seroreactive	N	%
Ehrlichia canis only	28	14.2	*Bartonella vinsonii (berkhoffii)* + *E. canis*	1	0.5
Babesia canis only	10	5.1	*B. vinsonii* + *E. canis* + *B. canis*	1	0.5
Anaplasma spp only	9	4.6	*B. vinsonii* + *E. canis* + *B. canis* + *Anaplasma spp*	1	0.5
E. canis + *B. canis*	48	24.4	*B. henselae* + *Anaplasma spp*	1	0.5
E. canis + *Anaplasma spp*	19	9.6	*B. henselae* + *Anaplasma spp* + *B.canis*	1	0.5
B. canis + *Anaplasma spp*	4	2.0	*B. henselae* + *E. canis* + *B. canis*	1	0.5
E. canis + *B. canis* + *Anaplasma spp*	45	22.8	*B. henselae* + *Anaplasma spp* + *E. canis* + *B. canis*	1	0.5

This study indicates that sick dogs from southeastern Brazil are frequently exposed to *E. canis*, *Anaplasma spp* and *B. canis*, whereas exposure to *B. henselae, B. vinsonii* (*berkhoffii*), *B. burgdorferi* and *D. immitis* is infrequent to non-existent. Exposure to multiple-organisms, known to be transmitted by *Rhipicephalus sanguineus*, is common in this dog population. The relative role of each organism to the disease pathogenesis in naturally infected dogs requires further study.

ABSTRACT #12

EVALUATION OF SEROLOGY AND CIRCULATING IMMUNE COMPLEXES IN DOGS NATURALLY INFECTED WITH *BORRELIA BURGDORFERI*. RE Goldstein and DZ Atwater College of Veterinary Medicine, Cornell University, Ithaca, NY.

The aims of this study were to compare available serologic tests and to identify associations between those tests and *Borrelia burgdorferi* (Bb) induced circulating serum immune complexes (CICs), in dogs infected with Bb, with or without signs of Lyme disease (LD). Serum samples from dogs positive on a screening test for Bb (3Dx® – IDEXX® Labs) at various veterinary clinics in the US, were analyzed for quantitative C6 (quantC6) antibodies (IDEXX® labs), Kinetic ELISA (KELA), and Western blot (both at Cornell University). Western blot results were scored (WBS) based on the presence and density of bands from 1 (negative) to 5.5 (very severe infection). Serum CICs were purified using polyethylene glycol centrifugation. Total CICs were measured by optical density. After purification and disassociation, Bb specific CIC associated antibodies were quantified by Lyme KELA (BbCICs), identified and scored by Western blot (WB CICs). All assays were performed independently in a blinded fashion. Because of insufficient quantities not all assays were performed on all samples. Clinical data from the veterinarian was scored as non-clinical, likely clinical (lameness, fever and/or lethargy) or unknown. Non-parametric statistics were utilized. Results are reported as median and range, $p < 0.05$ was considered significant.

Serum was obtained from 156 dogs, 96 non-clinical and 35 clinical for LD. Median (and range) serological values for all dogs assayed were: QuantC6 115 (10–417, n = 156), serum KELA 464 (69–716, n = 107), and median serum WBS was 5.0 (1.0–5.5, n = 107). Median serum CICs for all dogs was 2.0 (0.7–4.6, n = 103). Median Bb specific CICs for all dogs were 55 (0–500, n = 151) for BbCICs, and 1 (1–5, n = 133) for WB CICs. Significant differences between non-clinical and clinical dogs existed for Bb specific CICs. Median (and range) values for BbCICs were 31.5 (0–399) for non-clinical (n = 94) and 102 (0–500) for clinical dogs (n = 32) ($p = 0.03$). Median (and range) values for WB CICs were 1 (1–4) for non-clinical (n = 80) and 2 (1–5) for clinical dogs (n = 28) ($p = 0.03$). Positive correlations existed between quantC6 and: Serum KELA (all and non-clinical dogs), total CIC (all dogs), serum WBS (all, non-clinical and clinical dogs), BbCICs (all, non-clinical and clinical dogs) and WB CICs (all, non-clinical, clinical dogs). Between serum KELA and: Serum WBS (all, non-clinical and clinical dogs) and WB CICs (all, non-clinical and clinical dogs). Between: BbCICs and serum KELA (all, non-clinical and clinical dogs), serum WBS (all and non-clinical dogs) and WB CICs (all, non-clinical and clinical dogs).

QuantC6, serum KELA and serum WB correlate closely in dogs infected with Bb. Total and Lyme specific CICs can be purified and quantified from serum of clinical and non-clinical dogs. Lyme specific CIC concentrations (by KELA or WB) correlate well with serum KELA and quantC6 concentrations, and are increased in dogs with clinical signs of LD. Increased concentrations of Bb specific CICs may be a consistent finding in clinical canine Lyme disease.

ABSTRACT #13

SEROLOGY AND CIRCULATING IMMUNE COMPLEXES IN DOGS NATURALLY INFECTED WITH *BORRELIA BURGDORFERI* BEFORE AND AFTER DOXYCYLINE THERAPY. RE Goldstein and DZ Atwater College of Veterinary Medicine, Cornell University, Ithaca, NY.

The aims of this study were to compare available serologic tests, and *Borrelia burgdorferi* (Bb) induced circulating serum immune complexes (CICs), before and after doxycycline therapy in dogs infected with Bb, with or without clinical signs of Lyme disease (LD). Serum samples from dogs, positive on a screening test for Bb (3Dx® – IDEXX® Labs) at various veterinary clinics in the US, were included in the study. The samples were obtained before (pre), and approximately 5 months after (post), 1 month of doxycycline therapy (10–20 mg/kg/day). Samples were analyzed for quantitative C6 antibodies (quantC6) (IDEXX® labs), Kinetic ELISA (KELA), and Western blot (both at Cornell University). Western blot results were scored (WBS) based on the presence and density of bands from 1 (negative) to 5.5 (very severe infection). Serum CICs were purified using polyethylene glycol centrifugation. Total serum CICs were measured by optical density. After purification and disassociation, Lyme specific CIC associated antibodies were quantified by Lyme KELA (BbCICs), identified and scored by Western blot (WB CICs). All assays were performed independently in a blinded fashion. Because of insufficient quantities not all assays were performed on all samples. Clinical status from the veterinarian was scored as non-clinical, likely clinical (lameness, fever and/or lethargy) or unknown for each dog. Non-parametric statistics were utilized. Results are reported as median and range, $p < 0.05$ is considered significant.

Serum was obtained from 156 dogs. Ninety six were scored as non-clinical and 35 as clinical for LD signs on the pre visit. Of the non-clinical dogs 1 was assessed as clinical on the post visit. Of the clinical dogs 21 were assessed as non-clinical and 1 as clinical on the post visit. All serologic tests exhibited a significant decrease ($p < 0.001$) in the post sample when compared to the pre including quantC6 (n = 156), serum KELA (n = 107), serum WBS (n = 107). CIC concentrations also dropped ($p < 0.001$) including total CICs (n = 103), BbCICs (n = 151) and WB CICs (n = 133). No association was identified for any parameter between the change in value (post-pre) and the clinical status of the dogs. Positive correlations existed between the magnitude of change (post-pre) and the following: QuantC6 and: Serum KELA (all, and clinical dogs), total CIC (all dogs), serum WBS (all and non-clinical), BbCICs (all, non-clinical and clinical dogs) and WB CICs (all, non-clinical). Serum KELA and: BbCICs (all, and non-clinical dogs), serum WBS (all, non-clinical and clinical dogs) and WB CICs (all and non-clinical dogs). BbCICs and: Total CIC (all dogs), serum WBS (all and non-clinical dogs) and WB CICs (all, non-clinical and clinical dogs).

QuantC6, serum KELA, serum WBS, and Bb induced CICs, all decreased 5 months following doxycycline therapy in clinical and non-clinical dogs infected with Bb. The magnitude of the decrease in CICs was correlated to the decrease in quantC6 and serum KELA. Repeated serology may be a method of predicting decreased CICs following treatment of dogs infected with Bb.

ABSTRACT #14

BORRELIA BURGDORFERI AND *ANAPLASMA PHAGOCYTOPHILUM*: POTENTIAL IMPLICATIONS OF CO-INFECTION ON CLINICAL PRESENTATION IN THE DOG. Melissa Beall[1], Ramaswamy Chandrashekar[1], Matt Eberts[2], Katie Cyr[1], John Crawford[3], Celine Mainville[1], Barbara Hegarty[3], Edward Breitschwerdt[3]. [1]IDEXX Laboratories, Westbrook, ME. [2]Lakeland Veterinary Hospital, Baxter, MN. [3] College of Veterinary Medicine, North Carolina State University, Raleigh, NC.

Ixodes species are responsible for transmitting *Borrelia burgdorferi* (*Bb*), the causative agent of Lyme disease, and *Anaplasma phagocytophilum* (*Aph*), the cause of granulocytic anaplasmosis, to cats, dogs, horses and human beings. To study the potential clinical impact of co-infection, a random population of 731 dogs from Baxter, Minnesota, an area endemic for Lyme disease and anaplasmosis, were tested for *Bb* and *Aph* antibodies and a subset of sick and healthy dogs were tested for DNA evidence of *Anaplasma* infection. Patient data, including clinical signs at the time of sample collection, were recorded. Sick dogs were defined as having signs consistent with *Aph* infection, including fever, lethargy, lameness, and/or thrombocytopenia. Serum samples were tested using an in-clinic ELISA, Snap® 4Dx™, for the presence of antibodies against *Aph*, *Bb*, and *Ehrlichia canis* (*Eca*); and *Dirofilaria immitus* (*Di*) antigen. A positive Snap® 4Dx™ test for *Bb* (C6 peptide) was considered indicative of active infection. Two hundred seventy-seven EDTA-anti-coagulated blood samples were processed for genomic DNA and analyzed by PCR to detect active *Aph* infection. Results of the serosurvey indicated that 216 (29%) dogs had *Aph* antibodies, 80 (11%) had *Bb* antibodies, whereas 188 (25%) dogs had both *Aph* and *Bb* antibodies. *Eca* antibodies and *Di* antigen were each detected in 11 (1.5%) dogs. Of 89 sick dogs, *Aph* antibodies were detected in 22 (25%), *Bb* in 8 (9%), and both *Aph*

and *Bb* in 38 (43%). *Aph* DNA was amplified from 7 of 222 (3%) healthy dogs in the survey population, and 16 of 51 (31%) sick dogs. Based upon serology, co-infection with *Aph* and *Bb* is more likely to induce illness in dogs as compared to infection with either organism alone ($p = 0.0014$, Fishers Exact Test, one-tailed) and based upon PCR, *Aph* DNA is more prevalent in sick dogs than healthy dogs ($p < 0.0001$, Fishers Exact Test, one-tailed).

ABSTRACT #15

THROMBOCYTOSIS IN CATS: A RETROSPECTIVE STUDY OF 51 CASES (2000–2005). F Rizzo, SW Tappin, S Tasker. Department of Clinical Veterinary Sciences, University of Bristol Small Animal Hospital, Langford, Bristol, UK.

To the authors' knowledge, published retrospective studies of thrombocytosis in cats are not available. The purpose of this retrospective study was to investigate thrombocytosis in cats presenting to a referral hospital. Cases were evaluated in order to identify possible relationships between signalment, clinical presentation, diagnosis and prognosis.

Feline haematology profiles of patients presented to the University of Bristol Small Animal Hospital from January 2000 to October 2005 were evaluated for thrombocytosis (defined as a platelets count of $>700 \times 10^9$/l and confirmed on smear evaluation). Thrombocytosis was found in 79 cats (4.64% of the hospital feline population in the same period), with values ranging from 703 to 1895 $\times 10^9$/l. Signalment, clinical presentation, concurrence of other haematological abnormalities, diagnoses and outcome were evaluated in 51 cases in which complete medical records were available. Other variables (retrovirus infection status, thyroxine levels, haemoplasma PCR, Toxoplasma antibody titres) were also evaluated. No association was found between the presence of thrombocytosis and breed or gender. Gastrointestinal signs were the most common clinical presentation (26/51), with vomiting and anorexia being the most common clinical signs. Neurological signs were the second most represented category (10/51). Concurrent haematological abnormalities were present in most cases (39/51), the most common of which was lymphopenia (17/51). A final diagnosis was reached in 45/51 cases. Based on the final diagnosis reached, cats were grouped both according to the DAMNITV classification and according to the body system affected. Amongst the DAMNITV classification, inflammatory/infectious conditions were most commonly associated with thrombocytosis (16/45), and no particular condition was overrepresented in this category. Neoplastic conditions were the second most common represented group (6/45). According to the DAMNITV classification, many cases fell into the miscellaneous category (20/45). According to classification by body system, gastrointestinal involvement was most represented (13/45) with hepatic lipidosis (3 cases) being the most common disease in this category. Endocrine cases were the second most common category (10/45), with hyperthyroidism commonly seen (9 cases). No association was found between the severity of thrombocytosis and outcome. Ten cases died or were euthanased and only 2 of these had a severely elevated platelet count (1016 and 1497 $\times 10^9$/l respectively) whilst the other 8 cases had only mild elevations (ranging from 745 to 907 $\times 10^9$/l).

ABSTRACT #16

DALTEPARIN CAUSES SIGNIFICANT DOSE RELATED CHANGES IN TISSUE FACTOR AND KAOLIN ACTIVATED WHOLE BLOOD THROMBOELASTOGRAPHY (TEG) PARAMETERS. LR Jessen[1], B Wiinberg[1], AL Jensen[1], M Kjelgaard-Hansen[1], AT Kristensen[1]. [1]Small Animal Clinical Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Low molecular weight heparin (LMWH) is increasingly used in veterinary medicine for both treatment and prophylaxis of thromboembolic disease. Since conventional plasma clotting assays are only minimally influenced by LMWH, the golden standard for monitoring LMWH therapy is the anti-Xa assay. However, despite a good correlation between the anti-Xa activity in plasma and the dose of LMWH, the anti-Xa activity has been demonstrated to be a poor predictor of antithrombotic efficacy and bleeding risk in human patients treated with LMWH. Thromboelastography (TEG) enables global assessment of hemostatic function in whole blood and could potentially help predict effect of and optimize the treatment with LMWH in dogs.

The aim of this study was to evaluate the ability of recombinant human tissue factor (TF) and kaolin activated TEG to detect hemostatic alterations following in vitro heparinization of canine whole blood with dalteparin (Fragmin®) corresponding to doses of 0, 12.5, 50 and 200 IU/kg.

Citrated whole blood was collected from 7 clinically healthy dogs and stored at room temperature for 30 minutes until TEG analyses. Four separate samples from each dog were subsequently mixed with diluted dalteparin, giving final concentrations of 0.156, 0.625 and 2.5 IU/ml whole blood respectively. One sample served as control and was not mixed with dalteparin. TEG analyses were performed on all samples using both TF [1 : 50,000] and kaolin as activators. Four TEG parameters; reaction time (R), clotting time (K), angle (α), and maximum amplitude (MA) were recorded.

Results from TF and kaolin activated TEG were analyzed separately. One dog was excluded from statistical analyses in the TF group due to missing values at the dalteparin concentration of 2.5 U/ml whole blood. One dog was excluded from statistical analyses of the α values in the TF group due to a missing value. A Friedman analysis was applied to identify any significant change of R, K, α and MA between dalteparin concentrations. Statistical significance was set at $p < 0.05$.

When using TF as activator increasing concentrations of dalteparin significantly and dose-dependently prolonged R time ($p < 0.001$) and K time ($p < 0.001$) and significantly decreased α ($p < 0.003$) and MA ($p < 0.001$). Kaolin as activator only significantly and dose-dependently prolonged R time ($p < 0.0064$).

In conclusion, TEG appears to be an effective method for detection of hemostatic alterations following in vitro heparinization of canine WB with dalteparin. Furthermore TF activated TEG was more sensitive than kaolin activated TEG to the effects of dalteparin in the concentrations evaluated in this study. Prospective clinical studies are needed to evaluate the clinical applicability of TF activated TEG for the prediction of effect and monitoring of treatment with dalteparin.

ABSTRACT #17

SERUM UREA: CREATININE RATIO IN HEMOLYTIC DISEASE. R.G. Lobetti[1], M. de Scally[1], A Leisewitz[2] [1]Bryanston Veterinary Hospital, Bryanston, South Africa. [2]Department of Veterinary Tropical Diseases, University of Pretoria, South Africa.

The normal serum urea: creatinine (UC) is 10–15, with an increase in the ratio associated with acute renal azotemia, prerenal azotemia, or disrupted urinary tract. In an azotemic animal, a UC ratio ≥ 20 is indicative of prerenal azotemia, whereas a ratio of <20 is indicative of intrinsic renal disease. Thus serum urea is more likely to increase due to prerenal factors than serum creatinine, whereas both parameters are equally likely to increase with renal disease. Hemolytic disease can result in both elevated urea and renal disease and it has been speculated that the increased serum urea is from the hemoglobin load on the liver.

The purpose of this study was to determine serum urea, creatinine, and hemoglobin concentrations, UC ratio, and glomerular filtration rate (GFR) in dogs with babesiosis and dogs with experimentally induced hemoglobinemia. Four groups of dogs were used: 25 dogs with severe babesiosis (group 1), 13 control dogs (group 2), 6 dogs with induced hemoglobinemia and hypoxia (group 3), and 6 dogs with induced hemoglobinemia (group 4). Group I were dogs with natural infection; group II were dogs presented for elective ovariohysterectomy; group 3 were experimental dogs that over a 4 day period were bled to a hemocrit of 12% and had a total of 300 grams homologous hemoglobin infused; and group 4 had over a 4 day period a total of 300 grams homologous hemoglobin infused. GFR was determined by serum cystatin-C concentration in groups 1 and 2 by creatinine clearance in groups 3 and 4. The study was approved by the Ethics and Research Committees of the University of Pretoria.

Results showed that the median serum urea was 11.8 mmol/l (group 1), 4.3 mmol/l (group 2), 4.3 mmol/l (group 3) and 4.35 mmol/l (group 4). Median serum creatinine was 67 µmol/l (group 1), 75 µmol/l (group 2), 78.5 µmol/l (group 3) and 84 µmol/l (group 4); median serum hemoglobin was 1.3 g/l (group 1), 0.8 g/l (group 2), 9 g/l (group 3) and 3 g/l (group 4). Median UC ratio was 41.35 (group 1), 15.36 (group 2), 14.18 (group 3) and 13.6 (group 4). GFR was normal in all 4 groups. Group 1 showed a statistically significant difference in serum urea and UC ratio ($p < 0.001$).

This study showed that dogs with severe babesiosis had both elevated serum urea and UC ratios, however, experimental dogs with only severely elevated serum hemoglobin levels did not show an increase in either the serum urea or the UC ratio. Renal function was normal in all 4 groups of dogs. Thus this increased UC ratio in dogs with babesiosis and therefore hemolytic disease does not appear to be from an increased hemoglobin load on the liver as previously speculated.

ABSTRACT #18

USE OF THE CANINE HEMOLYTIC ANEMIA OBJECTIVE SCORE (CHAOS) TO PREDICT SURVIVAL IN DOGS WITH IMMUNE-

MEDIATED HEMOLYTIC ANEMIA. MF Whelan[1], EA Rozanski[1], TE O'Toole[1], S Crawford[1], J Holm[2] and SM Cotter[1]. [1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA. [2]Angell Animal Medical Center, Boston, MA.

Immune-mediated hemolytic anemia (IMHA) is a disease of variable severity. Multiple retrospective studies have identified prognostic indicators and attempted to evaluate therapies. Scoring schemes are routinely used in human medicine to predict outcome, prioritize resources, and compare treatment protocols. The goal of this study was to evaluate in a retrospective manner dogs with IMHA, and to identify a number of clinical factors that may be prognostic for development of a scoring scheme. An objective scoring system could then be utilized in assigning prognosis, and also to provide the basis of prospective comparison of differing treatment regimens between similarly affected dogs.

A scoring scheme was created by assigning point value to clinical parameters that were considered prognostic indicators. The indicators included were age and body temperature, the presence of autoagglutination, and concentrations of bilirubin and albumin. The highest possible score signifying degree of illness was 7.

IMHA was defined using previously established criteria including a regenerative anemia of unknown cause, with spherocytosis or autoagglutination, or a positive Coomb's test in the absence of autoagglutination. The medical records of cases of dogs with the diagnosis of IMHA that were hospitalized and treated at the Cummings School of Veterinary Medicine from 2003–2005 were retrospectively evaluated. Cases with incomplete evaluations, incomplete medical records, or that were euthanized at the time of diagnosis were excluded. An established list of clinical parameters were given a number score, and each of the cases received a final cumulative score. Outcome was reported as survival to discharge, death or euthanasia. Mann-Whitney Test was used to compare differences between survivors and non-survivors.

Sixty-four dogs met the inclusion requirement, including 47 survivors and 17 dogs that either died or were euthanized. The median CHAOS of the survivors was significantly lower than non-survivors. [Survivors, 3 (range 0–6) non-survivors, 5 (range 2–7), $p < 0.01$].

CHAOS	0	1	2	3	4	5	6	7
% survival	100	100	85	86	79	43	50	0

In this retrospective evaluation, a simple scoring scheme was found to distinguish between survivors and non-survivors. A prospective application of the canine hemolytic anemia objective score (CHAOS) is ongoing and is required to provide validation. However, we conclude and propose that this may be a reasonable criteria for determining severity of illness in dogs with IMHA and may help stratify studies designed to examine outcome.

ABSTRACT #19

RETROSPECTIVE EVALUATION OF D-DIMER AS AN INDICATOR OF COAGULATION STATUS IN 323 DOGS ADMITTED TO A VETERINARY TEACHING HOSPITAL. EA Spangler[1], KE Russell[2]. [1]Dept. of Pathobiology, College of Veterinary Medicine, Auburn University, AL. [2]Dept. of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

D-dimer is produced during the breakdown of cross-linked fibrin, and thus is a specific marker for activation of coagulation and fibrinolysis. In dogs, high D-dimer concentrations have been associated with thromboembolic disease and DIC, but are also seen in a variety of disease processes in which there is no clinical evidence of thrombosis. In this retrospective study, a quantitative measurement of plasma D-dimer (Instrumentation Laboratories) was examined as one component of a panel of coagulation tests in a large population of dogs with diverse underlying diseases. The goals of this study were to determine whether or not a correlation between D-dimer concentration and any other coagulation parameter could be identified in sick dogs, and to assess the contribution of D-dimer to the identification of dogs with DIC. Laboratory data were reviewed for dogs that underwent coagulation testing at Texas A&M University from June 2004 to September 2005. Only the first day of coagulation testing results for each patient were examined, and patients were included if at least 4/5 of the following tests were completed: PT, aPTT, AT, fibrinogen and platelet count. A total of 323 dogs were included in the study, and plasma D-dimer was measured in all cases. Plasma from 30 healthy dogs was used for assay validation and to establish reference intervals. D-dimer concentration was significantly higher in sick dogs than in healthy dogs ($p = .004$). Forty-six percent of sick dogs ($n = 149$) had a D-dimer concentration that was more than 25% above the reference interval, and in 6.8% ($n = 10$) of those dogs no other coagulation abnormality was detected. No significant correlation between D-dimer concentration and any other single coagulation parameter was found. The population of sick dogs was stratified to identify dogs with probable DIC based on evaluation of the PT, aPTT, AT and platelet count. Dogs were defined as having probable DIC if 3 or more values were more than 25% above (PT; PTT) or below (AT; platelet count) the reference interval. This population included a total of 25 dogs. Dogs with probable DIC showed a significant increase in D-dimer concentration relative to both healthy dogs and the non-DIC population of sick animals ($p < .0001$), although there was overlap between all categories. Two dogs in the probable DIC population had D-dimer concentrations within the reference interval. Based on ROC curve analysis a cut-off value for D-dimer of >1000 ng/ml has a sensitivity of 76% and specificity of 77% for the identification of dogs with DIC. The finding of a D-dimer concentration below 1000 ng/ml excludes most animals that do not have DIC (NPV = 96%). This study support the conclusion that D-dimer is informative for the identification of dogs with thrombotic disease, but emphasize that it must be considered in the context of clinical signs and as one component of a panel of tests that evaluate coagulation status.

ABSTRACT #20

ASSOCIATION OF DRUG-SERUM PROTEIN ADDUCTS AND ANTI-DRUG ANTIBODIES IN DOGS WITH SULFONAMIDE HYPERSENSITIVITY. S. N. Lavergne, E. Volkman, R. Danhof, L.A. Trepanier. School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA.

Sulfonamide antimicrobials (e.g. sulfamethoxazole, SMX) are used in both human and veterinary medicine to prevent and treat various infections. However, delayed sulfonamide hypersensitivity reactions (HS) can occur in both humans and dogs with a similar clinical pattern: a delayed onset (5–14 days); clinical signs including fever, cutaneous eruption, hepatopathy, or blood dyscrasias; faster onset and more severe presentation with re-exposure; and recovery after drug withdrawal. Drug-serum protein adducts and anti-drug antibodies have been found in humans with sulfonamide HS. The purpose of this study was to determine whether similar immune markers were also present in dogs with sulfonamide HS.

We evaluated 34 dogs with HS to SMX, sulfadiazine, or sulfadimethoxine, and 10 dogs tolerating a therapeutic course of these sulfonamides without adverse reaction, for drug-serum adducts (by immunoblotting) and anti-drug antibodies (by ELISA). Drug-serum adducts were found in 10/20 HS dogs (50%), but in no tolerant dogs. Anti-drug antibodies were detected in 17/34 HS dogs (50%), but in only one tolerant dog (10%), with absorbance values significantly higher in HS dogs ($P = 0.011$). Furthermore, there was a significant association between the presence of sulfonamide-serum adducts and anti-sulfonamide antibodies ($P = 0.009$). Interestingly, 53% of sulfonamide-sensitive dogs with antibodies against the sulfonamide of exposure also had antibodies cross-reacting with at least one other commonly prescribed sulfonamide. These results corroborate those found in SMX-sensitive humans, suggesting a possibly similar pathogenesis. These data also support the potential use of drug-serum adducts and anti-drug antibodies as markers for sulfonamide HS in dogs.

ABSTRACT #21

ANTI-PLATELET AND ANTI-NEUTROPHIL ANTIBODIES IN DOGS WITH SULFONAMIDE-ASSOCIATED BLOOD DYSCRASIAS. S. N. Lavergne, N. Dresher, L. Grishaber, L.A. Trepanier. School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA.

Sulfonamide antimicrobials, such as sulfamethoxazole (SMX), are used for opportunistic infections in human and veterinary medicine. However, sulfonamide hypersensitivity (HS) can complicate therapy in both dogs and humans, with a similar clinical presentation, and clinical signs such as fever, skin eruptions, hepatopathy, or blood dyscrasias (thrombocytopenia, neutropenia). Several humoral markers have been found in humans with SMX HS, including anti-drug antibodies and drug-dependent anti-platelet antibodies. The purpose of this study was to determine whether markers of antibody-mediated immunity were also present in dogs with sulfonamide HS, manifested as blood dyscrasias.

We evaluated 20 dogs with thrombocytopenia and/or neutropenia associated with sulfonamide antimicrobials, using assays for antibodies against platelet and neutrophil membrane proteins (by flow cytometry); antibodies against myeloperoxidase (using ELISA); and anti-neutrophil cytoplasmic antibodies (ANCA, using immunoblotting). Controls included healthy sulfonamide naïve dogs. Anti-platelet antibodies were detected in 12/16 HS dogs (75%) with thrombocytopenia; these antibodies bound only in the presence of drug in 5 cases. Anti-platelet antibodies in all 12 dogs were

still able to bind canine platelets that lacked the IIb subunit of the fibrinogen receptor, indicating that, unlike in humans, this subunit is not a target of these auto-antibodies. Six out of 20 HS dogs (30%) had antibodies to myeloperoxidase, and 17/20 (85%) were positive for ANCA. This study demonstrates that dogs with sulfonamide-associated blood dyscrasias have antibodies to both platelet and neutrophil antigens. Additional work will determine the specificity of these antibody markers, and whether they are directly involved in the pathogenesis of drug HS.

ABSTRACT #22

PCR ANALYSIS OF C-KIT JUXTAMEMBRANE DOMAIN MUTATION IN EIGHT CASES OF CANINE MAST CELL TUMOR TREATED WITH IMATINIB MESYLATE (GLEEVEC). N Ishida, M Isotani, M Tominaga, H Yagihara, K Tamura, M Bonkobara, T Washizu. Nippon Veterinary and Animal Science University, Tokyo, Japan.

It has been reported that there are mutations in c-kit in some canine mast cell tumor cells. The mutations frequently occur in the intracellular juxtamembrane domain (ICJD) of the gene as internal tandem duplication (ITD). This causes the constitutive phosphorylation of the gene product, resulting in the development of mast cell tumor (MCT). The kinase inhibitor imatinib mesylate (Gleevec) has been successfully used to treat the human cancer patients with similar c-kit mutations. In this study we evaluated the therapeutic efficacy of Gleevec in canine MCT and analyzed mutations within the ICJD of c-kit gene.

Gleevec was administered to 10 cases of MCT (9 to 15 yrs) at a dose of 10 mg/kg for 1 to 3 weeks. The size of the tumor was evaluated before and after the administration of Gleevec. Tumor cells were collected by fine needle aspiration before the medication, and genomic DNA was isolated. PCR amplifications were performed using these genomic DNA and the primer set that located in ICJD. The mutation of ITD was detected as increased size of PCR products.

The tumor size was dramatically reduced or became non palpable in 5 cases after the administration of Gleevec. Increased size of PCR product in addition to the product of wild type was observed in 2 cases among these 4 cases, suggesting the existence of ITD. These findings indicate that Gleevec is extremely effective for certain cases of MCT and PCR screening is useful to select the candidates to treat with Gleevec.

ABSTRACT #23

PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR AND ANGIOPOIETIN-2 LEVELS IN DOGS WITH HEPATOCELLULAR CARCINOMA, HEMANGIOSARCOMA, AND MAMMARY GLAND TUMOR. Yuka Kato, Kazushi Asano, Toshiaki Mogi, Kazuya Edamura, Atsuhiko Hasegawa, Shigeo Tanaka Nihon University, Kanagawa, Japan.

Angiogenesis plays an important role in tumor growth and metastasis and is regulated by several angiogenesis factors, including vascular endothelial growth factor (VEGF) and angiopoietin (Ang) family. High blood levels of VEGF and Ang-2 have been demonstrated in human patients with malignant tumors. The purpose of this study was to evaluate plasma VEGF and Ang-2 levels in dogs with tumors.

Plasma samples were obtained from 19 healthy beagles and 45 tumor-bearing dogs [6 hepatocellular carcinomas (HCC), 12 hemangiosarcomas (HSA), and 27 mammary gland tumors (MGT)]. Plasma concentrations of VEGF and Ang-2 were measured by enzyme-linked immunosorbent assay (R & D Systems). Plasma VEGF and Ang-2 levels were compared between healthy dogs and dogs with HCC, HSA, and MGT. All data were expressed as median values (ranges).

Plasma VEGF level was undetectable (<0.2 pg/mL) in all the healthy dogs, whereas plasma VEGF levels were 1.365 pg/mL (<0.2–782.38 pg/mL), <0.2 pg/mL (<0.2–7.18 pg/mL), and 1.94 pg/mL (<0.2–895.67 pg/mL) in dogs with HCC, HSA, and MGT, respectively. On the other hand, plasma Ang-2 levels were 3.365 ng/mL (1.66–6.15 ng/mL), 2.13 ng/mL (0.67–4.45 ng/mL), and 1.47 ng/mL (0.38–6.14 ng/mL) in dogs with HCC, HSA, and MGT, respectively, in contrast to 0.93 ng/mL (0.62–1.43 ng/mL) of plasma Ang-2 level in healthy dogs. The plasma VEGF and Ang-2 levels in each tumor group were significantly higher than those in healthy dogs ($P < 0.01$). In dogs with MGT, the plasma VEGF level showed a significant difference between malignant and benign tumor groups, while plasma VEGF and Ang-2 levels showed a significant difference between postoperative metastasis and no metastasis groups ($P < 0.05$).

This study demonstrated that plasma VEGF and Ang-2 increased in canine HCC, HSA, and MGT. In particular, plasma VEGF and Ang-2 can be used for determining prognosis in canine MGT.

ABSTRACT #24

DEVELOPMENT OF AN ARTIFICIAL ANTIGEN-PRESENTING CELL TO SUPPORT EX VIVO EXPANSION OF CANINE T CELLS FOR ADOPTIVE IMMUNOTHERAPY. Nicola Mason, Megan Suhoski, Carl June. Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA.

Adoptive immunotherapy with native antigen-specific T cells or genetically re-directed T cells promises to be a powerful modality in the treatment of cancer and infectious disease. A pre-requisite for adoptive immunotherapy and efficient retroviral gene transfer into peripheral T cells is the ability to rapidly expand functional T cells ex vivo. To this end we have generated an artificial antigen-presenting cell (aAPC) that supports robust ex vivo expansion of canine T cell subsets. The human erythroleukemic cell line, K562, was stably transduced with a self-inactivating lentiviral vector pCLPS expressing human FcγRII (CD32) or human IL-15 IRES CD32. Cells expressing high levels of CD32 were sorted by flow cytometry and cloned. Resulting KT32 and KT32/IL-15 clones were further transduced with pCLPS expressing canine (c)CD86 and bulk sorted for high cCD86 expression to create KT32/cCD86 and KT32/IL-15/cCD86 aAPCs. Carboxy-Fluorescein Succinimidyl Ester (CFSE) labelled canine PBLs were co-cultured with irradiated KT32, KT32/IL-15, KT32/cCD86 or KT32/IL-15/cCD86 cells at PBL:aAPC of 4 : 1, 2 : 1 and 1 : 1 in the presence of 0, 0.1, 1 and 10 μg/ml soluble α-canine CD3. Cell counts and size were determined on day 4 of culture and every 2 days thereafter. Proliferation profiles of T cell subsets were determined by flow cytometry on day 6 of culture. When the mean cell size of PBLs returned to 100–150 fl, cells were re-stimulated with irradiated aAPCs as described. At a 1 : 1 ratio in the presence of 1 μg/ml α-CD3, KT32/cCD86 and KT32/IL-15/cCD86 cells supported robust activation, proliferation and sustained expansion of PBLs throughout four rounds of stimulation (13.1 and 13.7 population doublings of PBLs respectively). In contrast, PBLs stimulated with α-CD3 alone, KT32+α-CD3 or KT32/cCD86 and KT32/IL-15/cCD86 in the absence of α-CD3 underwent less than 2.5 population doublings in the first stimulation and failed to expand upon re-stimulation. CFSE proliferation profiles of $CD4^+$ and $CD8^+$ T cell subsets revealed that cCD86 expression on aAPC increased the responder frequency ((RF) percentage of cells in culture undergoing at least one cell division) of both $CD4^+$ and $CD8^+$ T cell subsets compared to KT32 cells alone (KT32+α-CD3 RF: $CD4^+$ 11%, $CD8^+$ 42%; KT32/cCD86+α-CD3 $CD4^+$ 65%, $CD8^+$ 86%). PBLs co-cultured with KT32/cCD86+α-CD3 or KT32/cCD86/IL-15+α-CD3 showed comparable RFs of $CD8^+$ cells, however the presence of IL-15 doubled the $CD8^+$ proliferative capacity ((PC) average number of daughter cells per responding cell). In summary, we have generated an aAPC system that supports robust expansion of canine T cells ex-vivo, in the absence of exogenous IL-2. This achievement provides the first steps toward making adoptive immunotherapy with artificially expanded, native or genetically modified, autologous canine T cells a reality that may be employed in the treatment of canine cancer and infectious disease.

ABSTRACT #25

IDENTIFICATION OF TUMOUR MARKERS IN A NEWLY ESTABLISHED CELL-LINE ALLOWING DEVELOPMENT OF THERAPEUTIC STRATEGIES FOR PROSTATE CANCER. Winkler, S,[1] Murua Escobar, H.[2], Eberle, N.[2], Meyer, B[1]., Reimann-Berg, N[1]., Fork, M.[2], Nolte, I[2]. and Bullerdiek, J[1]. [1]Center of Human Genetics, University of Bremen, Bremen, Germany [2]Small Animal Clinic, School of Veterinary Medicine, Hannover, Germany.

Although prostate carcinomas are rare, there is an increasing number of dogs developing this locally invasive malignancy with a high metastatic potential. Due to the poor prognosis and the rare number of cases, in vivo models for the development of therapeutic approaches are of significant importance. In various human carcinomas the aberrant expression of *HMGA* is a well known fact. Several studies demonstrated that this altered expression is related to tumour differentiation and metastatic potential.

HMGA-proteins are highly conserved during evolution. In previous investigations we characterised the canine *HMG* genes and described the close similarity between canine and human genes and proteins. In tissue specific cell-lines, the application of an adenovirus carrying the *HMGA* gene in antisense orientation abrogated the effects mediated by over expression of *HMGA*. These studies indicated, that the infection with *HMGA* antisense constructs lead to cell death in *HMGA* expressing cell-lines.

Recently, we established a cell-line derived from a canine prostate carcinoma with a highly rearranged karyotype. 20 NOD-SCID mice were injected with an amount of either 1×10^6 or 1×10^5 of these cells by subcutaneous or peritoneal injection, respectively, showing their ability to induce tumour growth. Real-time quantitative RT-PCR showed expression of high levels of *HMGA2* in both the cell-line and the xenografts. FISH-screening for *HMGA2* showed no amplification of the gene. These results

could serve as first steps for the development of gene therapeutic strategies for the treatment of cancers expressing elevated levels of *HMGA* using antisense strategies.

ABSTRACT #26

LEVOVIST-ENHANCED CONTRAST HARMONIC IMAGING OF CANINE ABDOMINAL MASSES. Kenji Kutara, Kazushi Asano, Kazuya Edamura, Hisashi Shibuya, Tsuneo Sato, Atsuhiko Hasegawa, Shigeo Tanaka; Nihon University, Kanagawa, Japan.

Contrast harmonic imaging (CHI) is a cutting edge diagnostic ultrasound technique for the evaluation of tissue perfusion in various ischemic diseases and vascularity of tumors in human medicine. The purpose of this study was to assess the CHI findings of abdominal masses in dogs by using Levosist.

The subjects of this study comprised 16 dogs, including 10 with hepatic tumors [8 hepatocellular carcinomas (HCC) and 2 hepatic hemangiosarcomas (HHS)], 4 with splenic masses (1 malignant histocytoma, 1 hematoma, 1 nodular hyperplasia, and 1 plasmacytoma), and 2 with bladder masses [1 transitional cell carcinoma (TCC) and 1 polypoid cystitis]. For CHI, Levovist (300 mg/mL, 0.1 mL/kg) was administered as a bolus venous injection. In each dog, the masses and surrounding tissues were examined in the transverse and longitudinal planes for CHI, and the data was recorded. In the liver, the early arterial and late vascular phases of CHI were evaluated for 1 min after Levovist administration, and the post vascular phase was assessed from 5 min after the administration. In the spleen, the arterial phase of CHI was evaluated for 30 sec after Levovist administration, and the parenchymal phase was assessed from 5 min after the administration. In the urinary bladder, CHI was evaluated for 1 min after Levovist administration. After CHI, the masses were surgically removed from each dog, and all the resected masses were diagnosed histopathologically.

In canine HCC, the CHI finding of the early arterial phase revealed the enhancement of a fine network of blood vessels in the surrounding region and within the tumor in all the 8 dogs (100%). Moreover, the post vascular phase demonstrated a defect in the whole tumor and enhancement of the surrounding hepatic tissues in 7 dogs (87.5%). In 2 dogs with HHS, the characteristic CHI findings were observed; the early arterial and late vascular phases showed a rim contrast enhancement pattern, and the post vascular phase revealed that the whole tumor lacked contrast enhancement, and the surrounding hepatic tissues was clearly enhanced.

In the arterial phase of CHI for dogs with splenic masses, the hematoma case showed slight enhancement of the tumor in contrast to the obvious enhancement of the whole tumor in the other 3 cases. In the parenchymal phase, the mass was partially enhanced in the splenic nodular hyperplasia case, whereas there was no enhancement of the whole tumor and an obvious enhancement of the surrounding splenic tissues in the other 3 cases. On the other hand, in the cases of canine urinary bladder masses, similar enhancement of the masses was observed in both TCC and polypoid cystitis.

In conclusion, CHI may be of clinical value for the diagnosis of canine HCC and HHS. However, further clinical studies are required to evaluate the diagnostic usefulness of CHI in canine splenic and urinary bladder masses.

ABSTRACT #27

ADUVANT EPIRUBICIN IN THE TREATMENT OF SPLENIC HEMANGIOSARCOMA IN DOGS. SE Kim, JM Liptak, TT Gall, JP Woods. Ontario Veterinary College, Guelph, ON.

Hemangiosarcoma (HSA) is a highly malignant neoplasm of vascular endothelial origin that is characterized by early distant metastasis and poor survival rates, despite surgical treatment and chemotherapy. Doxorubicin-based protocols are recommended for the adjuvant treatment of dogs with HSA, however dose-dependant cardiotoxicity may limit its use in some dogs. Epirubicin is an anthracycline chemotherapy agent with similar mechanisms of action and antitumor activity as doxorubicin, but substantially decreased cardiotoxicity when compared to doxorubicin. The purposes of this retrospective study were to compare the survival time of dogs with splenic HSA treated with splenectomy and adjuvant epirubicin to dogs treated with surgery alone; evaluate the type and severity of epirubicin-induced toxicities; and identify predictors of survival.

The medical records at the Ontario Veterinary College were reviewed retrospectively for dogs with histologically confirmed splenic HSA from May 1997 to September 2004. Dogs were divided into 2 groups: splenectomy alone and splenectomy with adjuvant epirubicin. For dogs treated with epirubicin, chemotherapy was initiated within 3 weeks of splenectomy and targeted to receive epirubicin at a dose of 30 mg/m^2 every 3rd week for a total of 6 treatments. Dose numbers, intervals, and reductions, and type and severity of toxicities were recorded. Kaplan-Meier survival analysis with log rank was used to compare survival times and Cox proportional hazards regression analysis was used identify prognostic factors.

Fifty-nine dogs were included in the study: 41 dogs with splenectomy alone and 18 dogs with splenectomy and adjuvant epirubicin. While only 2 out of 18 dogs completed the targeted course of 6 cycles, 50% of the dogs received 4 or more doses. Seventy-nine percent of the doses resulted in toxicity, but the vast majority of these were mild and self-limiting. No dogs developed cardiac arrhythmias or signs of heart failure. The overall median survival time (MST) was significantly longer in dogs treated with splenectomy and epirubicin (144 days) compared to splenectomy alone (86 days; $P = 0.04$). The MST for dogs with stage I disease (345 days), regardless of treatment, was significantly longer than dogs with either stage II (93 days; $P = 0.04$) or III disease (68 days; $P = 0.007$). The MST for dogs with stage III disease treated splenectomy and epirubicin (135 days) was significantly longer than dogs with stage III disease treated with splenectomy alone (54 days; $P = 0.01$). Inappetence, duration of clinical signs, thrombocytopenia, neutrophilia, and mitotic rate were negative prognostic factors.

For dogs with splenic HSA, adjuvant epirubicin results in similar survival times to historical reports of adjuvant doxorubicin-based protocols. Cardiotoxicity was not diagnosed in any dog treated with epirubicin. Dogs with stage III splenic HSA had a significant survival benefit with adjuvant epirubicin.

ABSTRACT #28

A RETROSPECTIVE ANALYSIS OF COARSE FRACTIONATION (0-7-21) EXTERNAL BEAM RADIATION THERAPY OF CANINE ORAL MELANOMA (88 CASES). J. Paul Woods, ACG Abrams-Ogg, Dianna E Saam, Stephen Kruth, Karen E Bateman, Kim Stewart, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

Melanoma, the most common oropharyngeal cancer in dogs, is characterized by local invasion and early widespread metastases. Surgical excision has been the primary means of local control; however, radiation has been increasingly utilized with various radiation protocols. The purpose of this retrospective study was to assess the (0–7–21 day) radiation therapy protocol for treatment of canine oral melanoma.

The records of dogs presented to the Ontario Veterinary College 1990 to 2005 with histologically confirmed oral melanoma treated with coarse (0–7–21 day) radiation therapy were retrospectively entered into the study. Staging included size of primary tumour, aspirate or biopsy of submandibular lymph node, and 3-view thoracic radiographs. Radiation therapy was delivered by a Cobalt 60 teletherapy unit. The treatment volume included the primary tumour site and mandibular lymph nodes. Dogs received 2,400 cGy in 3 equal fractions, delivered on day 0, day 7, and day 21.

Eighty-eight dogs were entered into the study consisting of 16 male, 33 male (neutered), 3 female, 36 female (spayed); mean age 11.1 years (median 11.4 years; range 10 months to 17 years); of various breeds (including 23 mixed breed, 10 poodles, 9 cocker spaniels, 4 Chow Chow); at different oral sites (39 maxilla, 33 mandible, 15 palate/tongue, 1 unreported). The overall median survival was 242 days (range 18–2,154 days). By WHO stage:

WHO Tumour Stage	Number of dogs	Survival median (days)	Survival range (days)
I	27	549	22 - 2,154
II	23	207	18 - 935
III	38	170	21 - 1,018

Survival between Stages I versus II, and Stages I versus III were statistically different ($p < 0.01$).

Tumour response (reported for 52 dogs) consisted of: 15 complete remission (29%), 18 partial remission (35%), 13 stable disease (25%), and 6 progressive disease (11%). All dogs completed radiation therapy without delays in therapy. Side effects were reported in 18 dogs which consisted of: alopecia (4 dogs), hypopigmentation (3 dogs), tumour necrosis (2 dogs), tumour bleeding (1 dog), erythema (1 dog), and mild pruritis (1 dog). Post mortems were performed on 13 dogs which revealed local disease in 10 dogs (2 dogs had only local disease), and metastases in 11 dogs (3 dogs had only metastases).

Canine oral melanomas are responsive to radiation and hypofractionated regimens are well tolerated with only mild toxicity; however, the most effective radiation fractionation schedule is yet to be determined for local control. Further therapy is required for systemic control.

ABSTRACT #29

EFFECT OF AGE ON SYSTEMIC ANTIBODY RESPONSE FOLLOWING RABIES AND INFLUENZA VACCINATIONS IN HEALTHY HORSES. T Muirhead[1], JT McClure[1], J Wichtel[1], D. McFarlane[2], DP Lunn[3]. [1]Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada, [2]Olkahoma State University, Stillwater, OK; [3]Colorado State University, Fort Collins, CO.

The immunogenicity of vaccination in aged horses has not been critically evaluated. To evaluate the effect of age on systemic antibody response to vaccination, 34 aged healthy horses (≥20 years) and 29 younger adult horses (4–12 years) of varying breeds were initially vaccinated with killed rabies (Imrab®) and influenza vaccines (Calvenza®). Horses in each age group were randomly allocated to receive either rabies or influenza booster vaccine 4 weeks after the initial vaccination. Experimental animals had no history of influenza vaccination and respiratory infection during the 12 months prior to enrolment, and no history of rabies vaccination. Serum specific immunoglobulins were measured prior to vaccination, and at 4, 8 and 24 weeks after initial vaccination. Rabies serum neutralization assay (RSNA) and ELISA for equine influenza virus specific antibody sub-types IgGa, IgGb, IgG(T) and IgA were performed.

A general linear model was used to evaluate the effect of age on titer difference (the difference between pre- and post-vaccination titer for each period). Age did not affect rabies titer difference. Horses receiving a rabies booster vaccine had a greater increase in titer at 8 and 24 weeks after initial vaccination when compared to those that did not receive a rabies booster vaccination ($p < 0.001$), the latter having titers considered inconsistent with adequate protection. Older horses had a greater increase in influenza IgA titer at 4, 8, and 24 weeks ($p = 0.001, 0.035, 0.010$, respectively) when compared to the younger horses; there was no booster vaccination effect. Younger horses had a greater increase in IgGb titers at 8 and 24 weeks ($p = 0.041, 0.010$, respectively) when compared to older horses. Horses receiving an influenza booster vaccination had a greater increase in IgGb titers at 8 and 24 weeks ($p = 0.001$ and 0.003, respectively) when compared to horses that did not receive an influenza booster vaccination. Younger horses had a greater increase in IgGa titers at all time periods when compared to older horses ($p < 0.001$). Horses receiving an influenza booster vaccination had a greater increase in IgGa titers at 8 and 24 weeks ($p = 0.001$ and 0.051, respectively) when compared to horses that did not receive an influenza booster vaccination. The titers recorded for IgG(T) were considered below detection limits in all horses.

In summary, both young and old horses mounted an immune response to rabies and influenza vaccination. The increase in antibody response in aged horses after influenza vaccination was significantly lower when compared to younger horses, except for IgA. Aged horses mounted a primary immune response to rabies vaccination equivalent to younger horses. Based on the findings in this study, the currently recommended single-dose strategy for vaccination of rabies-naïve horses should be re-evaluated.

ABSTRACT #30

ALTITUDE MAY AFFECT THE INCIDENCE AND SEVERITY OF EXERCISE-INDUCED PULMONARY HEMORRHAGE IN THOROUGHBRED RACEHORSES. MN Saulez[1], AJ Guthrie[1], KW Hinchcliff[2], D Macdonald[3]. [1]Equine Research Center, Faculty of Veterinary Science, University of Pretoria, South Africa. [2]College of Veterinary Medicine, The Ohio State University, Columbus, OH. [3]The National Horse Racing Authority, South Africa.

Exercise-induced pulmonary hemorrhage (EIPH) occurs frequently in horses undergoing strenuous exercise. The prevalence of EIPH has been well documented in several countries and risk factors reported. Although EIPH-related epistaxis is reported to occur more often at sea level, the effect of altitude on prevalence of EIPH as detected by tracheobronchoscopy is not known. Plausible reasons do exist for suspecting that exacerbated hypoxemia during maximal exercise at high altitude could increase the prevalence of EIPH. Our hypothesis was that racing at high altitude would increase the prevalence and severity of EIPH in Thoroughbred racehorses. We therefore investigated the incidence and severity of EIPH in South Africa, a racing jurisdiction that does not permit race day administration of furosemide, and in which horses race at both sea level and at high altitude.

A prospective, cross-sectional study of pre-enrolled Thoroughbred racehorses competing in flat races at high altitude (1450 m above sea level) and at sea level was performed between 1 August and 19 November 2005. Tracheobronchoscopic examinations were performed within 2 hours after racing and recorded onto digital video disc and the presence and severity of EIPH was graded using a previously described system. Data was analyzed using a Chi-square test, Cochran's linear, and Student's t-test. $P < .05$ was considered significant.

Tracheobronchoscopic examinations were performed on 1,014 racehorses (mean ± SD age 4.3 ± 1.1 years {altitude} and 3.9 ± 1 years {sea level}), competing at 5 race venues in 28 race meets over a race distance of 1,488 ± 414 m (altitude) vs. 1,421 ± 345 m (sea level). The severity of EIPH for 411 horses examined at high altitude and 603 horses examined at sea level was: grade 0 (49.6 vs. 41.5%), grade 1 (33.3 vs. 30.3%), grade 2 (9 vs. 13.1%), grade 3 (6.3 vs. 10.4%), grade 4 (1.7 vs. 4.6%) respectively. At sea level, EIPH was more prevalent ($P = 0.002$) with a greater proportion of racehorses having more severe EIPH ($P < 0.001$). EIPH-related epistaxis was present in 6 of 35 (17.1%) horses with a grade 4 EIPH. Overall, racehorses examined at sea level were younger ($P < 0.001$) and competed over shorter distances ($P = 0.005$).

Racing at high altitude does not appear to be associated with increased prevalence or severity of EIPH. We conclude that as yet unidentified factors contribute to this increased risk of EIPH at sea level, despite horses being younger and racing shorter distances.

ABSTRACT #31

EVALUATION OF LUNG FUNCTION AND AIRWAY CYTOLOGY IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION IN LONG-TERM REMISSION. M Miskovic, L Couëtil, C Thompson. Purdue University School of Veterinary Medicine, West Lafayette, IN.

The purpose of the study was to determine if horses with recurrent airway obstruction (RAO) have evidence of airway inflammation and peripheral airway obstruction at 1, 2–3, and 5–6 years after diagnosis while being maintained in low-dust environments.

A case-control study was performed. Horses with RAO were evaluated at 1 year (n = 9, time 1), 2–3 years (n = 7, time 2), and 5–6 years (n = 8, time 3) after diagnosis. These horses had been initially treated for RAO, and were then maintained in low-dust environments with no further medical management. Age-matched horses were used as healthy controls. A physical examination, standard lung mechanics, forced expiration maneuvers, and bronchoalveolar lavage (BAL) were performed on each horse. Data are presented as median [range].

The clinical score (CS, range 0–21) of the RAO horses was higher than the control horses at time 2 (cases CS = 3.0 [2–5], controls CS = 2.0 [0–3], $P = 0.021$) and time 3 (cases CS = 2.5 [2–4], controls CS = 2.0 [0–3], $P = 0.049$). Standard lung mechanics data were not significantly different between the groups at any time point. Forced expiration maneuvers showed evidence of peripheral airway obstruction in the RAO-affected horses but not the control horses at all time points (time 3: cases $FEF_{95\%}$ = 5.35 L/s [2.2–16], controls $FEF_{95\%}$ = 23.15 L/s, [15.4–26.7], $P = 0.0011$). BAL results showed a decreased number of total nucleated cells (TNC) and lymphocytes (lymph) in the RAO-affected horses compared to the control horses at time 2 (cases TNC = 301/μL [184–482], controls TNC = 392/μL [354–592], $P = 0.025$; cases lymph = 75/μL [26–315], controls lymph = 190/μL [127–280], $P = 0.048$) and time 3 (cases TNC = 237/μL [44–492], controls TNC = 388/μL [332–564], $P = 0.028$; cases lymph = 48/μL [4–147], controls lymph = 159/μL [74–234], $P = 0.0055$).

The clinical score of RAO-affected horses was statistically higher than the control horses. This difference was not considered clinically relevant. Horses with RAO do not have persistent neutrophilic airway inflammation years after being maintained in low-dust environments. In fact, they have lower total nucleated cell counts and lymphocyte counts. However, peripheral airway obstruction is persistently detected. The obstruction is likely due to airway remodeling. Further work should be done to characterize the pulmonary lymphocyte phenotype of RAO-affected horses and determine its effects on inflammation and remodeling.

ABSTRACT #32

EVALUATION OF DI-TRI-OCTAHEDRAL SMECTITE NEUTRALIZATION OF ALPHA, BETA AND BETA-2 TOXINS OF *CLOSTRIDIUM PERFRINGENS*. J Boggs Lawler[1], DM Hassel[1], JL Traub-Dargatz[1], R Magnuson[2], PM McCue[1], DR Hyatt[3], RP Ellis[4]. [1]Department of Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO. [2]Animal Population Health Institute,

Colorado State University, Fort Collins, CO. [3]Veterinary Diagnostic Laboratory, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO. [4]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO.

Clostridial-associated enterocolitis is a sporadic disease of neonatal foals and adult horses. However, it is associated with a high case-fatality rate and substantial economic losses. The severity of disease appears to be related to the particular Clostridial isolate, and thus, the specific exotoxin produced. Higher levels of *Clostridium perfringens* alpha, beta, and beta-2 exotoxins have been identified in the feces of clinically affected animals as compared to healthy controls. Di-tri-octahedral (DTO) smectite has been shown to be effective at neutralizing toxins; however, the effect on *C. perfringens* exotoxins has not been evaluated.

An *in vitro* study was performed to evaluate the efficacy of a commercially available DTO smectite (Bio-Sponge™) at neutralizing *C. perfringens* alpha, beta, and beta-2 exotoxins. For comparison, the effectiveness of distilled water at neutralizing toxins was evaluated as a control. Additives (DTO smectite or distilled water) were mixed with standard amounts of each toxin in serial concentrations ranging from 1:4 to 1:131,072. Samples were incubated at room temperature for 30 minutes. Clarified supernatants were placed on indirect ELISA plates, thus enabling measurement of the amount of exotoxin present after incubation.

Results showed that DTO smectite was effective in neutralizing *C. perfringens* alpha, beta and beta-2 exotoxins. Thus, DTO smectite (Bio-Sponge™) may have clinical application for reducing the severity of disease associated with Clostridial enterocolitis. *In vivo* studies are necessary to evaluate clinical outcomes.

ABSTRACT #33

CLINICAL ALTERATIONS IN HORSES WITH BRONCHOALVEOLAR LAVAGE EOSINOPHILIA. M. Riihimäki[a], Inger Lilliehöök[b], John Pringle[a] [a]Swedish University of Agricultural Sciences, Department of Clinical Sciences, Section of Equine Internal Medicine, Box 7018, 750 07 Uppsala, Sweden. [b]Swedish University of Agricultural Sciences, Department of Biomedicine and Veterinary Public Health, Section of Clinical Pathology, Box 7038, 750 07 Uppsala, Sweden.

There are few studies of the background and clinical significance of eosinophilia in bronchoalveolar lavage in horses. To document history, clinical and laboratory diagnostic findings, treatment and outcome of pulmonary eosinophilia we have reviewed retrospectively medical records from all horses bronchoalveolar lavage (BAL) sample taken from two referral hospitals in Sweden from April 2002 to June 2005.

Results: Totally 272 horses with BAL record were studied and 14 had pulmonary eosinophilia (>5%, mean 14.1%) in BAL. The horses with eosinophilia had a lower mean age than the entire group undergoing BAL (4.9 yr vs 8.4 yr), and stallions and standardbred trotters were over-represented. Several horses had been referred solely due to exercise intolerance rather than clear respiratory signs. The horses with the highest numbers of pulmonary eosinophils returned to normal BAL cytology without specific treatment. In comparison to all horses undergoing BAL, those with eosinophilia generally had higher total cell counts (489×10^6 vs 303×10^6), lower neutrophil percent (3.5% vs 12.5%) and higher mast cell percent (4.3% vs 3.0%) in BAL. On those horses with a complete blood count eosinophilia was not present, nor were there any clinically significant changes to other white blood cell parameters or to plasma fibrinogen.

Conclusions: Racehorses that lack obvious clinical signs of respiratory disease but have exercise intolerance can have marked pulmonary eosinophilia as the sole detected laboratory abnormality. To better define the significance of this pulmonary eosinophilia we are currently investigating immunological mechanisms behind eosinophilia in selected portion of our horses, with immunohistochemistry and PCR in lung tissue and BAL material.

ABSTRACT #34

DOMPERIDONE CAUSES AN INCREASE IN PLASMA ENDOGENOUS ACTH IN HORSES WITH PARS INTERMEDIA TUMORS. JE Sojka, LP Jackson, GE Moore. Purdue University, West Lafayette, IN.

Equine pituitary pars intermedia dysfunction (PPID, Equine Cushing's Disease) affects the melanotroph cells of the pars intermedia. Melanotrophs possess D_2 receptors and are subject to inhibition by dopaminergic neurons. The purpose of this study was to investigate the effect of domperidone, a dopamine antagonist, on endogenous ACTH secretion in horses with and without histologically-confirmed tumors of the pars intermedia.

Seven horses were used in the study: 5 with PPID and 2 control. The average ages of the PPID and control horses were 22.2 (range 17–35) and 14.5 (12 and 17) years respectively. One and one half grams domperidone (Equidone®, Equi-Tox, Inc.) was given per os immediately after 5 ml blood was collected into silicone-coated EDTA tubes. Blood was again collected at +4 and +8 hours. The plasma was separated within 15 minutes of collection and frozen until assay. Endogenous ACTH was determined via chemiluminescent immunoassay. Horses were then euthanized and determined to have PPID if there was gross and/or histologic evidence of adenoma in the pituitary pars intermedia.

Time 0 ACTH concentrations in the 5 PPID horses were 37.8, 25.8, 30.8, 271.0, and 77.0 pg/ml respectively. Thus, 3 of the 5 had baseline ACTH values within our reference range (10–59 pg/ml) and would not have been identified as PPID using a one-time ACTH measurement. In all instances the +4 hour ACTH concentration was above our reference range in PPID horses, but not increased in the control animals. The +4/Time 0 ratio value was calculated by dividing the ACTH concentration at the +4 time point by the baseline value.

ACTH Concentration (pg/ml)

PPID	Baseline	+4 hours	+ 8 hours	+4 hours/Time 0 ratio
Average	77.5	217.2	139.2	4.26
St. Dev.	104.0	203.2	153.5	3.37
Range	25.8 -271.0	72.0-566.0	38.7-410	2.1-10.1
Control	Baseline	+4 hours	+ 8 hours	+4 hours/Time 0 ratio
Average	28.8	26.6	26.9	0.98
Range	20.5-37.1	24.6-28.6	25.2-28.7	0.77-1.2

In conclusion, in this small sample an exaggerated response to domperidone was only observed in PPID horses, and it proved more useful than a baseline ACTH measurement when differentiating PPID from control animals. The domperidone-response-test holds promise as an evocative test that can assist in the diagnosis of PPID in horses.

ABSTRACT #35

PCR RESULTS FOR EHV-1 FOLLOWING VACCINATION WITH MODIFIED LIVE VIRUS. K.P. Chaney[1], H.C. Schott II[1], N. Pusterla[3], and R. Maes[2]. [1]Department of Large Animal Clinical Sciences. [2]Diagnostic Center for Population and Animal Health, Michigan State University, East Lansing, MI. [3]Veterinary Medical Teaching Hospital, University of California at Davis, Davis, CA.

PCR methods are being increasingly used for detection of pathogens in tissue and fluid samples. Although a highly sensitive tool for detection of viral, bacterial, or protozoal DNA, PCR test results are sometimes difficult to interpret in relation to disease, especially with pathogens that may become latent or have been recently administered in vaccines. Specifically, we have obtained positive PCR results for EHV-1 from nasopharyngeal swabs collected from horses with neurological disease other than EHV-1 myeloencephalopathy and from clinically normal horses that have recently been vaccinated against EHV-1. These findings are clearly of concern with the recent increased attention and perhaps increased occurrence of EHV-1 myeloencephalopathy.

To investigate whether intramuscular vaccination with a modified live EHV-1 vaccine could induce transient positive PCR results in either blood or secretions collected on a nasopharyngeal swab, a pilot study was performed. A modified live EHV-1 vaccine was administered to four of six co-mingled horses. Blood and nasopharyngeal swabs were collected from these six horses, as well as two additional control horses housed at a distance more than 500 meters away from the other six horses. Samples were collected twice prior to vaccination and once weekly for 4 weeks following vaccination and submitted for PCR testing for EHV-1 by two independent laboratories utilizing different PCR methodologies.

Although EHV-1 in the vaccine was readily detected by PCR as a positive control, both laboratories reported negative PCR results on all eight horses for blood and nasopharyngeal swabs both before and throughout the 4-week sampling period following vaccination. The findings of this pilot study would have been of clinical importance even if only one sample collected after vaccination tested positive. Unfortunately, these negative data do not exclude the possibility that a positive result would be found if a larger number of vaccinated horses would be tested. Thus, the question of whether administration of vaccines could lead to transient "false positive" PCR test results was not answered in this pilot study. In addition, results could also differ following vaccination of weanlings or immunocompromised horses.

ABSTRACT #36

TEMPORAL DETECTION OF EQUINE HERPESVIRUS INFECTIONS OF A COHORT OF MARES AND THEIR FOALS. SA Bell, UBR Balasuriya, IA Gardner, PA Barry, WD Wilson, GL Ferraro, NJ MacLachlan, School of Veterinary Medicine, University of California, Davis, CA.

The presence of equine herpesviruses (EHV) 1–5 in the nasal secretions (NS) and peripheral blood mononuclear cells (PBMC) of a cohort of 12 mares and their foals from birth to 6 months of age was determined using virus-specific PCR. EHV-2, 4, and 5 were all detected in the NS of horses in the group, but only the presence of EHV-4 was associated with overt respiratory disease. EHV-2 and 5 infections were both common, but EHV-2 had a greater overall prevalence in NS than EHV-5 ($p = .02$). The majority of foals also shed EHV-2 in their nasal secretions earlier than EHV-5 ($p = .0005$). Apparent latent EHV-2 and 5 infections were detected in the PBMC of 75% of the foals at approximately 6 months of age. Phylogenetic relationships among the various strains of EHV-2 and 5 were examined by comparing sequences of a homologous 402 base pair section of the glycoprotein B gene. The strains of EHV-2 shed in the NS of individual horses were more genetically heterogeneous and had more polymorphisms than the strains of EHV-5 (90.2% vs 97.3% sequence identity respectively). One-month-old foals consistently shed strains of EHV-2 that were identical to those infecting their dams whereas older foals often shed virus strains that were different from those of their dams, suggesting early transmission of EHV-2 may occur from mare to foal. Although herpesvirus infections were ubiquitous in this cohort of horses, there were distinct clinical consequences and clear epidemiological differences among infections with the different viruses.

ABSTRACT #37

CYTOKINE PROFILES OF PERIPHERAL BLOOD MONONUCLEAR CELLS ISOLATED FROM SEPTIC AND HEALTHY NEONATAL FOALS. JR Gold[a], GA Perkins[a], HN Erb[b], DM Ainsworth[a]. [a]Dept of Clinical Sciences and [b]Dept of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY. USA.

In human and equine neonates, there is evidence that the immune system is biased toward a Th-2 response and that Th-1 cells have a reduced capacity to produce interferon-γ (IFNγ). Presently, little is known about the cytokine response in septic foals.The purpose of this study was to compare the gene expression of interleukin 1β (IL-1β), IL-4, IL-6, IL-8, toll-like receptor 4 (TLR-4), IFNγ in septicemic and healthy foals.

A prospective study of neonatal foals admitted to the Cornell University Hospital for Animals during the spring of 2004 and 2005 was performed. Blood samples were collected from foals at the time of admission (T0) and then 24 (T24) and 72 (T72) hours later. Septicemia was diagnosed in 21 foals that had a sepsis score ≥11. All foals were treated with antibiotics, 1 liter of plasma[a] and supportive therapies. Healthy foals born at the CU Equine Park in the spring of 2005 served as controls and blood was obtained prior to, 24 and 72 hours after the IV administration of plasma (1 L). Peripheral blood mononuclear cells were isolated from blood samples using Ficoll density gradients, enumerated and frozen −80° C until analyzed. RNA was isolated, cDNA was synthesized and the gene expression of IL-4, IL-6, IL-8, IL-1*B*, IFNγ, TLR-4 and β-actin was measured using real-time RT-PCR. Wilcoxan rank sum tests were performed. Statistical significance was considered at $p \leq 0.05$ and a Bonferroni adjustment was used (0.0083).

In the healthy foals, there were no significant changes in the gene expression of the cytokines measured over time. In septicemic foals, the gene expression of IL-4 increased from T0 to T24. At T0, the gene expression of TLR-4 was up regulated ($p = 0.007$) and IL-4 was down regulated in the septic foals compared to the healthy foals ($p = 0.0007$). In the septic foals at T24 the gene expression of IL-6 ($p = 0.0006$) was decreased compared to the healthy foals. At T0, septicemic foals that died ($n = 3$) had a 15-fold higher expression of IL-6 ($p = 0.005$) than those that survived ($n = 18$).

Although septic foals responded to bacterial infections by up regulating TLR4 expression and down-regulating IL-4 gene expression, they failed to up-regulate the gene expression of IFN-γ. Limited data ($n = 3$) suggest that an overzealous IL-6 response is associated with reduced survival but this requires further investigation.

ABSTRACT #38

ACTH, CORTISOL AND VASOPRESSIN LEVELS OF SEPTIC (SURVIVORS AND NONSURVIVORS) IN COMPARISON TO NORMAL FOALS. JR Gold[a], TJ Divers[a], MH Barton[b], SV Lamb[a], NJ Place[a], HO Mohammed[a], FT Bain[c]. [a]Cornell University, Dept of Clinical Sciences, College of Veterinary Medicine, Ithaca, NY. USA. [b]Department of Large Animal Medicine, University of Georgia, Athens, GA. USA. [c]Hagyard Equine Medical Institute, Lexington, KY. USA.

There is little information on the hypothalamic-pituitary-adrenal axis in septic foals. The purpose of this study was to determine plasma ACTH, cortisol and vasopressin (AVP) concentrations in septic foals and compare them to normal age-matched foals. Blood was collected once from 28 normal foals less than 14 days of age and from 46 septic foals at the time of admission to tertiary care referral centers. Septic foal selection was based on a sepsis score of >11 and/or a positive blood culture. ACTH and cortisol values were measured in all foals on EDTA plasma by chemiluminescent immunoassay. Radioimmunoassay measured AVP in 25 septic and 13 normal foals. ACTH, cortisol, ACTH/cortisol ratio and AVP values were compared between groups by ANOVA with significance set at $P < 0.05$.

In the septic foal group, 28 foals survived to discharge. Septic foals had significantly higher ACTH, cortisol, ACTH/cortisol ratio and AVP values, compared to normal foals. The ACTH/cortisol ratio and AVP concentration were significantly greater in non-surviving foals. A positive correlation ($r^2 = 0.66$) existed between ACTH and AVP values in septic foals.

In conclusion, septic foals had increased ACTH, cortisol, ACTH/cortisol ratio and AVP values compared to normal foals, which is an expected endocrine response to critical illness. However, the increased ACTH/cortisol ratio in non-surviving septic foals may indicate hypothalamic-pituitary-adrenal axis dysfunction at the level of the adrenal gland. Increased AVP values in septic non-survivors could have been a result of hypotension and/or extreme physiologic stress.

ABSTRACT #39

THE EFFECT OF UTERINE TORSION ON MARE AND FOAL SURVIVAL: A RETROSPECTIVE STUDY 1985-2005. Kristin .P. Chaney[1], Susan J. Holcombe[1], Michele M. LeBlanc[2], Joe G. Hauptman[1], Rolf M. Embertson[2], P.O. Eric Mueller[3], Warren L. Beard[4]. [1]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI. [2]Rood and Riddle Equine Hospital, Lexington, KY. [3]Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA. [4]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH (Dr. Beard is currently at Kansas State University, Department of Clinical Sciences, College of Veterinary Medicine, Manhattan, KS).

Uterine torsion in mares occurs in mid to late gestation.Previous reports suggest that the prognosis for survival of mares and foals following uterine torsion is 60%–70% and 30%–70%, respectively. The purpose of this study was to determine the effect of duration of clinical signs, stage of gestation when uterine torsion occurred, parity, physical examination findings of the mare and method of correction, on the prognosis for survival of the mare and foal, as well as future reproductive health of the mare.

Medical records of sixty-three mares from four referral hospitals were reviewed and follow-up phone calls were made to complete data collection. Data were analyzed using a chi-square for the discreet data and a t-test for the continuous data, $p < 0.05$.

Stage of gestation when uterine torsion occurred was associated with survival of the mare and foal. Overall mare survival was 84% (53/63): when uterine torsion occurred at 10 months or less of gestation, 97% (36/37) of mares survived, compared to 65% (17/26) survival rate when uterine torsion occurred at greater than 320 days of gestation. Overall foal survival was 54% (29/54): when uterine torsion occurred at 10 months or less of gestation, 72% (21/29) of foals survived compared to 32% (8/25) survival when uterine torsion occurred at greater than 320 days of gestation. Of the thirty mares discharged from the hospital carrying a live fetus, twenty-five foals lived beyond the neonatal period. Method of correction was associated with foal survival. When uterine torsion occurred at less than ten months of gestation, ventral midline celiotomy was associated with decreased foal survival compared to standing flank laparotomy. Method of uterine torsion correction in mares greater than 320 days of gestation was biologically significant because 75% of mares survived following ventral midline celiotomy compared to 40% of mares that were rolled to correct uterine torsion. Sixty-seven percent of mares were successfully rebred following correction of uterine torsion

In conclusion, stage of gestation when uterine torsion occurs should be considered when advising clients regarding the prognosis for survival of mares and foals.

ABSTRACT #40

LONG-TERM FOLLOW UP OF EXPERIMENTALLY INDUCED INFECTION WITH *ANAPLASMA PHAGOCYTOPHILUM* IN HORSES: CLINICAL AND PCR FINDINGS. P Franzén[1] , A Aspan A[2], A Egenvall[1], A Gunnarsson[2], E Karlstam[2], J Pringle[1]. [1]Swedish University of Agricultural Sciences, Uppsala, Sweden [2]National Veterinary Institute, Uppsala, Sweden.

The purpose of this study was to evaluate if *Anaplasma phagocytophilum* can persist in horses after acute disease and, if so, describe occurrence of any clinical sign/s.

Five healthy horses were experimentally inoculated intravenously with infected blood originating from a Swedish horse with naturally occurring Equine Granulocytic Anaplasmosis (EGA). They were followed for 98 to 129 days post inoculation (p.i.) with complete clinical exams daily and blood samples mostly every other day for PCR testing.

Acutely, all horses developed typical clinical signs of EGA and recovered spontaneously whereas the PCR turned positive before onset of clinical signs and remained positive on average 17 consecutive days, as previously reported (Franzén et al 2005). During days 22–66 two of the horses were PCR negative on all samples. The other three horses were intermittently PCR positive on three, two and two occasions respectively. After day 66 p.i. four of five horses continued to have periodically positive PCRs. One horse was positive days 128–129 p.i., immediately prior to euthanasia. Clinical examinations throughout the period after recovery from acute disease revealed some changes related to long term catheter use, but no other changes that could be related to the persistence of infection by the EGA agent.

In conclusion, we show it is possible to detect bacterial DNA from *A. phagocytophilum* in horses for at least 129 days after experimental infection. However, such persistence of the bacteria in this study was not associated with detectable clinical signs.

ABSTRACT #41

THE EFFECTS OF NOREPINEPHRINE AND A COMBINED NOREPINEPHRINE AND DOBUTAMINE INFUSION ON SYSTEMIC HAEMODYNAMICS AND INDICES OF RENAL FUNCTION IN THE NORMOTENSIVE NEONATAL THOROUGHBRED FOAL. Hollis AR[1], Ousey JC[2], Palmer L[3], Stoneham SJ[3], Allen WR[2] and Corley KTT[1]. [1]The Royal Veterinary College, London; [2]The Equine Fertility Unit, Newmarket; [3]Rossdale and Partners, Newmarket.

Norepinephrine is a potent vasopressor which is useful during management of hyperdynamic shock. The expected haemodynamic response to an infusion of norepinephrine is an increase in arterial blood pressure. However, norepinephrine may adversely affect renal haemodynamics, reducing blood flow and thus urine output. In septic humans and sheep, a combined infusion of dobutamine and norepinephrine resulted in a higher urine flow than with norepinephrine alone.

8 Thoroughbred foals, 32 to 63 hours of age, were studied. All were born at term and considered to be healthy on the basis of clinical examination, haematology and baseline measurements of cardiac output (lithium dilution) and arterial blood pressure. The foals were sedated with 5–10 mg of intravenous diazepam and instrumented with jugular venous, dorsal metatarsal arterial and urinary catheters for the study. The foals were allowed to stand and nurse from the dam, and given a recovery period of 1 hour from the administration of diazepam. The foals were then restrained in lateral recumbency on a foal mat and given an infusion of norepinephrine (0.1 mcg/kg/min), a combined norepinephrine (0.1 mcg/kg/min) and dobutamine (5 mcg/kg/min) infusion and a control dose of saline, in a double blind, randomised study. Each infusion was maintained for 30 minutes, and measurements were performed during the last 20 minutes of infusion. The washout period was at least 40 minutes between infusions. Heart rate, arterial blood pressure and cardiac output (by lithium dilution) were measured, and systemic vascular resistance, stroke volume, cardiac index and stroke volume index calculated. Renal function was estimated by urine output, endogenous creatinine clearance and the fractional excretion of electrolytes. Repeated measures and one-way ANOVA tests were used to compare these parameters between each drug and placebo.

Compared to saline, both norepinephrine and the combination of norepinephrine and dobutamine increased arterial blood pressure and systemic vascular resistance, and decreased heart rate and cardiac index. The combination resulted in higher pressures than norepinephrine alone. There was no significant difference in urine output or creatinine clearance with either infusion compared to saline.

This dose of norepinephrine has no effect on urine output or creatinine clearance despite changing the global haemodynamics of normotensive neonatal foals. This effect is unique to this species, and warrants further investigation.

ABSTRACT #42

COAGULATION PROFILE AND PLASMA D-DIMER CONCENTRATION IN SEPTIC NEWBORN FOALS. L Armengou, L Monreal, M Navarro, I Tarancón, D Segura. Servei de Medicina Interna Equina, Facultat de Veterinària, Universitat Autònoma de Barcelona, Barcelona, Spain.

The objective of this study was to determine plasma D-dimer values and coagulation parameters in septic newborn foals in order to assess the usefulness of the D-dimer test as a diagnostic tool.

Eighty one sick newborn foals admitted to the hospital were used. Of these, 40 neonates were septic (septic group), and the other 41 neonates were included in the non-septic group. Additionally, 13 healthy newborn foals constituted the control group. Citrated plasma samples were obtained on admission and during hospitalization. Clotting times (PT, aPTT), antithrombin III (AT-III) activity and D-dimer concentration were determined, the last one using both immunoturbidimetric and latex-agglutination techniques.

Mean values ±SD for samples taken on admission are shown in the table below. Significant differences were found for all parameters between the septic and the control group, and for most parameters (all but PT) between the septic and the non-septic group. Significant differences were also found for all parameters (except for D-dimer) between the non-septic and the control foals.

	PT (s)	aPTT (s)	AT-III (%)	D-dimer (ng/ml)
Septic group	19.5±7.9	83.3±30.2	112.4±28.8	2604.0±5280.5
Non-septic group	17.4±6.7	64.5±19.1	136.3±26.1	495.9±668.1
Control group	12.4±0.7	45.7±5.8	173.7±28.5	504.4±299.1

Antithrombin III activity was significantly lower in foals that died or were euthanized. Twenty-five foals (20 septic) were in DIC. D-dimer levels were significantly increased ($p < 0.01$) in DIC foals. The D-dimer value showed a high positive predictive value (85%) to diagnose sepsis, and a high negative predictive value (86%) to rule out DIC. A high correlation between the two D-dimer assays was observed ($r = 0.79$).

In conclusion, septic foals were in a severe hypercoagulation state, and 50% of them were in DIC. Although AT-III activity was the hemostatic parameter with the highest prognostic value, the D-dimer concentration was of clinical usefulness to diagnose septic neonates and neonates in DIC. Reliable D-dimer values can be obtained using the latex-agglutination technique, which is potentially useful in emergency settings.

ABSTRACT #43

COMPARISON OF FOUR METHODS TO ASSESS COLOSTRAL IgG CONCENTRATION. M Chigerwe, JW Tyler, JR Middleton, DW Nagy, J Dill. University of Missouri, Columbia, MO.

A study was undertaken comparing 4 methods to assess colostral IgG in dairy cows. Colostrum was collected from 67 dairy cows within 2 hours of parturition. Methods used included 2 commercially available hydrometers, an electronic refractometer and measurement of the weight of first milking colostrum. Test results were compared with colostral IgG concentration determined by radial immunodiffusion. The sensitivity and specificity of each method in the detection of low colostral IgG concentration (<50 g/L) was calculated across the range of measured outcomes for the 4 predictor variables. Test endpoints were selected in an effort to maximize both sensitivity (Se) and specificity (Sp) and classify less than one-third of colostrum sources as having inadequate IgG concentration. Hydrometer #1 had a Se of 0.62 and a Sp of 0.83 in the detection of low colostral IgG concentration using a instrument determined endpoint of 60 g/L. Hydrometer #2 had a Se of 0.28 and a Sp of 0.87 in the detection of low colostral IgG concentration using a instrument determined endpoint of 65 g/L. The electronic refractometer had a Se of 0.62 and a Sp of 0.85 using an endpoint of 20 Brix (%). First milking colostrum weight had Se of 0.38 and a Sp of 0.78 using an endpoint of 8 kg. Hydrometer #1 and the electronic refractometer had equivalent test performance which was superior to either hydrometer #2 or colostrum weight in the detection of low colostral IgG concentrations. Additional data collection is in progress.

ABSTRACT #44

THE EFFECT OF FEEDING HEAT-TREATED COLOSTRUM ON SERUM IMMUNOGLOBULIN CONCENTRATIONS IN DAIRY CALVES. D Hagman, J Johnson, S Godden, T Molitor, T Ames, M

Bandrick, M Becker, K Steffenhagen Dept. of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.

In recent years, interest in heat-treatment of bovine colostrum has increased as a method for preventing transmission of infectious diseases, such as Bovine Leukosis Virus (BLV) and *Mycobacterium avium* ssp. *paratuberculosis (MAP)* (Johne's disease). However heating colostrum at traditional pasteurization times and temperatures denatures approximately 30% of colostral immunoglobulins and can produce large viscosity changes, resulting in lower serum IgG concentrations in calves and unacceptable feeding characteristics, respectively. In a recent laboratory study we developed a low-temperature long-time approach to heat-treating colostrum (60 °C × 60 min) that preserves important colostral antibodies while successfully eliminating pathogens such as Salmonella spp., *E. coli*, and *Mycoplasma bovis* and *MAP*. However, before this practice can be recommended for adoption by the industry, it is critical that on-farm validation be completed to verify that the practice can be successfully implemented on commercial farms without having a detrimental effect on passive transfer in neonatal calves.

Fifty newborn Holstein calves on a commercial dairy farm were enrolled into 2 treatment groups: raw pooled colostrum (control) or heat-treated pooled colostrum (treated). Blood samples were collected from calves at zero hours (pre-colostrum) and 24 hours (post-colostrum) of age for measurement of serum total protein (TP, gm/dl) and immunoglobulin G (IgG, mg/ml) concentrations. Raw and heat-treated colostrum samples underwent testing for total IgG concentration (mg/ml) and total bacteria count (TPC, cfu/ml).

Total IgG concentration was not different between heat-treated vs raw colostrum samples, but total bacteria counts were significantly reduced in heat-treated colostrum samples. Serum total protein and IgG concentrations were not different between the two groups of calves for the zero hour sample. However, calf serum TP and total IgG concentrations were significantly greater at 24 hours for the heat-treated group (TP = 6.3 +/− 0.5 gm/dl; IgG = 22.3 +/− 4.6 mg/ml) as compared to the control group (TP = 5.9 +/− 0.7 gm/dl; IgG = 17.5 +/− 5.5 mg/ml) ($P < 0.05$).

These preliminary results suggest that feeding heat-treated colostrum can reduce the risk for pathogen exposure while at the same time maintaining and even increasing absorption of colostral immunoglobulins. Further research, in the form of a large scale field study, will be needed to describe the economic and health benefits of feeding heat-treated colostrum to newborn dairy calves.

ABSTRACT #45

ORAL INOCULATION OF NEONATAL CALVES WITH A TOXIGENIC STRAIN OF *CLOSTRIDIUM DIFFICILE*. A Rodriguez[1]; H Staempfli[1]; T Duffield[2]; K Schulz[1] & JS Weese[1]. Departments of Clinical Studies[1], & Population Medicine[2], Ontario Veterinary College, University of Guelph, ON, Canada.

Clostridium difficile is an important cause of enteric disease in humans and a variety of other animal species. The role of this pathogen in neonatal calf diarrhea (NCD) is unclear, however a recent study reported an association between the presence of *C. difficile* toxins in feces and NCD. The role of this pathogen is important to discern both in terms of animal health, and because *C. difficile* could be a zoonotic pathogen. The objective of this study was to determine whether the oral administration of a calf-derived toxigenic strain of *C. difficile* would result in colonization of the intestinal tract, production of *C. difficile* toxins, and development of diarrhea in neonatal calves.

A controlled experimental infection study was conducted with fourteen 6-to-24 hour-old male Holstein calves fed colostrum. Animals were isolated and maintained in individual pens, fed fresh whole milk and examined daily until the end of the study. Calves received three doses of 10^7–10^8 colony-forming units of *C. difficile* organisms (n = 8) or sterile culture broth (n = 6) every 12–16 hours during the first 2–3 days of age. Animals were monitored for diarrhea twice daily. Fecal samples were collected daily. Calves were euthanized on day 6 of the study or after the onset of diarrhea, whichever came first. Necropsy was performed and intestinal samples were collected for *C. difficile* culture and toxin A/B ELISA. PCR ribotyping was performed to genetically compare the inoculated and recovered strains. Laboratory analysis was conducted blindly.

All 6 control calves and 3 of 8 inoculated calves had diarrhea ($p = 0.03$). Toxins A/B were not detected in feces of inoculated calves however were detected in 1 fecal and 1 intestinal sample from 2 control calves. *C. difficile* was isolated from 1/6 control and 8/8 infected calves ($p = 0.003$). Ribotyping analysis indicated that the inoculated strain survived intestinal transit. In conclusion, the oral administration of *C. difficile* to calves resulted in fecal shedding, but not in the presence of fecal toxins A/B or major signs of diarrhea. While this does not exclude the possibility that *C. difficile* is an important cause of NCD, further study is required to evaluate its role in disease and the pathophysiology of disease.

ABSTRACT #46

EVALUATION OF TWO COLOSTRUM REPLACER PRODUCTS IN DAIRY CALVES. DM Foster[1], GW Smith[1], TR Sanner[2], GV Busso[1]. [1]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, and [2]Rocky Creek Veterinary Services, Olin, NC.

A well-managed colostrum program is the most important step in reducing disease in neonatal calves. However, despite this knowledge, failure of passive transfer (FPT) is still extremely common in the dairy industry. Some of the risk factors that lead to FPT include poor colostrum quality (low colostral immunoglobulin concentration), excessive bacterial contamination of colostrum, or lack of a reserve of frozen colostrum. Colostrum can also transfer several infectious organisms including *Salmonella*, *Mycobacterium avium* subsp *paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus. In the last few years, colostrum replacers have increased in popularity and are designed to be an alternative to colostrum on farms that have poor colostrum quality, limited colostrum reserves, or to break the cycle of transmission for certain infectious diseases. However, it is important to make sure these products are effective as a colostrum replacer and are capable of achieving adequate passive transfer in calves.

In this study, 80 Holstein bull calves from a single dairy were randomly assigned to 1 of 4 groups at birth. Group 1 (n = 20) calves were given 4 quarts of colostrum via esophageal feeder within 6 hours of birth and served as the control group for this study. Group 2 (n = 20) received 2 packages of a colostrum replacer product (product A) within 6 hours of birth. Group 3 (n = 20) received 1 package of a different colostrum replacer product (product B), and group 4 (n = 20) received 2 packages of product B within 6 hours of birth. Blood samples from all calves were collected 24 hours after colostrum administration and analyzed for serum total protein and immunoglobulin (IgG) concentrations.

Calves in group 1 had significantly higher serum total protein levels (mean = 6.3 g/dL) and IgG concentrations (mean = 29.1 g/L) than any of the other 3 groups (group 2—mean total protein = 4.5 g/dL, mean IgG = 7.1 g/L; group 3—mean total protein = 4.9 g/dL, mean IgG = 11.6 g/L; group 4—mean total protein = 5.6 g/dL, mean IgG = 16.6 g/L). However, the percentage of calves with adequate passive transfer was not significantly different between groups 1 (90%), 3 (75%) and 4 (95%). Only 10% of calves in group 2 achieved adequate passive transfer which was significantly less than group 1.

Product B represents a safe and effective colostrum replacer in herds considering an alternative to natural bovine colostrum. In contrast, Product A failed to routinely provide adequate passive transfer when fed according to label directions.

ABSTRACT #47

ULTRASONOGRAPHIC ASSESSMENT OF BOVINE FETAL WELL BEING DURING LATE PREGNANCY IN NORMAL, COMPROMISED AND CLONED PREGNANCIES. Sébastien Buczinski, Gilles Fecteau, Réjean C. Lefebvre, Lawrence C. Smith. Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.

Assisted reproductive technologies or late pregnancy illness are associated with various disorders of the fetus or its annexes such as premature death, abortion, respiratory problems or hydrops. The objective of this study was to describe the ultrasonographic appearance of the late term fetus and uterus in normal, compromised (dams affected by various diseases) and cloned pregnancies.

Holstein cows in the last month of pregnancy were allocated in three groups: G1 (n = 10) which were non complicated pregnancies in which parturition was induced for teaching purposes, G2 (n = 10) in which dams suffers from various diseases that could compromise fetal well being, G3 (n = 11) in which heifers were carrying clone calves. All pregnancies were assessed by transabdominal ultrasonographic examination with a 3.5 MHz probe. Fetal heart rates (FHR) measured 3 to 5 times during examination, fetal movements, fetal annexes appearance (maximal fluid depth, allanto-amniotic membrane thickness) as well as placentoma size were studied. Evolution of the pregnancy and outcome of the newborn were also recorded.

Mean gestational length were 272.6 d (G1), 273.6 d (G2) and 267.6 d (G3). Survival rate were 9 of 10 calves in G1, 5 of 10 calves in G2, and 3 of 11 calves in G3. Average fetal heart rate did not differ significantly between groups (Fisher's F-test, p = 0.91). Gestation age did not affect FHR which was 124.6 beats per min (bpm) when performed less than 4 weeks before birth, 114.0 bpm less than 3 weeks before birth, 115.2 bpm less than 2 weeks before birth and 116.1 bpm less than one week before birth. There was no correlation between FHR and fetal activity. However, when fetal inactivity was observed, it was associated with fetal death (n = 2, G2). Fetal hyperactivity may be a sign of fetal distress since all fetuses who were moving throughout the total duration of the ultrasonographic examination had a fatal outcome (born dead or perinatal death). Hyperechoic particles imaged repeatedly in amniotic and allantoid fluids were observed in three

fatal cloned pregnancies. Estimated placentoma area was larger in G3 (35.5 cm^2) when compared with G1 (25.9 cm^2) and G2 (27.9 cm^2). Allantoamniotic membrane was measured in 2 cases of hydrallantois (G3) at 2.0 and 2.2 cm. This was thicker than the size of this membrane in G1 (mean = 0.69 cm, n = 9) or G2 (mean = 0.5 cm, n = 8).

These early results are promising to assess fetal distress in the late term pregnancy. Fetal hyperactivity or prolonged fetal inactivity, permanent imaging of hyperechoic particles in fetal fluids were suggestive of fetal distress in this study. They should be included in a bovine fetal biophysical profile. Allantoamniotic membrane measurement could be an interesting tool to early detect hydrallantois in cloned pregnancies.

ABSTRACT #48

ULTRASONOGRAPHIC ANATOMY OF THE BOVINE EYE. GD Hallowell[1], TJ Potter[1], IM Bowen[2] [1]Royal Veterinary College, University of London, UK. [2]School of Veterinary Medicine and Science. University of Nottingham, UK.

Transpalpebral ocular ultrasonography is a useful diagnostic technique that is applied in other species. However, the normal ultrasonographic appearance of the bovine eye and intraocular dimensions have not been described, thus interpretation of images from cattle with ocular disease, where ocular dimensions and anatomy can change, is problematic. The purpose of the study was to describe the ultrasonographic appearance and measurements of the normal bovine eye, and to describe differences between Holstein-Friesian (HF) and Jersey (Je) breeds.

Sixty transpalpebral ocular ultrasonographic examinations were performed on 30 adult (mean age 61 ± 22 months) HF lactating dairy cows. In addition, 14 examinations were performed on 7 adult non-lactating Je cows (74 ± 13 months). None of the cows had significant ophthalmological abnormalities. The cattle were restrained in a crush without sedation or local analgesia. Transpalpebral ultrasonographic images were obtained with a 10-MHz linear transducer in both horizontal and vertical imaging planes. Intraocular structures were measured using standardised views. Comparisons of ocular dimensions between breeds were made using an unpaired Student's t-test or a Mann-Whitney test where appropriate. Data are presented as mean ± SD.

The ultrasonographic appearance of the structures within the bovine eye is similar to other species although the ciliary artery was frequently identified, appearing as a 0.33 ± 0.04 cm diameter hypoechoic area. The anterior to posterior diameter of the globe was significantly greater in HF (3.46 ± 0.09 cm) compared to Je (3.27 ± 0.19 cm; $p = 0.001$), although the diameter of the posterior segment was smaller in HF (1.46 ± 0.09 cm) compared to Je (1.62 ± 0.04 cm; $p = 0.0009$). There was no difference between the measured diameters in horizontal or vertical imaging planes. The diameter of the lens was significantly greater in Je (1.92 ± 0.11 cm) compared to HF (1.78 ± 0.09 cm), while there was no difference in its width between the two breeds (1.35 ± 0.08 cm and 1.24 ± 0.04 cm respectively). Ultrasonographic measurements of the bovine lens are similar to measurements previously reported (diameter 1.78–1.87 cm and width 1.2 cm) from cadaveric material. The cornea was thinner in Je (0.17 ± 0.02 cm) compared to HF (0.20 ± 0.02 cm; $p = 0.0062$) but both are similar to measurements obtained previously from cadaveric material (0.15–0.2 cm). There was no difference in sizes of the anterior chamber between breeds (HF: 0.33 ± 0.05 cm; Je: 0.36 ± 0.07 cm) although both breeds were larger than reported previously from cadaveric material (0.25 cm). There was no difference in the combined retina and choroidal thickness between breeds (HF: 0.18 ± 0.02 cm, Je: 0.22 ± 0.03 cm) and correlates well with cadaveric measurements (0.2 cm).

Transpalpebral ultrasonography could be successfully performed in unsedated dairy cows. Measurements obtained were similar to those reported for cadaveric specimens. Some significant differences were noted in ocular measurements between these two breeds, thus these values should not be extrapolated to other breeds without further evaluation.

ABSTRACT #49

ULTRASONOGRAPHIC COMPARISON OF THE MAMMARY GLAND IN COWS WITH EXPERIMENTALLY INDUCED VERSUS NATURALLY OCCURING *E. COLI* MASTITIS. JK Nagy[1], DE Morin[2], PD Constable[3], SK Kneller[2], MW Thomas[2]. [1]College of Veterinary Medicine, University of Missouri, Columbia, MO. [2]College of Veterinary Medicine, University of Illinois, Urbana, IL. [3]School of Veterinary Medicine, Purdue University, West Lafayette, IN.

Ultrasound is used to diagnose milk flow disorders in dairy cows and detect abscesses and fibrosis in mammary glands with chronic mastitis. However, ultrasonographic changes accompanying acute clinical mastitis are not well described and the diagnostic and prognostic utility of ultrasound in cows with acute clinical mastitis has not been reported. The objective of this study was to characterize ultrasonographic changes in the mammary gland, teat, and prefemoral lymph node accompanying experimentally-induced *E. coli* mastitis and compare with ultrasonographic findings in cows with naturally-occurring *E. coli* mastitis. Five healthy Holstein cows inoculated to induce *E. coli* mastitis and ten cows with acute clinical *E. coli* mastitis were used. Healthy cows were inoculated in one front mammary gland with 10^6 cfu of *E. coli* after each of two consecutive milkings. Pre-determined locations on the mammary gland and teat were evaluated for echotexture, edema, and debris in the secretion before inoculation and every six hours for 96 hours. Lymph node thickness, teat wall thickness, and teat cistern diameter were also measured.

Ultrasonographic findings at the peak of experimental illness were compared with those for cows with naturally-occurring mastitis. Debris was observed in the teat cistern of all cows. Cows with experimentally-induced mastitis developed subcutaneous edema but had minimal change in glandular echotexture. In contrast, glandular hyperechogenicity was the most prominent finding in cows with naturally-occurring mastitis. The teat wall was thicker and the prefemoral lymph node tended to be larger in cows with experimentally-induced disease.

Our results suggest that pathophysiological changes accompanying experimental *E. coli* mastitis differ from those accompanying naturally-occurring *E. coli* mastitis. The main finding in cows with naturally-occurring mastitis was glandular parenchymal hyperechogenicity, which is difficult to quantify. Any future studies investigating the prognostic utility of ultrasonography should be performed in cows with naturally-occurring disease.

ABSTRACT #50

EASTERN EQUINE ENCEPHALITIS (EEE) IN SOUTH AMERICAN CAMELIDS: 9 CASES. R Nolen-Walston[1], S Rushton[2], C Rodriguez[1], D Bedenice[1], F Del Piero[3]. [1]Tufts Cummings School of Veterinary Medicine, N. Grafton, MA. [2]North Carolina Veterinary Diagnostic Laboratory, Raleigh, NC. [3]School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, PA.

Eastern Equine Encephalitis virus (EEE) causes severe, often fatal polioencephalitis in a wide range of mammalian species, but has not been previously reported in South American Camelids. The purpose of this study was to report and describe the clinical and pathologic findings of EEE in alpacas and llamas. A retrospective multicenter study was performed to include confirmed cases of EEE in camelids in North America prior to 2006. Cases were included if the patient had clinical signs of intracranial disease, and either definitive presence of EEE virus in the CNS post-mortem or significant antibody titers with exclusion of major alternate differential diagnoses.

Nine cases (8 alpacas and 1 llama) were identified, aged 3.5 weeks to 12 years (mean 2.27 years). Of these 7/9 were ≤1 year of age, with 4/9 ≤10 weeks old. Three cases were female animals, and 6 males. All affected animals were from the East Coast, and were seen exclusively in late summer and fall. The mortality rate was 89% (8/9), with mean time from onset of clinical signs to death of 2 days (0.5–6 days). Euthanasia was performed in 6/9 cases; 2/9 died naturally, with 1/9 surviving.

Clinical signs were consistent with deficits associated with intracranial disease. Lethargy was noted in all patients (9/9), as well as ataxia (7/7), mentally inappropriate behavior (4/5), seizures (4/7), recumbency (5/8), torticollis/opisthotonus (5/7), and vestibular signs (3/6). Increased rectal temperatures (≥39.2°F [102.5°F]) was noted in 7/8 animals, with a mean temperature of 40.1°C [103.7°F]). No other consistent abnormalities were present on physical examination. Treatment administered was predominantly focused on supportive care and therapy for major differential diagnoses, but had no appreciable effect on outcome. Complete blood counts and/or serum chemistry analysis was performed on 6/9 cases and was largely unremarkable. Analysis of CSF was performed on 2/9 cases, demonstrating increased protein levels.

A complete post mortem examination was performed in 6/8 non-survivors. The 8 fatal cases of EEE were confirmed by alphavirus detection in the CNS, either by indirect immunohistochemistry (6/8) using a specific monoclonal antibody and/or specific transcriptase polymerase chain reaction (4/8). Both techniques resulted to be sensitive and specific. The surviving case (and 4/4 of the fatal cases tested) was seropositive for antibodies via plaque reduction neutralization testing. Other common causes of encephalitis were ruled out using standard techniques. Histopathologic findings included polioencephalitis with microglial activation, astrocytosis, lymphocytic perivascular cuffing, neutrophils and edema with alphavirus within the cytoplasm of neurons, fibers and glial cells. No virus was detected within extraneural tissues. EEE should be considered a differential diagnosis

for young and adult camelids that reside in endemic areas and show signs consistent with central neurologic disease.

ABSTRACT #51

COMPARISON OF INSULIN AND NON-INSULIN TREATED CAMELIDS WITH HYPERTRIGLYCERIDEMIA: 33 CASES. L. Waitt and C. Cebra; Oregon State University, College of Veterinary Medicine, Corvallis, OR.

Medical records from 25 alpacas and 8 llamas with hypertriglyceridemia were reviewed retrospectively. No age predilection was apparent. 23/33 of the camelids were female, and only 8 were lactating or pregnant. Common concurrent problems included endoparasitism (5/33) and neonatal sepsis (4/33). The camelids were divided into groups based on the severity of their hypertriglyceridemia and whether they had been treated or not treated with insulin. Groups 1 and 2 consisted of camelids with triglyceride concentrations that did not exceed 500 mg/dl. Group 1 was not treated with insulin and Group 2 was treated with insulin. Group 3 contained camelids that had triglyceride concentrations >500 mg/dl and were treated with insulin. Mean triglyceride concentrations did not differ significantly between Groups 1 and 2 on admission (156.5 ± 166.5 mg/dl vs. 259.0 ± 179.0 mg/dl; $p = 0.284$) but triglycerides dropped significantly after insulin treatment in the Group 2 camelids (61.3 ± 36.3 mg/dl; $p = 0.013$) and were significantly lower than the Group 1 camelids over the period of treatment (250.8 ± 200.4 mg/dl; $p = 0.033$). Group 3 camelids also had a significant decrease in triglyceride concentration with insulin treatment (2351.9 ± 2079.2 mg/dl pre vs. 1053 ± 1137.1 mg/dl post; $p = 0.008$). There was no control untreated population for this group as every lipemic hospitalized camelid was treated with insulin. These findings suggest that all ages and genders of camelids with a variety of disease states are susceptible to hypertriglyceridemia and that insulin treatment decreases triglyceride concentrations in camelids with moderate and severe hypertriglyceridemia. They therefore suggest that insulin may be useful in treatment.

ABSTRACT #52

ALBENDAZOLE TOXICITY IN NINE ALPACA CRIAS. AM Gruntman, RD Nolen-Walston. Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

Albendazole is a broad-spectrum benzamidazole anthelmintic widely used in llamas and alpacas with an anecdotal dosing range of 10-15 mg/kg, similar to that suggested for ruminants. Albendazole consistently causes bone marrow hypoplasia and intestinal crypt epithelial necrosis in pigeons and doves at suggested doses. Idiosyncratic reactions have been reported in other species. Albendazole's mechanism of toxicity is to affect the rapidly proliferating cells of the intestine and bone marrow, similar to classic radiomimetic toxic lesions. This report focuses on nine alpaca crias (1–7 months of age) with a history of oral albendazole therapy.

The 9 affected crias were the only animals in a herd of 60 to receive oral albendazole for presumed cestodiasis, and all animals treated either died or required extensive hospitalization. Each cria received 900 mgs (33–100 mg/kg/day) of oral albendazole (Valbazen) once daily for four contiguous days. Two crias were found dead within 24–72 hours of receiving the last dose of albendazole, and were presented for postmortem examination. The remaining 7 animals presented over the course of 3 days with severe watery diarrhea and moderate-marked neutropenia (mean 758 cells/ul; range 32–3264 cells/ul). The dose of albendazole administered did not appear to correlate directly with the severity of clinical signs. Despite aggressive therapy, within 4 days 5/7 animals died or were euthanized secondary to sepsis or multiple organ failure. The surviving animals' neutrophil counts decreased from 216 and 3264 cells/ul to 0 and 8 cells/ul respectively by 4 days after admission, and therapy with filgrastim (Neupogen) 125ug SQ q24h, a human granulocyte colony-stimulating factor (G-CSF), was initiated. After 7 days of therapy the neutrophil counts increased to 5600 and 3264 cells/ul respectively and Neupogen therapy was discontinued. One surviving animal developed a severe thrombocytopenia (5000 platelets/ul) 10 days after admission that was treated with a whole blood transfusion. The surviving animals were hospitalized for a total of 17 and 24 days respectively. Both required broad-spectrum antimicrobial therapy with one patient requiring imipenem (6 mg/kg) and subsequently combined therapy with vancomycin (15 mg/kg, IV, q6h), enrofloxacin (10 mg/kg, IV, q12h), metronidazole (15 mg/kg, IV, q6h), and fluconazole (5 mg/kg, IV, q24h) to treat persistent pyrexia and presumed sepsis. Extensive diagnostic investigation and blood culture did not reveal the source of the infection.

Postmortem examinations were performed on 6/7 deceased animals revealing necrosis of the intestinal villi and crypt epithelium as well as generalized bone marrow hypoplasia and necrosis. Diagnostics did not reveal any other causes for the diarrhea.

This case suggests that alpacas have an increased sensitivity to albendazole, similar to that seen in passerine birds. The effect of repeated daily dosing cannot be discounted as a factor increasing the likelihood of toxicity, but it is clear that relatively low doses are sufficient to cause high mortality rates in alpacas.

ABSTRACT #53

SERUM AND CEREBROSPINAL FLUID CONCENTRATIONS OF FENBENDAZOLE AND OXFENDAZOLE IN ALPACAS AFTER FIVE DAILY ORAL DOSES OF 50MG/KG FENBENDAZOLE 10% SUSPENSION. MD Miesner, DE Anderson, D Linden, W Walker, T Specht, DM Rings, J Lakritz. Ohio State University, Dept. of Veterinary Clinical Sciences, Columbus, OH.

Clinical *Parelaphostrongylus tenuis* (meningeal worm) therapy consists of reducing CNS inflammation and killing migrating larvae. In most situations, avermectin-type dewormers are considered effective at killing migrating larvae before reaching the CNS, but are ineffective on CNS established larvae. Clinical evidence suggests benzimidazoles are therapeutic in clinical cases, but data to support CNS penetration of benzimidazoles in alpacas does not exist.

The purpose of this study was to evaluate serum and cerebrospinal fluid concentrations of fenbendazole (FBZ) and its metabolite, oxfendazole (OXF), in alpacas after multiple daily doses at 50 mg/kg FBZ. The majority of FBZ is converted to OXF which has greater anthelmintic activity and both accumulate after repeated daily doses. Six healthy alpacas (2 male; 4 female) in good health were housed in stalls in pairs. The alpacas were weighed, and given fenbendazole (50 mg/kg) after acquiring a pre-dose blood sample. Thereafter, the animals were given a single oral dose at 24, 48, 72, and 96 h. Jugular venipuncture and aseptic LS-spinal fluid collection were performed daily preceding treatment. Serum and CSF were harvested and frozen at −20C until analyzed. Serum and CSF FBZ and OXF were determined using HPLC. Serum concentration of FBZ was > 1 ug/mL by 48 h and continued to rise. OXF in serum exceeded 1 ug/mL by 24 h and exceeded 3 ug/mL by 96 hr. Cerebrospinal fluid FBZ concentrations ranged from 10–40 ng/mL over the 5 day study, whereas; OXF concentrations exceeded 100 ng/mL by 24 h and achieved 300 ng/mL by 5 days.

ABSTRACT #54

ENHANCED PERIPARTURIENT TRANSMISSION OF BOVINE LEUKOSIS VIRUS IN COLOSTRUM – DEPRIVED CALVES. DW Nagy, JW Tyler, SB Kleiboeker. College of Veterinary Medicine, University of Missouri, Columbia, MO.

The objective of this study was to determine if colostrum from BLV positive cows was protective for calves. Twelve colostrum - deprived Holstein calves 6 weeks of age or older and 12 colostrum fed Holstein calves were used in the study. All calves were born to BLV infected cows. All colostrum – deprived calves were housed in individual stalls away from the farm with no animal contact. No procedures that potentially transmit BLV were preformed on the calves. All colostrum deprived calves had 6 weekly ELISA and PCR tests for BLV antibody and provirus completed. All colostrum – fed calves were housed on the farm of origin and processed in a manner that was routine for the farm. Colostrum – fed calves were fed colostrum derived from BLV+ cows and had positive serum ELISA test results after ingestion of colostrum. Thereafter, colostrum – fed calves had ELISA and PCR tests for BLV antibody and provirus performed every other week until 2 consecutive negative ELISA tests were achieved or until 1 positive PCR tests were achieved.

The proportion of calves that converted to BLV + status was calculated for each group. In the colostrum – deprived group, calves were classified as infected when a positive test result by either assay was obtained. In the colostrum fed group, calves were classified as infected or true positives when a positive PCR was obtained. The proportion of calves becoming infected with BLV was compared among the 2 treatment groups using a Fischer's exact test.

In the colostrum deprived group, 4/12 (33%) calves converted to BLV + status. Each BLV positive, colostrum – deprived calf had a minimum of 2 positive ELISA tests and 1 positive PCR tests by the end of the study. In the colostrum fed group, 0/12 (0%) calves converted to BLV + status. The

proportion of calves that became infected with BLV was significantly higher in the colostrum deprived group ($p = 0.001$).

It is impossible to determine the exact timing of viral exposure in the colostrum deprived calves. Based on the rate of transmission and the disparity between the colostrum – fed and colostrum – deprived groups, it is unlikely that the exposure occurred *in utero*. The difference in transmission rates between colostrum–fed and colostrum–deprived calves eliminates colostrum transmission as a source of infection. Hence, we are left with the conclusion that parturition results in exposure to BLV and administration of colostrum containing antibodies to BLV greatly ameliorates the risk of infection.

ABSTRACT #55

mRNA LEVELS AND BINDING SITES FOR α_2- ADRENOCEPTOR SUBTYPES IN MUSCLE LAYERS OF THE ILEUM AND SPIRAL COLON OF DAIRY COWS. E.C. Ontsouka, J.W. Blum, A. Steiner, M. Meylan; University of Berne, Switzerland.

Different types of adrenergic receptors have been shown to modulate the activity of the digestive tract of ruminants, whereby α_2-receptors, especially the subtype α_{2AD}, play an important role in the regulation of gastrointestinal motility. The aim of this study was to measure maximal binding (B_{max}) and mRNA levels of α_2-adrenoceptors (AR) in ileal and colonic muscle layers of healthy dairy cows.

Full-thickness specimens from the ileum and spiral colon were obtained from 6 healthy dairy cows immediately after slaughter. The muscle layers were isolated by scraping off the mucosa and sub-mucosa from the full-thickness samples. mRNA levels of α_2-AR subtypes were measured by real-time reverse transcriptase PCR and expressed relative to the mean mRNA levels of glyceraldehyde phosphate dehydrogenase (GAPDH), ubiquitin and ribosomal 18S. The α_2-AR binding studies were performed using the selective α_2-AR antagonist (^{3}H)-RX821002 and subtype selective ligands as competitors, namely BRL 44408 (α_{2AD}), imiloxan (α_{2B}), MK-912 (α_{2C}), prazosin (α_{2B}), rauwolscine (α_{2C}), and phentolamine (α_1 and α_2).

mRNA levels of α_{2AD}, α_{2B} and α_{2C}-AR did not differ between ileal and colonic muscle layers. In both locations, mRNA levels of α_{2AD}-AR were greater ($P < 0.05$) than of α_{2B}- and α_{2C}-AR, representing 92%, 6% and 2%, respectively, of the total mRNA. Competition of (^{3}H)-RX821002 binding with BRL44408, imiloxan and MK-912 were best fitted by a one-site model. B_{max} of α_{2AD}- and of α_{2C}-AR were greater ($P < 0.05$) than that of α_{2B}-AR. B_{max} and mRNA levels were significantly correlated only for α_{2AD}-AR ($r = 0.8$, $P < 0.05$). The ratio B_{max}/mRNA levels for α_{2C}-AR was similar to that for α_{2B}, but higher ($P < 0.05$) than for α_{2AD}-AR.

In conclusion, α_2-AR in the intestinal muscle layers were represented by a mixture of α_{2AD}-AR and α_{2C}-AR, while α_{2B}-AR was least abundant. mRNA levels for individual receptor subypes did not *a priori* mirror the corresponding density of binding sites, as mRNA levels for α_{2AD}-AR were distinctly higher than those for both α_{2B}- and α_{2C}-AR. There were no significant differences in mRNA and protein levels in smooth muscle tissues from the ileum and spiral colon of healthy dairy cows. The results of this study will help to clarify the role of adrenoceptor subtypes in α_2-adrenergic mechanisms regulating intestinal motility in cattle.

ABSTRACT #56

mRNA LEVELS AND DENSITIES OF 5-HYDROXYTRYPTAMINE-4 RECEPTORS IN THE GASTROINTESTINAL SMOOTH MUSCLES OF HEALTHY DAIRY COWS. E.C. Ontsouka, J.W. Blum, A. Steiner, M. Meylan; University of Bern, Switzerland.

Serotonin (5-hydroxyryptamine; 5-HT) is involved in gastrointestinal tract (GIT) motor functions through binding to specific receptors, including the 5-HTR_4 subtype, located on enteric neurons and on smooth muscle cells of the intestinal wall. The mRNA levels and densities of 5-HT_4 receptors (5-HTR_4) were determined in smooth muscle layers from several locations from the abomasum to the spiral colon of the GIT of healthy dairy cows to test the hypotheses that (i) mRNA levels and densities of 5-HTR_4 differ among locations in the GIT and that (ii) expression levels and receptor densities are significantly associated.

Full-thickness specimens from the fundus abomasi, pylorus, ileum, cecum, proximal loop of the ascending colon (PLAC), and external loop of the spiral colon (ELSC) were obtained from 8 healthy dairy cows immediately after slaughter. The muscle layers were isolated by scraping off the mucosa and sub-mucosa. The mRNA levels of 5-HTR_4 were measured by real-time RT-PCR and expressed as the percentage of the housekeeping gene glyceraldehyde phosphate dehydrogenase (GAPDH). Binding studies were performed using the 5-HTR_4 antagonist (^{3}H)-GR113808.

The competition binding of (^{3}H)-GR113808 to suspended membranes from fundus abomasi, pylorus, cecum and ELSC by unlabelled GR113808 was best fitted by a two-sites receptor model, whereas it was best fitted by a one-site receptor model in the ileum and PLAC. The inhibitory constant (K_i) varied from 1.1×10^{-12} M to 1.4×10^{-9} M at high, and 8.6×10^{-8} to 8.2×10^{-7} M at the low affinity binding sites. The specific binding of [^{3}H]GR113808 to bovine intestinal tissues could not be fully saturated even using up to 10 nM [^{3}H]GR113808. Receptor densities of 5-HTR_4 using 0.02 to 1 nM [^{3}H]GR113808 did not differ among fundus abomasi, ileum and ELSC ($P = 0.17$). The dissociation constant (K_D) also did not differ significantly in these three locations ($P = 0.23$). Hill coefficients were higher for the ileum (0.95) than for the fundus abomasi (0.86) and the ELSC (0.78). The mRNA levels and densities of 5-HTR_4 binding sites were not correlated within these three locations ($r = 0.14$; $P = 0.71$).

In conclusion, binding sites for 5-HTR_4 are present in the smooth muscle layers throughout the GIT of dairy cows. The effects of activation of the 5-HT_4 receptor subtype may differ among gastrointestinal locations due to variable levels of low and high affinity binding sites. These results suggest that 5-HTR_4 may be involved in the control of bovine GI motility as in other species, and thus may also play a role in the pathogenesis of motility disorders such as abomasal displacement and/or cecal dilatation.

ABSTRACT #57

SERUM INSULIN-LIKE GROWTH FACTOR-I CONCENTRATION IN DIABETIC AND ACROMEGALIC CATS. Rebecca I.M. Berg[1], Richard W. Nelson[1], Edward C. Feldman[1], Phillip H. Kass[1], and Kent R. Refsal[2]. [1]School of Veterinary Medicine, University of California, Davis, CA. [2]College of Veterinary Medicine, Michigan State University, East Lansing, MI.

Chronic excess secretion of growth hormone (GH) in cats can result in acromegaly, a disease characterized by insulin-resistant diabetes mellitus, proliferation of bone and soft tissues, and organomegaly. Historically, measurement of serum GH was used to establish the diagnosis. Unfortunately, validated feline GH assays are not available in the United States. Anabolic effects of GH are mediated through insulin-like growth factor-I (IGF-I) and increased serum IGF-I concentrations have been reported in cats with acromegaly. The aim of this study was to determine the usefulness of serum IGF-I concentration for identifying acromegaly in diabetic cats.

Serum IGF-I concentration was quantified in 38 healthy cats, 15 cats with well-controlled diabetes, 40 cats with poorly-controlled diabetes attributed to concurrent disease, problems with the insulin treatment regimen, or both, and 19 cats with poorly-controlled diabetes and acromegaly. The diagnosis of acromegaly was based on presence of large body size, large head, prognathia inferior, organomegaly, stable or increasing body weight despite persistent and often severe hyperglycemia, inability to improve hyperglycemia despite administration of large quantities of insulin (median dose, 1.9 U/kg; range, 1.0 to 4.3 U/kg), identification of a pituitary macrotumor by computed tomography or at necropsy, and increased plasma GH concentration in 7 cats in which it was measured. Serum IGF-I was measured using a commercially available radioimmunoassay (Nichols Institute, San Clemente, CA). Sensitivity of the assay was 2.1 nmol/l and intra- and inter-assay percent coefficient of variation ranged from 6.0 to 7.9% and 6.5 to 9.5%, respectively.

Mean (±SD) serum IGF-I concentration was not significantly different among healthy cats (52 ± 20 nmol/l), well-controlled diabetic cats (53 ± 18 nmol/l), and poorly-controlled diabetic cats (64 ± 29 nmol/l). Serum IGF-I concentration was significantly ($p < 0.0001$) increased in acromegalic diabetic cats (152 ± 64 nmol/l), compared with healthy cats, well-controlled diabetic cats, and poorly-controlled diabetic cats. The reference interval for serum IGF-I concentration established using the mean IGF-I concentration ± 2 SD in healthy cats was 12 to 92 nmol/l. The calculated sensitivity and specificity for serum IGF-I concentration as a diagnostic test for acromegaly was 84% and 91%, respectively. There was no correlation between serum IGF-I concentration and duration of insulin treatment prior to measuring IGF-I in well-controlled and poorly-controlled diabetic cats.

Results of this study support measurement of serum IGF-I concentration as a screen for acromegaly in diabetic cats.

ABSTRACT #58

INTRAVENOUS ARGININE STIMULATION TEST IN CATS WITH TRANSIENT AND NON-TRANSIENT DIABETES MELLITUS.

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Up to 50% of cats with diabetes mellitus (DM) become transiently diabetic after initiation of insulin treatment. Previous studies have shown that evaluation of insulin levels during a glucagon tolerance test is unable to differentiate between transient and non-transient DM. In humans beta cells show progressive deterioration in their responsiveness to various secretagogues in a sequential manner. Responsiveness towards arginine seems to persist the longest. Due to the similar pathophysiology of human type 2 and feline DM the same may be true for cats. Therefore the objectives of the study were (1) to establish the intravenous arginine stimulation test (ivAST) in healthy cats and to compare the results to diabetic cats and (2) to evaluate if differences in the response to arginine exist between transient and non-transient cats.

The ivAST was performed in 7 healthy male castrated (2.4 ± 0.2 years; 4.9 ± 0.4 kg) and 10 diabetic cats (4 female neutered, 6 male castrated; 11.9 ± 3.6 years; 5.6 ± 4.4 kg) before initiation of insulin therapy. Inclusion criteria for the diabetic group were a follow-up period of at least 16 weeks and lack of severe concurrent illnesses. After taking a baseline blood sample, arginine (0.2 g/kg) was infused into the cephalic vein. Blood samples were taken after 2, 4, 7, 9, 15, 25 and 30 min. The integrated 30-min responses of glucose, insulin and glucagon to arginine injection were analyzed by calculating the area under the curve (AUC) above baseline (AUC_{30}). Glucose was assayed by a hexokinase method (Cobas Integra Analyser, Roche, Basel), insulin and glucagon by previously validated RIA (Linco porcine insulin kit and MP Biomedicals glucagon kit). After the ivAST, the cats were fed a high protein diet and treated with insulin (Caninsulin) using a routine protocol. Five of the 10 cats became transiently diabetic within 12 weeks of insulin treatment.

Whereas glucose AUC_{30} did not differ between diabetic and healthy cats, median baseline glucose was significantly higher in the diabetic group (15.85 vs. 4.23 mmol/l; $p < 0.05$). The insulin AUC_{30} was significantly lower in diabetic compared to healthy cats (50.7 vs. 366.9 µU/ml*min; $p < 0.05$). In contrast, the glucagon response to arginine was significantly higher in diabetic cats (AUC_{30} 12636 vs. 4005 pg/ml*min; $p < 0.05$). AUC_{30} for glucose, insulin and glucagon did not differ between the transient and the non-transient group. Because the peak insulin response to arginine usually occurred within 9 min after arginine, AUC_9 was also calculated. However, there was no significant difference in AUC_9 for any parameter between transient and non-transient diabetic cats.

As expected diabetic cats had a lower insulin response in the ivAST compared to healthy cats. Interestingly their glucagon response was significantly higher. This has already been shown in other species. Further the ivAST does not seem to be an adequate tool to prospectively differentiate between a transient and a non-transient course of DM in cats.

ABSTRACT #59

SWITCHING TO AN ULTRA-LOW CARBOHYDRATE DIET HAS A SIMILAR EFFECT ON POSTPRANDIAL BLOOD GLUCOSE CONCENTRATIONS TO ADMINISTERING ACARBOSE TO HEALTHY CATS fed A HIGH CARBOHYDRATE DIET. R Singh[1], JS Rand[2], JM Morton[2]. [1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Khon Kaen University, Thailand. [2]Centre for Companion Animal Health, School of Veterinary Science, University of Queensland, Australia.

Recent evidence suggests that acarbose can improve glycemic control in diabetic dogs and cats when combined with insulin therapy. However it is unknown whether the benefits of adding acarbose to a high carbohydrate diet can also be achieved by simply feeding an ultra-low carbohydrate diet. The objective of this study was to assess the effect of the alpha-glucosidase inhibitor, acarbose, in decreasing postprandial glucose concentrations when administered to healthy cats fed diets with either high or ultra-low carbohydrate content, and fed as one meal or multiple meals each day.

Twelve non-obese, adult, neutered and clinically healthy cats (6 M, 6 F) with mean weight of 3.64 kg were used in a four-period cross-over study. Following consumption of a maintenance cat food for 2 weeks, baseline metabolic testing was performed. Three cats were then randomly allocated to each of four diet-treatment sequences. The diet-treatment combinations were two test diets, either ultra-low [6.6% metabolizable energy (ME)] or high (57.9% ME) in carbohydrate, each with and without added acarbose. Within each diet, postprandial glucose concentrations were assessed twice, once when diets were fed as a meal and, subsequently when the diet was fed as multiple meals. The acarbose dose was 25 mg/cat fed once daily for meal-fed cats and 12.5 mg/cat every 12 hours for cats fed multiple meals. All diets were fed for 2 weeks prior to metabolic testing in the 3rd week.

Amongst cats fed the high carbohydrate diet, the 12-hour mean AUCglucose and mean glucose concentration was significantly lower when acarbose was administered ($P < 0.001$). In meal fed cats receiving the high carbohydrate diet and acarbose once daily, acarbose action was only significant in the first 12 hr. There was a trend for acarbose to have a greater glucose lowering effect with the high carbohydrate diet compared to the low carbohydrate diet (significance of interaction term $P = 0.075$).

We conclude that acarbose significantly decreased postprandial hyperglycaemia in cats fed a high carbohydrate diet. High carbohydrate diets resulted in significantly higher (24-hours) postprandial glucose concentrations than ultra-low carbohydrate diets.

ABSTRACT #60

DOES SUBCLINICAL HYPERTHYROIDISM EXIST IN CATS? J. Wakeling, J. Elliott, H. Syme; Royal Veterinary College, London.

Hyperthyroidism (HTH) in cats has strong parallels with toxic nodular goitre (TNG) in humans. A well-defined sub-clinical phase exists in TNG where thyroid hormone concentrations are within the reference range but thyroid stimulating hormone (TSH) concentration is sub-normal. The aim of this study was to determine if a sub-clinical phase of HTH could be identified in cats that were apparently euthyroid when first examined but that were subsequently diagnosed with HTH.

Heparinized plasma samples that had been collected (and frozen at −80°C) from aged cats (>8 years) were used for this study. Only cats with total thyroxine (T4) <40 nmol/l (reference range 19–55 nmol/l) were considered for inclusion thus excluding cats with occult HTH. TSH concentrations were measured using the DPC Immulite chemiluminescent canine TSH assay. T4 and TSH concentrations from three groups of cats were compared: Group 1 were apparently healthy on the basis of history, physical examination and complete plasma biochemistry, Group 2 were diagnosed with chronic renal failure (CRF) on the basis of elevated creatinine concentration (>2.0 mg/dl), urine specific gravity <1.035 and compatible clinical signs, these cats were followed for at least six months following sample collection and did not develop signs of HTH. Group 3 were initially considered to be euthyroid but were subsequently diagnosed with HTH on the basis of elevated T4, 1–3 years after their initial visit. Group 3 cats were initially blood sampled either as part of a 'geriatric screen' or due to suspected mild CRF. T4 and TSH were also measured >3 years prior to diagnosis of HTH where samples were available. Results are reported as median [range]. Comparisons of T4 and TSH concentrations between groups were made using Kruskall Wallis and Mann Whitney U tests. The proportion of cats with TSH <0.03 ng/ml was compared by the Fisher exact test.

T4 concentration was significantly lower in Group 1 (21.7 [8.2–32.7] nmol/l; n = 20) and Group 2 (18.0 [5.4–34.6] nmol/l; n = 20) compared with cats from Group 3 (28.5 [17.3–37.3] nmol/l; n = 13; p = 0.001). TSH concentration was significantly higher in cats in Group 1 (0.07 [<0.03–0.20] ng/ml; $P < 0.001$) and Group 2 (0.05 [<0.03–0.11] ng/ml; $P<0.001$), compared with cats in Group 3 (<0.03 [<0.03–0.04] ng/ml). In Group 3 time from sampling to diagnosis of HTH was 651 [422–1071] days. The proportion of cats with TSH <0.03 ng/ml was significantly different in Groups 1 and 2 compared with Group 3 ($P = 0.01$; 3/20, 3/20 and 12/13, respectively). When TSH was measured >3 years (1211–3040 days) prior to the diagnosis of HTH, TSH was <0.03 ng/ml in only 1/5 cats (<0.03–0.09 ng/ml).

Feline TSH is <0.03 ng/ml in the majority of HTH cats for 1–3 years prior to diagnosis of HTH, even when T4 concentrations are well within given reference range. This suggests the existence of a prolonged sub-clinical phase in HTH cats. It is possible that some of the cats with TSH <0.03 ng/ml in Groups 1 and 2 also had sub-clinical HTH. These results confirm the need for a sensitive feline specific TSH assay to differentiate cats with sub-clinical HTH from the normal population.

ABSTRACT #61

DELAYED GASTRIC EMPTYING MAY CONTRIBUTE TO PROLONGED POSTPRANDIAL HYPERGLYCEMIA IN MEAL-FED CATS. M Coradini, JS Rand, JM Morton, LJ Filippich. Centre for Companion Animal Health, School of Veterinary Science, University of Queensland, Australia.

Following ingestion of a meal, postprandial hyperglycemia in cats persists for 20–24 hrs, which is much longer than for dogs and human beings, and the reasons for this are unknown. The objectives of this study were 1) to describe the patterns of postprandial plasma glucose, D-lactate, and L-lactate concentrations, and gastric emptying time in meal-fed cats and 2) to assess the effects of meal volume on gastric emptying time.

Eleven non-obese, adult, neutered and clinically healthy cats (6 M, 5 F) were fed a commercially available, high carbohydrate diet (carbohydrate 54% metabolizable energy (ME), protein 24% ME, fat 22% ME) for 2 weeks. In the third week, on two occasions separated by 4 days, fasted cats were fed a meal of 50 kcal/kg and consumed at least 90% within 30 mins. On the first occasion, cats underwent repeated ultrasound examinations over 26 hrs to determine gastric emptying time. On the second occasion, plasma glucose, D-lactate and L-lactate concentrations were measured over 24 hrs. To assess the effect of volume of food eaten on gastric emptying time, 2 weeks later, 5 of the same cats were fed a meal of the same composition but half the volume (25 kcal/kg) and a second series of ultrasound examinations was performed. Data were analysed using generalised estimating equations and Wilcoxon's matched-pairs signed-rank test.

Glucose concentrations were significantly higher than baseline from 1 to 18 hrs after feeding ($P < 0.001$), reaching a peak at 10.7 ± 5.3 hrs (mean $\pm$ SD) after the meal. In 2 of the 11 cats, glucose concentrations had not returned to baseline at 24 hrs. Median time to gastric emptying when cats were fed their total daily energy intake in a single meal was 24 hrs (range 16–26 hrs). Eight of the 11 cats had food detected at 22 hrs and 4 cats still had food detected in the stomach at 24 hrs. In contrast, times to gastric emptying were substantially shorter when cats were fed 50% of their daily intake in a single meal (median 14 hrs; range 12–14 hrs). D- and L-lactate concentrations did not change substantially after feeding.

These results confirm that following a high carbohydrate meal, cats have prolonged postprandial hyperglycemia. They also demonstrate that gastric emptying time is prolonged following such a meal. This suggests that prolonged gastric emptying time contributes to the prolonged postprandial hyperglycemia observed in meal-fed cats. These findings should also be considered when making recommendations for feeding cats prior to procedures that require the stomach to be empty, such as anesthesia. They indicate that fasting for 12 hrs, as is commonly recommended, does not ensure complete gastric emptying, and explains why cats occasionally vomit food despite not having eaten in the previous 12 hrs. Complete gastric emptying is much more likely if only half of the cat's daily requirements are fed followed by a 14 hr fast.

ABSTRACT #62

USE OF THE MEDTRONIC MINIMED CONTINUOUS GLUCOSE MONITORING SYSTEM FOR ASSESSMENT OF DIABETES MELLITUS IN 40 DOGS. L.J. Davison[1], L.A. Slater[2], F. Gaudiano[2], D.B. Church[2], M.E. Herrtage[1], J.M.E. Ristic[3] and B. Catchpole[2]. [1]Department of Veterinary Medicine, University of Cambridge, UK. [2]Royal Veterinary College, London, UK. [3]Axiom Laboratories, Devon, UK.

Serial blood glucose curves are important in assessing the response to insulin therapy in canine diabetic patients. The use of a minimally invasive continuous glucose monitoring system (MiniMed™ CGMS) has recently been reported in diabetic dogs for this purpose. This study describes the use of the CGMS in 40 canine diabetic patients, comparing the glucose concentrations generated using this device to a conventional blood glucose curve performed simultaneously.

Forty diabetic dogs undergoing serial blood glucose curves for stabilisation purposes were included in the study, and underwent either one ($n = 33$) or two glucose curves ($n = 7$). Venous blood glucose concentrations were measured using a commercial glucometer (ESPIRIT, Bayer) every 2–4 hours for up to 72 hours, with glucometer values being verified in selected samples at an external diagnostic laboratory. The interstitial fluid glucose concentration was measured simultaneously using a 22-gauge subcutaneous sensor attached to the CGMS device, which automatically records a glucose value every 5 minutes.

The sensor was well tolerated by all patients and >2000 hours of CGMS and blood glucose data were collected during a total of 47 glucose curves. In addition to the minimum 3 samples per 24 hours required in each patient to calibrate the CGMS device, 752 independent blood glucose measurements were made using the glucometer (range 6–32 per patient). When these measurements were compared to the CGMS interstitial fluid glucose concentration at each timepoint, an overall correlation coefficient of $r = 0.85$ ($p < 0.05$) was obtained, although this varied between patients. There was good correlation between glucose measured by the glucometer and by the external laboratory ($r = 0.96$ $p < 0.05$). Discrepancies between the blood and interstitial fluid glucose concentrations occurred most commonly in the final hours of recording and in some cases ($n = 4$) immediately after feeding. The sensor had to be replaced during the recording period due to a declining signal or technical issues in 5 patients. Breaks in the CGMS trace during recording occurred in 8 patients. The working range of the CGMS is 2.2 mmol/l–22.2 mmol/l and glucometer values outside this range were obtained during the recording period on at least one occasion during 32 of the 47 curves.

These data suggest that the Medtronic MiniMed CGMS is a valuable tool for the management of canine diabetes and that there is a good correlation between interstital fluid and blood glucose concentration in the majority of canine diabetic patients. Caution must be exercised, however, when relying on interstital fluid measurements alone, since discepancies between the CGMS and blood glucose can occur, sensors can fail and little useful data are obtained if the blood glucose is outside the working range of the CGMS device. Further studies are required to establish the reasons for differences between blood and interstital fluid glucose concentrations in some patients.

ABSTRACT #63

COMPENSATION FOR OBESITY-INDUCED INSULIN RESISTANCE IN DOGS: CAUSAL WEB ANALYSIS OF THE ASSOCIATIONS OF LEPTIN AND GLP-1. Kurt R Verkest[1], Linda M Fleeman[1], Jacquie S Rand[1], John M Morton[1], Katsumi Ishioka[2]. [1]Centre for Companion Animal Health, School of Veterinary Science, the University of Queensland, Brisbane, Australia; [2]Nippon Veterinary and Animal Science University, Tokyo, Japan.

Obesity affects aspects of glucose homeostasis such as insulin secretion and insulin sensitivity. Hormones secreted by adipocytes like leptin mediate the metabolic consequences of obesity. Incretin hormones like glucagon-like peptide-1 (GLP-1) increase insulin secretion in response to changes in blood glucose concentration and have been proposed to regulate insulin secretion in fasting, overweight dogs. The aim of this study was to examine hormonal mechanisms by which adiposity alters glucose homeostasis, plasma insulin concentration, and insulin sensitivity in spontaneously overweight dogs.

Plasma glucose, insulin, GLP-1, and leptin concentrations were sampled in 123 client-owned dogs after a 24-hour fast. Body condition score was recorded using a 9-point scale. Insulin sensitivity was assessed using the product of fasting plasma insulin and glucose concentrations (HOMA), which was validated for use in dogs against Bergman's Minimal Model derived insulin sensitivity index. Univariate regression assessed the effect of adiposity on plasma glucose concentration. Path analysis assessed possible causal relationships, whereby the importance of each relationship in a causal web was tested using multivariable linear regression.The effect of adiposity and plasma leptin concentration on insulin sensitivity and plasma GLP-1 concentration; the effect of adiposity, plasma leptin concentration, plasma glucose concentration, and plasma GLP-1 concentration on plasma insulin concentration; and the effect of adiposity, plasma glucose concentration, and plasma insulin concentration on plasma leptin concentration were examined.

Fasting plasma glucose concentration was not influenced by adiposity ($p = 0.38$). Insulin sensitivity was decreased by adiposity ($p < 0.001$) and tended to decrease with higher leptin concentration ($p = 0.06$). Plasma insulin concentration was increased by adiposity ($p < 0.001$) and plasma leptin concentration ($p = 0.04$) but not by either plasma glucose concentration ($p = 0.25$) nor plasma GLP-1 concentration ($p = 0.95$). Plasma GLP-1 concentration was increased by adiposity ($p = 0.03$), but adiposity accounted for only 3.2% of the variance in plasma GLP-1 concentration. Plasma leptin concentration was increased by adiposity ($p < 0.001$), but not by either plasma glucose concentration ($p = 0.19$) nor plasma insulin concentration ($p = 0.12$).

We conclude that in this study (1) Fasting plasma glucose concentration in dogs was not affected by adiposity; (2) Insulin resistance was increased by adiposity in dogs, and this increase appeared to be partially mediated by leptin; (3) Increased insulin resistance in overweight dogs was compensated by increased insulin secretion so that fasting plasma glucose was not affected by either adiposity nor insulin resistance; (4) GLP-1 did not appear to regulate insulin secretion in fasted dogs.

ABSTRACT #64

CLINICAL EFFICACY AND SAFETY OF LEVENTA™ FOR TREATMENT OF HYPOTHYROID DOGS. SF Brennan[1], S Daminet[2], P. de Fornel-Thibaud[3], K. Gommeren[2], CT Mooney[1], D Rosenberg[3]. [1]Small Animal Clinical Studies, University College Dublin, Ireland. [2]Dept Small Animal Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium. [3]Internal Medicine Unit, National Veterinary School of Alfort, Maisons-Alfort, France.

The objective of this non-controlled clinical study was to evaluate the efficacy and safety of a novel liquid formulation of levothyroxine sodium (Leventa™ 1 mg/mL) in hypothyroid dogs.

Twenty client-owned dogs with naturally occurring hypothyroidism (appropriate clinical signs and circulating serum concentrations of free thyroxine (fT4) (after equilibrium dialysis) $\leq$5.4 pmol/L and canine thyroid stimulating hormone (TSH) $\geq$0.68 ng/mL) were treated with Leventa™ orally q24h at a starting dose rate of 20 µg/kg body weight. Dose adjustment

was performed every 4 weeks based on both clinical and thyroid hormone response. At each visit, blood samples were collected for peak (4 to 6 hours post-treatment) serum total thyroxine (TT4) and TSH concentrations. Once the clinical signs had improved and the hormonal status normalized (TT4 35–95 nmol/L), the dose was fixed and the dog considered as a responder. These dogs were then monitored 9 and 22 weeks later. Dogs in which a suitable dose could not be fixed after a maximum of 12 weeks of treatment were considered as treatment failures. Six dogs have not yet completed the trial.

The clinical condition was improved or resolved in all dogs after 4 weeks of treatment at the original starting dose. Metabolic signs (lethargy, exercise intolerance) resolved first, whereas dermatological signs (alopecia, hyperpigmentation) took longer. The dose rate was fixed in 15 dogs after 4 weeks, a further 2 dogs after 8 weeks and one dog after 12 weeks of treatment. Sixteen dogs remained on the starting dose of 20 g/kg q24h whereas two dogs required a dose increase to 30 µg/kg q24h. All of the dogs responded to treatment.

The mean ± SD peak TT4 and TSH concentrations at each visit are summarized in the table:

	pre-treatment (n=20)	at dose fixing (n=18)	fixing +9 weeks (n=15)	fixing +22 weeks (n=9)
peak TT4 (nmol/L)	8.2 ± 3.1	53.7 ± 18.5	49.8 ± 22.5	60.4 ± 18.2
TSH (ng/mL)	2.6 ± 2.2	0.2 ± 0.3	0.3 ± 0.3	0.1 ± 0.1

Once the dose rate had been fixed, clinical signs of hypothyroidism did not recur. Mild to moderate skin reactions of unknown significance, were reported in 3 dogs. This apart, no treatment-related adverse events were observed.

A rapid response to treatment was seen in all cases with few if any suspected adverse effects. A daily dose rate of 20 µg/kg was suitable for most dogs, with a maximum dose of 30 µg/kg being required in only 2 animals. Leventa™, a novel liquid formulation of levothyroxine sodium, is effective and safe in controlling clinical signs and normalizing thyroid hormone concentrations in dogs with hypothyroidism.

ABSTRACT #65

SERUM LEPTIN AND INSULIN CONCENTRATIONS IN DOGS WITH HYPOTHYROIDISM. M Mazaki-Tovi[1], Y Feuermann[2], E Yas-Natan[1], E Klement[1], A Farkas[3], A Kol[1], A Shamay[2]. [1]School of Veterinary Medicine, the Hebrew University of Jerusalem, Israel. [2]Institute of Animal Science, Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel. [3]American Medical Laboratories, Herzliya, Israel.

Hypothyroidism is one of the most common endocrinopathies in dogs. This disease is typically manifested by obesity. A less prominent metabolic consequence of hypothyroidism is glucose intolerance, which is commonly associated with obesity. Adipose tissue secretes a variety of hormones which affect insulin sensitivity. The most thoroughly investigated is leptin, which has an insulin sensitizing effect; and its serum concentration is positively correlated to body fat content. This study was designed to evaluate whether insulin resistance in hypothyroidism is attributed to the increased body fat content solely, and if it is associated with any alteration in serum leptin concetration. To this end, serum insulin and leptin concentrations of dogs with hypothyroidism were compared to those of healthy dogs, with consideration of the disparity in body fat content.

Ten (5 neutered females, 4 intact and 1 neutered males) dogs newly diagnosed with naturally-occurring hypothyroidism and 9 (3 neutered females, 4 intact and 2 neutered males) healthy dogs were included in the study. All dogs underwent complete physical examination and body condition score (BCS) evaluation (1–9 scale). Fasted blood samples were collected from all dogs for complete blood count, serum biochemical profile, glucometer test, serum total T_4 and canine thyroid stimulating hormone concentrations. Serum insulin and leptin concentrations were measured using radioimmunoassay tests previously validated for dogs.

Serum insulin concentrations ranged from 36 to 58 pmol/l with a mean (±SE) of 45.6 (±2.6) pmol/l in the healthy dogs and from 38 to 210 pmol/l with a mean of 94.7 (±16.3) pmol/l in the hypothyroid dogs. Serum leptin concentrations ranged from 0.07 to 8.00 ng/ml with a mean of 3.00 (±1.04) ng/ml in the healthy dogs and from 1.67 to 32.94 ng/ml with a mean of 16.18 (±5.20) ng/ml in the hypothyroid dogs. Log transformation was used for serum insulin and leptin concentrations since these parameters showed normal log distribution on a P-P plot. Linear regression of the transformed data, controlling for BCS and age, showed that serum insulin was significantly higher in the hypothyroid dogs ($p = 0.009$), while no significant difference in serum leptin between the hypothyroid and normal dogs was found. There was no significant difference in blood glucose concentration between the hypothyroid (mean ± SE, 3.5 ± 0.3 mmol/l) and normal (3.9 ± 0.3 mmol/) dogs.

In conclusion, hypothyroid dogs showed hyperinsulinemia, which can not be explained by obesity alone. In contrast, the increased serum leptin concentration in the hypothyroid dogs was probably mainly attributed to their increased body fat content. These findings warrant further investigation into the role of leptin in insulin resistance associated with hypothryroidism.

ABSTRACT #66

LIQUID THYROXYL® IS AN ALTERNATIVE TO SOLOXINE® FOR TREATING CANINE HYPOTHYROIDISM. M Rick, RF Nachreiner, KR Refsal, PA Schenck. Diagnostic Center for Population and Animal Health, Michigan State University, Lansing, MI, USA.

Liquid levothyroxine-sodium is known to be very unstable and until recently, oral levothyroxine-sodium was commercially available only in pill form. This study was done to compare the bioavailability of Soloxine® with the first commercially available liquid levothyroxine-sodium supplement Thyroxyl®.

Eight healthy intact male mix-breed dogs (0.9–5 years, 13–26 kg) were thyroidectomized and fed a pre-measured commercially available diet daily at 8am. Dogs were given 22 µg/kg (in increments of 0.05 mg) of either oral thyroid replacement for one week at 8am and 8pm. The last day of treatment, no evening dose was given. To estimate absolute bioavailability, injectable levothyroxine sodium was given once as a 0.11 µg/kg iv bolus immediately after reconstitution. Serial jugular blood samples were taken following the administration of the last morning dose or post iv bolus at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24, 36, 48 and 72 hours. Serum samples were frozen until analysis. Thyroxine (T4) was measured in one batch by validated commercially available radioimmunoassay (DiaSorin® Solid Phase RIA). A washout of one week between products was applied to allow return of laboratory hypothyroidism. Samples were taken at the end of this period to establish values for baseline endogenous T4 and to demonstrate return of laboratory hypothyroidism. Samples were also taken at 0, 3 and 6 hours post dosing on treatment days 5 and 6 to demonstrate steady-state. Calculations were performed using Microsoft Excel and Minitab 12.0.

All 8 dogs completed the study. Four dogs developed primary hypoparathyroidism and were treated with Calcitriol® to ensure normocalcemia throughout the study. One dog was excluded from calculations because of demonstrated coprophagia. Time at maximum concentration (Tmax) of T4, maximum concentration (Cmax), steady-state concentration (Css), mean residence time (MRT), elimination half-life (T½), area under the curve (AUC), relative bioavailability (RB), time at minimum concentration (Tmin), minimum concentration (Cmin) and absolute bioavailability of oral product (f) were established.

There was a statistically significant difference for both MRT and T½ among dogs, showing that the clearance of L-thyroxine varies among dogs. There were no significant differences between the two different treatments for any of the values (Tmax, Cmax, Css, MRT, T½, AUC, Tmin, Cmin or f (2 way ANOVA)). Average RB of Thyroxyl® was 130% of that of Soloxine®. These results show that liquid Thyroxyl® is not different to Soloxine®. The ease of medication storage, dosage-alterations and administration might make Thyroxyl® beneficial over Soloxine®. Long-term stability needs to be further researched.

ABSTRACT #67

IN NORMAL DOGS, ACTH STIMULATION TEST RESULTS ARE ALTERED BY TIME IN CAGE, ACTH DOSE, PREVIOUS STIMULATION TESTS, AND STEROID THERAPY. A. Milgrom[1], E.N. Behrend[2], C. Natanson[1], and S. Solomon[1] [1]Critical Care Medicine Department, National Institutes of Health, Bethesda, MD, and [2]Department of Clinical Sciences, Auburn University, Auburn, AL.

ACTH-stimulation test results are used as primary criterion for diagnosing a relative cortisol deficiency in critically ill subjects, yet lack standardized definition. The purpose of this study was to evaluate if commonly encountered, clinical variables exist that alter measurable ACTH-stimulated cortisol concentrations.

Two groups of six normal, conscious male mixed breed dogs were housed individually in cages during a 96-hour study. Each group was divided into three subgroups randomized for treatment with dexamethasone: control (no steroids), low-dose (mean 0.4 mg/kg/d), and high-dose (mean 7.9 mg/kg/d). At each time, low dose ACTH (Cosyntropin) was administered (1 mcg/kg IV) and a blood sample drawn 60 min later; immediately thereafter high dose ACTH (5 mcg/kg IV) was administered and another blood sample drawn again after 60 min. A high frequency group received these stimuli at baseline (prior to treatment) and at 4, 11, 24, 48, 72, and 96 hrs. A low

frequency group received these stimuli at baseline and at 11, 48, and 96 hrs. Treatment periods were classified as baseline, early (12–48 hr) and late (72–96 hr). Data were analyzed using ANOVA and multiple comparisons testing. $P \leq 0.05$ was considered significant.

Relative increases in serum cortisol concentration in response to ACTH were significantly greater in response to high-dose ACTH than to low-dose at all times. In all dogs, response to both ACTH doses increased significantly after being in a cage for 24 to 48 h (early treatment period) and then decreased toward baseline at 72 to 96 h in the late treatment period. In comparison, in the low-dose steroid group, this varied response over time was qualitatively similar but depressed compared to controls throughout. Furthermore, high-dose steroid treatment augmented the increase in adrenal responsiveness in the early treatment period compared to control, but then inhibited this response during the late treatment period similar to the low dose. Low frequency ACTH stimulation testing caused an increase in cortisol secretion in comparison to the controls, but high-frequency testing caused no further increase.

Our results suggest that in normal dogs response to ACTH may be cumulative over time. Low-dose steroid treatment may inhibit the cumulative effect while high-dose steroid treatment may initially stimulate cortisol secretion in the short-term but inhibit cortisol secretion after 48 hrs. Current methods of assessing cortisol concentrations in healthy patients are subject to a wide degree of variability from a multitude of factors. Further studies shall evaluate other clinically relevant factors such as sedation and mechanical ventilation to determine if these alter adrenal responses in diseases where relative adrenal deficiency is believed common, such as septic shock.

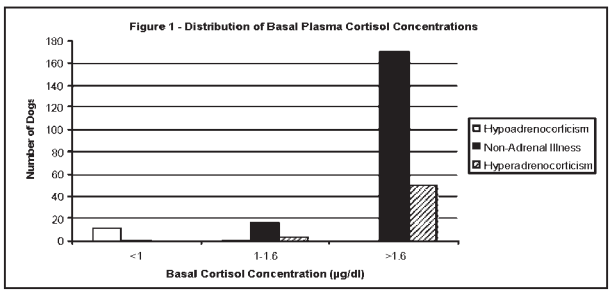
ABSTRACT #68

USE OF BASAL PLASMA CORTISOL CONCENTRATIONS TO RULE OUT A DIAGNOSIS OF HYPOADRENOCORTICISM IN DOGS. EM Lennon, KR Grace, A Friedenthal, SA Bissett, LS Moses, MT Correa, MG Papich, AJ Birkenheuer. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

The purpose of this study was to determine if basal plasma cortisol concentrations could be used to rule out hypoadrenocorticism in dogs. Previous studies have demonstrated that dogs with hypoadrenocorticism have low basal plasma cortisol concentrations; 98.8% of 309 dogs in five studies of hypoadrenocorticism had cortisol concentrations less than 1.7 µg/dl.

The records of 299 dogs that had undergone ACTH stimulation testing at North Carolina State University were reviewed. Cases were divided into 3 groups according to basal cortisol concentration: <1 µg/dl (n = 13), 1.0–1.6 µg/dl (n = 21), and >1.6 µg/dl (n = 220) (Figure 1). Forty-five cases were excluded due to administration of corticosteroids, ketoconazole, or o,p′-DDD prior to the ACTH stimulation test. The final diagnosis for each patient was then determined based on ACTH response and clinical findings.

None of the dogs with hypoadrenocorticism (0/13) had basal cortisol concentrations greater than 1 µg/dl. Comparatively, 97.9% (184/188) of dogs with non-adrenal illness and 100% (53/53) of dogs with hyperadrenocorticism had concentrations >1 µg/dl. Dogs with basal cortisol >1 µg/dl were significantly less likely ($p < 0.001$) to have hypoadrenocorticism compared to dogs with basal cortisol ≤1 µg/dl. In this study, the sensitivity and specificity of a basal cortisol >1 µg/dl to rule out hypoadrenocorticism were 98.4% and 100%, respectively. The data in this and other studies clearly indicate that a basal cortisol measurement can be used to rule out hypoadrenocorticism with a high sensitivity and specificity if the patient's plasma cortisol concentration is ≥1.7 µg/dl; otherwise an ACTH stimulation test is needed.



ABSTRACT #69

CHARACTERIZATION OF GLUCOCORTICOID DEFICIENT HYPOADRENOCORTICISM IN DOGS: A RETROSPECTIVE STUDY. A Thompson, C Scott-Moncrieff, J Anderson. Purdue University Veterinary Teaching Hospital, West Lafayette, IN.

Previous studies of canine hypoadrenocorticism report the incidence of glucocorticoid deficient hypoadrenocorticism (GDH) as <5%. The aim of this study was to determine the incidence of GDH in dogs presented to a referral hospital and to compare the clinical findings to the mineralocorticoid and glucocorticoid deficient (MGDH) form of hypoadrenocorticism. The veterinary medical database was searched for canine hypoadrenocorticism or Addison's disease at Purdue University from 1985–2005. Patients were included if the pre and 1 hour post-ACTH cortisol concentration were <2 µg/dl. Dogs were classified as MGDH if hyponatremia or hyperkalemia were present. GDH was diagnosed if sodium and potassium were within reference range. Dogs were excluded if they had received mitotane or been treated with more than one dose of corticosteroids prior to ACTH stimulation. Of 46 dogs that met the inclusion criteria, 35 (76%) had MGDH and 11 (23%) had GDH. Signalment, history, physical examination, minimum data base, treatment and outcome were compared between the two groups. GDH patients were older (GDH mean 7.0 years, MGDH mean 4.4 years, $p = 0.0046$) and had a longer duration of illness prior to diagnosis (GDH mean 1.4 months, MGDH 0.4 months, $p < 0.01$). Anemia (GDH mean HCT 32%, MGDH mean 45%, $p < 0.01$), hypoalbuminemia (GDH mean 2.14 g/dl, MGDH mean 3.0 g/dl, $p = 0.005$) and hypocholesterolemia (GDH mean 86.4 mg/dl, MGDH 137.5 mg/dl, $p = 0.0191$) were more common in GDH. Glucocorticoid deficient hypoadrenocorticism is more common than previously described. Anemia, hypoalbuminemia and hypocholesterolemia occurs more frequently in patients with GDH.

ABSTRACT #70

SERUM INHIBIN IMMUNOREACTIVITY IN NEUTERED DOGS WITH ADRENAL DYSFUNCTION. C Brömel, RW Nelson, EC Feldman, CJ Munro, PH Kass, A Esteller Vico, P Labelle, AJ Conley. University of California, School of Veterinary Medicine, Davis, CA.

Inhibin, a glycoprotein of the TGFbeta superfamily, is synthesized and secreted predominantly by ovarian granulosa and testicular Sertoli cells. Studies in humans show that inhibin is also secreted by the adrenal glands. Inhibin immunoreactivity (IR) has been documented in adrenocortical tumors and hyperplastic adrenals, but not in pheochromocytomas (PHEO). Inhibin is a marker for differentiating adrenocortical tumors from PHEO in humans and may play a role in endocrine tumorigenesis. Inhibin secretion in dogs with adrenal dysfunction has not been reported. The aim of this study was to compare inhibin IR in neutered healthy dogs and dogs with adrenal dysfunction and validate an inhibin assay for canine serum.

Sixty-three neutered dogs were studied and divided into five groups: healthy female spayed (FS, n = 11), healthy male castrated (MC, 10), and dogs with PHEO (8), pituitary-dependent (PDH, 20) or adrenal tumor hyperadrenocorticism due to cortical carcinomas (ACA, 14). Inhibin IR was measured in serum before and after ACTH stimulation and before and after treatment (if available) with a RIA using an antibody against 31 kD bovine inhibin (Dr. DM Robertson, Monash University, Australia). Data were analyzed with ANOVA, Mann-Whitney and Wilcoxon-signed- rank tests. Significance was set at $P < 0.05$.

The RIA was validated for sensitivity, dilutional parallelism and precision. The assay was sensitive for measuring canine inhibin IR to 0.05 ng/ml. Serial dilution of canine serum was parallel to the standard curve. Mean coefficients of variation for intra- and interassay precision were 7.1% and 11.7%, respectively. The median inhibin IR for both FS and MC healthy groups (pre and post ACTH) was 0.11 ng/ml (range, 0.05–0.29). Before treatment, the median (range) pre ACTH inhibin IR was 0.11 ng/ml (0.06–0.31) in PHEO, 0.20 ng/ml (0.06–0.58) in PDH, and 0.71 ng/ml (0.06–14.16) in ACA. Inhibin IR was greater in dogs with PDH and ACA than in PHEO ($P = 0.016$ and $P = 0.001$, respectively) and healthy dogs ($P = 0.0003$ and $P < 0.0001$, respectively), and greater in ACA than in PDH dogs ($P = 0.003$). It was similar in dogs with PHEO and healthy dogs. After treatment, median pre ACTH inhibin IR was similar in PHEO, PDH and ACA dogs (0.10, 0.11, and 0.11 ng/ml, respectively). Median pre and post ACTH inhibin IR were similar within all groups (before and after treatment). After adrenalectomy, post ACTH inhibin IR decreased in ACA dogs ($P = 0.001$).

The results indicate that serum inhibin IR is present in higher concentrations in dogs with hyperadrenocorticism (ACA and PDH) than in dogs with pheochromocytomas and healthy controls, and is higher in dogs with ACA than in PDH dogs. Baseline inhibin IR in serum may be useful for differentiating cortisol secreting adrenocortical carcinomas from pheochromocytomas.

ABSTRACT #71

ATRIAL SEPTAL DEFECTS IN AN EXTENDED FAMILY OF STANDARD POODLES. SG Gordon[1], AB Saunders[1], TW Fossum[1], DA Nelson[1], KM Meurs[2], RM Roland[1], and MW Miller.[1] [1]Department of Small Animal Clinical Science and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX and [2]Department of Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, Washington.

Atrial septal defect (ASD) is a relatively infrequently diagnosed canine congenital cardiac malformation, comprising less that 2% of reported defects. Breeds shown or suggested to be at increased risk include Boxer, Doberman pinscher, Samoyed and Newfoundland. Herein we report the clinical aspects of ASD in an extended family of Standard Poodle dogs.

To date we have evaluated 23 related dogs confirming the presence of an isolated septum secundum ASD in nine. Of the dogs with ASD, all were diagnosed between 1 and 7 years of age. Three presented for signs of left sided congestive heart failure and were 1, 1.5, and 5 years of age. All affected dogs had moderate to severe right atrial and ventricular enlargement and all shunts were predominantly left-to-right shunting lesions based on color-flow and spectral Doppler interrogation and intravenous saline contrast echocardiography. Estimates of the magnitude of volumetric shunting (QP/QS) based on radionuclide ventriculography was available in 5 dogs and ranged from 1.2–2.2 : 1. Even the dog with a QP/QS of 1.2 had substantial right ventricular cardiomegaly suggesting hemodynamically significant volumetric shunting. Echocardiographic 2D estimates of ASD size ranged from 0.6–2.2 cm. One dog has undergone successful catheter based repair and five additional dogs are scheduled for this same procedure. Echocardiographic evaluation of two dogs suggested an inadequate dorsal tissue rim for transcatheter repair and successful open heart repair was accomplished using cardiopulmonary bypass, right atriotomy and a gluteraldehyde tanned pericardial patch. All dogs that have undergone repair have had significant resolution (near normal) of their cardiac morphologic changes at 1 month. Of the three dogs with heart failure, clinical signs resolved in two resolved following repair and they require no cardiac medications. The third dog was euthanized due to malignant Histiocytosis; postmortem examination documented a 1.2 × 1.6 cm septum secundum ASD.

Initial evaluation of pedigree data has eliminated an X-linked recessive mode of inheritance as two affected females were able to produce unaffected male dogs. An autosomal dominant pattern appears to be most likely because the trait appears in each generation; however, evaluation of additional dogs is needed to confirm this. An autosomal recessive pattern cannot be ruled out, but this mode of inheritance would suggest a very high prevalence of the gene within the breed.

Ongoing evaluation of this family should allow determination of the mode of inheritance. Our data suggest that this breed should be considered at risk for ASD. Additionally, this may represent a large animal model of spontaneous septum secundum ASD.

ABSTRACT #72

USE OF A PERIPHERAL VASCULAR OCCLUSION DEVICE FOR CORRECTION OF PATENT DUCTUS ARTERIOSUS IN DOGS. DF Hogan, HW Green III, RA Sanders, JA Goodwin. Purdue University School of Veterinary Medicine, West Lafayette, IN.

Patent ductus arteriosus (PDA) is the most common congenital cardiac defect in dogs and usually results in congestive heart failure if left uncorrected. Interventional techniques using catheter-delivered devices have become widespread over the past decade. Although these techniques all have relatively high success rates individually, the anatomic uniqueness of the canine PDA and cost-conscious nature of veterinary medical clientele provide some limitations. There is a commercially available peripheral vascular occlusion device (VOD) that is similar but not identical in construction and shape to a PDA occlusion device for less than half the price. The VOD is constructed of nitinol wire mesh without internal thrombogenic material; therefore it is self-expanding and can be deployed through relatively small catheters. Vascular occlusion is achieved by the device filling the internal vascular lumen and induction of thrombosis. Deployment and retrieval is facilitated by a threaded delivery cable. Our goals were to assess the ability of the VOD to correct PDA in dogs and identify possible procedural complications.

Eight dogs (7 FI, 1 MI) with a mean (±SD) age and weight of 3.9 mo (1.7) and 7.2 kg (5.4), respectively were entered into the study. Seven dogs were of individual pure breeds and one was a mixed breed. All dogs had Miller angiographic anatomic type IIA (Krichenko type E) PDAs although 3 had minimal pulmonic narrowing. Mean (±SD) of the minimal ductal diameter, distal ductal diameter and length were 3.9 mm (2.6), 8.0 mm (4.2) and 13.9 mm (3.6), respectively. The VOD was deployed transarterially in 7 dogs and transvenously in one dog. The mean (±SD) VOD size was 10.3 mm (4.5) with a mean (±SD) fluoroscopy time of 7.9 min (4.4). Complete occlusion was achieved in 7/8 dogs with moderate residual flow in 1 dog. The mean time to complete occlusion (±SD) was 2.6 min (3.8) although 4/7 had immediate complete occlusion. Transthoracic echocardiography confirmed absence of ductal flow at 24 hours and 1 month post procedure in all 7 dogs with procedural occlusion. There was persistent moderate residual flow at 24 hrs and 1 month post-procedure in the remaining dog. There were no procedural complications.

The VOD appeared to perform well with excellent results. The procedure is relatively simple and can be performed from either a transarterial or transvenous approach. Appropriate sizing guidelines for the VOD are still premature but the range appears to be relatively large. The manufacturer suggests oversizing of the VOD by 130–150% of the vessel diameter. The mean (±SD) oversizing of the VOD in this study was 135.2% (22.4) with the lowest occurring in the dog with residual flow (108.5%). We conclude that the VOD is a viable alternative for interventional correction of the canine PDA.

ABSTRACT #73

PATENT DUCTUS ARTERIOSUS OCCLUSION WITH AN INVESTIGATIONAL AMPLATZER® CANINE DUCTAL OCCLUDER. TP Nguyenba, AH Tobias. University of Minnesota Veterinary Medical Center, Saint Paul, MN.

The Amplatzer® Canine Ductal Occluder (ACDO) is an investigational detachable multi-layered nitinol device with a short waist that separates a flat distal flange from a cupped proximal flange. The device, particularly its cupped proximal flange, is specifically designed to conform to canine ductal morphology. The purpose of this study was to evaluate the ACDO for patent ductus arteriosus (PDA) occlusion in dogs.

The study was conducted in 11 client-owned dogs with informed consent. Following femoral arterial catheterization, PDA dimensions were determined by angiography, and a guiding catheter was advanced into the main pulmonary artery via the aorta and PDA. The ACDO was advanced through the catheter, using an attached delivery cable, until the flat distal flange was deployed within the main pulmonary artery. The partially deployed ACDO, guiding catheter, and delivery cable were retracted as a single unit until the distal flange engaged the pulmonic ostium of the PDA. The delivery cable was then held stable while the catheter was retracted to deploy the ACDO waist across the pulmonic ostium and cupped proximal flange within the ductal ampulla. Proper ACDO positioning and stability were evaluated by back-and-forth maneuvering of the delivery cable and a small contrast injection made through the guiding catheter over the delivery cable. The delivery cable was then detached and removed along with the guiding catheter. An angiogram was performed at the conclusion of the procedure, followed by echocardiograms 24 hours and 3 months later.

Median weight and minimal ductal diameter on angiography were 9.7 kg (range 3.7–32.3 kg) and 3.43 mm (range 1.68–6.94 mm), respectively, and a variety of PDA morphologies were identified. Initial procedural success was achieved in 10 dogs, while dislodgment with pulmonary embolization of the device occurred in one case. No adverse effects due to device embolization were observed, and the PDA in this patient was later occluded successfully with a larger ACDO. The initial procedure in this one case differed from the others in that the selected device waist diameter was 1.21 times larger than the minimal ductal diameter, whereas for all other procedures, the device waist diameter was greater than 1.5 times the minimal ductal diameter (range 1.51–2.38). Following all successful ACDO placements, final angiograms showed trivial residual shunting in one case, and 24 hour post-procedure echocardiograms showed trivial residual shunting in another. However, no residual shunting has been identified in any of the 3 month post-procedure echocardiograms completed to date (n = 6).

Whereas research is ongoing, our data suggest that PDA occlusion in dogs with an appropriately sized ACDO is an extremely effective procedure across a wide range of body weights, minimal ductal diameters, and ductal morphologies.

ABSTRACT #74

EFFECT OF CARVEDILOL IN DOGS WITH DILATED CARDIOMYOPATHY: RESULTS FROM A PROSPECTIVE PLACEBO-CONTROLLED RANDOMIZED CLINICAL TRIAL. MA Oyama[1], DD Sisson[1], BJ Bulmer[1], R Prosek[1], MW Luethy[2], and V Luis Fuentes[3]. [1]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL; [2]Animal Emergency and Critical Care Center, Northbrook, IL; [3]Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH.

Increased adrenergic stimulation contributes to ventricular dilation, systolic dysfunction, and neurohormonal derangements in patients with dilated cardiomyopathy (DCM). Beta-adrenergic blocking agents, such as carvedilol, reduce remodeling and improve survival in humans with DCM, however, little is known regarding the safety and efficacy of such treatment in dogs with DCM. We sought to determine the effects of carvedilol on echocardiographic and neurohormonal parameters in dogs with DCM over a 4-month treatment period.

Twenty-three dogs with symptomatic DCM were entered into a prospective, placebo-controlled, double-blinded randomized study. Patients were evaluated by cardiac ultrasound, ECG, thoracic radiographs, and epinephrine, norepinephrine, aldosterone, renin activity, and atrial and B-type natriuretic peptide assay. Dogs were randomized to carvedilol or placebo in a 2 : 1 fashion and medications titrated to 0.3 mg/kg q12hrs over a 4-week period, followed by 3 months of chronic therapy. Primary study endpoints involved echocardiographic left ventricular volume and function. Secondary endpoints involved neurohormonal activation.

Sixteen dogs were randomized to carvedilol and 7 dogs to placebo. Baseline characteristics between groups were similar. One dog in each group died during the titration phase. After three months of chronic therapy, 13 dogs receiving carvedilol and 5 dogs receiving placebo were still alive. Carvedilol dogs experienced a statistically significant increase in left ventricular end-diastolic volume (LVVd change +8.6%; P = 0.009) and LV end-systolic volume (LVVs change +14.6%; P = 0.011) over the 4-month study period. LV volume of placebo dogs also increased, but these changes were not statistically significant (LVVd change +15.1%, P = 0.16; LVVs change +13.2%, P = 0.58). There was no difference in the mean change of LVVd and LVVs between carvedilol and placebo groups (LVVd P = 0.30; LVVs P = 0.50), suggesting that both groups experienced similar amounts of progressive LV enlargement. There was no difference in the absolute value or percent change in plasma ANP, BNP, renin activity, norepinephrine, epinephrine, or aldosterone between groups. In addition, there was no difference in the absolute value or percent change in radiographic heart size, systolic time intervals, blood pressure, heart rate, BUN, creatinine, sodium, or owner perceived quality-of-life between groups.

Carvedilol administration did not improve echocardiographic or neurohormonal parameters of heart function over 3 months of chronic oral treatment. The lack of effect may be related to severity of disease, carvedilol dose, and brevity of follow-up time. Statistical power of the present study was adversely affected by a high mortality rate and small sample size.

ABSTRACT #75

ACUTE CARDIOVASCULAR EFFECTS OF PIMOBENDAN IN DOGS WITH STABLE CONGESTIVE HEART FAILURE DUE TO CHRONIC DEGENERATIVE ATRIOVENTRICULAR VALVE DISEASE. RM Roland[1], SG Gordon,[1] A Bahr[2], MW Miller,[1] AB Saunders.[1] [1]Department of Small Animal Clinical Science and Michael E. DeBakey Institute, and [2]Department of Large Animal Clinical Science and Surgery, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX.

Chronic degenerative atrioventricular valve disease (CVD) is the leading cause of canine congestive heart failure (CHF) and in advanced stages is associated with systolic dysfunction. Pimobendan (Vetmedin®) is an orally active, rapidly absorbed inodilator demonstrating peak cardiovascular effects within 1–2 hours of administration in normal dogs. The hypothesis of this prospective study was that pimobendan (pimo) therapy on a background of conventional heart failure therapy (angiotensin converting enzyme inhibitors, diuretics, antiarrhythmics and additional afterload reducers as required) in dogs with stable CHF (furosemide dependant but not requiring contemporary dosage changes) due to CVD would result in acute (within 24 hours) favorable cardiovascular effects.

Seven small breed dogs (<18 kg) with CVD and stable CHF were enrolled in the study. Dogs were hospitalized for 3 days. On days 1 and 3, all dogs were evaluated with a physical examination, thoracic radiographs, complete blood work, ECG, indirect systemic blood pressure (SBP), echocardiogram and radionuclide ventriculography (RNV). Throughout the study all dogs were maintained on their current medications and doses. On the evening of day 2 and morning of day 3 pimo was administered at a dose of 0.25–0.3 mg/kg by mouth.

Measured parameters were compared with a paired Student's t test and a p value <0.05 was considered significant. Lack of significant differences in PCV, BUN or creatinine suggested overall volume status was unchanged between day 1 and day 3. Significant reductions in left ventricular internal dimension in diastole (LVIDd) and two dimensional left atrial to aortic root ratio and a trend towards a decrease in vertebral heart score (p = 0.07) suggested reduced overall cardiac size. Significant reductions of LVID in systole and LV short axis area in systole documented increased systolic function. No significant difference in SBP coupled with a significant increase in aortic TVI suggested balanced afterload reduction and enhanced forward cardiac output. Finally, significant reduction in the ratio of early mitral inflow to annular mitral E wave (E:Ea) suggested a reduction in left atrial pressure. Changes in RNV parameters including regurgitation fraction (RF), ejection fraction, peak emptying rate, peak filling rate and time to peak filling were insignificant overall but 5/7 showed significant reductions in RF. Finally, 5/7 and 2/7 dogs had prolapse and flail of their mitral valves respectively on day 1 of the study and these abnormalities were not changed after the administration of pimo. These data suggest when added to background of conventional heart failure therapy pimo produces favorable cardiovascular effects following two oral doses in stable CHF due to CVD with no adverse short term effects.

ABSTRACT #76

ASSOCIATION OF PIMOBENDAN WITH VENTRICULAR ARRHYTHMIAS IN DOGS WITH CONGESTIVE HEART FAILURE. SL Rosenthal, MJ Ferguson, BK Lefbom, WD Tyrrell. Chesapeake Veterinary Cardiology Associates, Towson, Maryland.

Cardiac arrhythmias are a common finding in dogs with congestive heart failure. These arrhythmias can be associated with syncope and even sudden death. Certain positive inotropic agents have been implicated in human congestive heart failure (CHF) patients to increase the incidence of tachyarrhythmias and sudden death. Pimobendan, a positive inotrope with vasodilating properties, has recently been advocated in the treatment of CHF in the dog. It is classified as a phosphodiesterase III (PDE III)inhibitor that reduces the breakdown of cAMP leading to potentiation of the adrenergic signal transduction, increased contractility, and vasodilation. Sensitization of the myofilaments to calcium is another proposed mechanism for the positive inotropic effects of pimobendan. Agents that inhibit PDE III (eg. milrinone and amrinone) have been implicated in the worsening of ventricular arrhythmias and an increased incidence of sudden death in humans. The objective of this study was to evaluate the potential proarrhythmic effects of pimobendan when added to conventional treatment for CHF canine patients.

8 dogs (4 Doberman pinchers, 1 each of Dalmatian, Greyhound, Labrador Retriever and Shiloh Shepherd) with CHF that were stable on therapy for CHF were used in the study population. Each dog had been treated with digoxin, furosemide, and enalapril prior to the addition of pimobendan. A serum biochemical profile, serum digoxin level and 24 hour ambulatory electrocardiogram (Holter) recording were performed as a baseline. 3/8 dogs (37.5%) of the dogs had a predominant rhythm of atrial fibrillation at the initiation of the study. The Holter recording was repeated approximately three weeks (range 14–24 days) after the initiation of therapy with pimobendan (mean dose 0.23 mg/kg twice daily, range 0.19–0.31 mg/kg). The frequency of ventricular premature contractions (VPC's) increased by a mean of 1096 per 24 hours (range −850 to +5775) or a mean increase of 102.1% per 24 hours (range −57%–+ 713%). 75% of the dogs had an increase in the number of VPC's, 62.5% of the dogs had an increased number of ventricular couplets, 50% of the dogs had an increased number of ventricular triplets, 25% of the dogs had and increased number of ventricular runs, and 25% had an increase in periods of ventricular tachycardia. The mean heart rate decreased in 62.5% of the dogs with a decrease in the mean from 119.9 beats per minute to 109.6 beats per minute.

The trend seen in these patients point toward an increased frequency of ventricular arrhythmias in dogs with congestive heart failure when pimobendan is added to traditional therapy for CHF. Causes of the increased ventricular ectopy can be attributed to the introduction of the pimobendan, a pharmacologic interaction with the other medications, or related to normal daily variation in Holter monitor recordings. Sudden death or syncope was not noted in any of the animals enrolled in this study, however, continued followup will be prudent to statistically rule this out.

ABSTRACT #77

DUAL-CHAMBER CARDIAC PACING IN 25 DOGS. HW Green III, DF Hogan, AE Vibert, RA Sanders. Purdue University School of Veterinary Medicine, West Lafayette, IN.

Symptomatic bradyarrhythmias resulting from atrioventricular (AV) blockade or sick sinus syndrome (SSS) are the primary indications for artificial cardiac pacing in dogs. Fixed-rate ventricular demand (VVI) pacing systems are still most commonly used dogs although dual chambered adaptive-rate pacing systems have been previously described and the potential benefits to cardiovascular performance demonstrated in humans and dogs. This retrospective study examines the outcome of dual-chamber (2-lead) pacemaker implantation in 25 dogs.

The most common indications were third-degree AV block (n = 10, 40%), SSS occurring alone or concomitantly with some AV block (n = 7, 28%), and high-grade second-degree AV block (n = 5, 20%). Mean body weight was 20.7 kg (5.8–65). Average procedural time was 2.0 hrs (1.35–2.75) compared to 1.1 hrs (0.5–1.5) for 12 single chambered systems. Minor complications occurred in 3/25 dogs (12%), including seroma formation, trivial tricuspid regurgitation and phrenic stimulation which was corrected with a simple mode switch. Major complications occurred in 4/25 dogs (16%); ventricular lead dislodgement in 2 dogs (8%), right atrial perforation with pericardial effusion in 1 dog (4%) and infection which required lead extraction in 1 dog (4%). Eighteen dogs (72%) did not experience any complications related to pacemaker placement and one dog displayed reduced clinical signs related to CHF when switched from a single to dual pacing system. Ten dogs (40%) have died or been euthanized since pacemaker implantation. Two deaths (8%) may be attributable to complications associated with the pacing system, four (16%) to advance mitral valve disease and 3 (12%) from non cardiac causes. With the exception of one dog that died 5 days post implantation, mean survival time of the remaining nine is 20 months (4–36 months). Fourteen dogs (56%) were alive the time of this writing with mean post implantation survival time of 24 months (7–54 months). One dog was lost to follow-up.

We conclude that 2-lead dual-chambered pacing systems, while requiring somewhat longer procedure times, were not associated with a higher complication rate than previous reports of single-lead pacing systems. Additionally, dual-chambered rate adaptive systems allow fine-tuning of artificial pacing and, at least theoretically, improved cardiac performance.

ABSTRACT #78

EFFECTS OF AGE AND HEART RATE ON RIGHT VENTRICULAR MYOCARDIAL VELOCITIES ASSESSED BY TISSUE DOPPLER IMAGING IN HEALTHY, NONSEDATED CATS. S Disatian, JM Bright, and J Boon. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Tissue Doppler imaging (TDI) is an ultrasound technique which may be used to evaluate right ventricular (RV) function in both human beings and cats. However, age and heart rate may affect TDI indices of RV function, and to our knowledge, data regarding the effects of age and heart rate on TDI indices of RV function are currently unavailable. This study was designed to determine the relationships between age and heart rate on RV myocardial velocities assessed by color TDI and to provide reference ranges for RV myocardial velocities in healthy nonsedated cats.

Fifty healthy nonsedated cats 3 months and older without clinical or laboratory abnormalities were studied. For analysis the cats were grouped by age: 3 to<6 months, 6 months to<3 years, 3 to<6 years, 6 to<12 years, and 12 years or older. Color TDI was done from a modified left apical four chamber image. RV myocardial velocities were measured at the tricuspid annulus on the RV wall. TDI measurements quantifying RV diastolic function included peak early diastolic velocity (E'), peak late diastolic velocity (A'), isovolumic relaxation time (IRT), deceleration rate of early diastolic wave (DR), the ratio of early diastolic to late diastolic velocities (E'/A' ratio), and the ratio of peak pulsed wave velocity of transmitral early diastolic flow to tissue Doppler peak early diastolic velocity (E/E' ratio). TDI measurements quantifying RV systolic function included peak systolic velocity (S'), isovolumic contraction time (ICT), regional myocardial ejection time (ET), and myocardial performance index ([ICT+IRT]/ET). Influence of age and heart rate on systolic and diastolic RV TDI variables was determined using statistical methods including simple linear regression and analysis of variance (ANOVA).

Significant weak negative correlation was found between age and peak early diastolic myocardial velocity ($r = -0.33$, $P = 0.036$). This relationship was not considered clinically important. No significant correlations between age and RV systolic TDI indices were found. Based on ANOVA ($P < 0.05$), none of the diastolic and systolic TDI parameters was significantly different between age groups. Both diastolic and systolic TDI parameters were not affected by heart rate with the exception of deceleration rate of the early diastolic wave (DR; $r = -0.32$, $P = 0.025$). In conclusion, age and heart rate do not have a clinically relevant effect on TDI indices of systolic or diastolic RV function in cats. Furthermore, reference values of normal RV TDI indices in nonsedated cats are reported.

ABSTRACT #79

TISSUE DOPPLER IMAGING DETECTS RADIAL AND LONGITUDINAL MYOCARDIAL DYSFUNCTION IN A CAT MODEL OF HYPERTROPHIC CARDIOMYOPATHY. V. Chetboul[1,2], S. Blot[3], V. Gouni[1], I. Barthelemy[3], C. Carlos Sampedrano[1], J.-L. Thibaud[3], R. Tissier[2], N. Granger[3], P. Bruneval[5], F. Gaschen[4], A.P. Nicolle[1], J.-L. Pouchelon[1,2]. [1]Cardiology Unit, National Veterinary School of Alfort, France. [2]INSERM U660 Maisons-Alfort, France. [3]Laboratoire de Neurobiologie, National Veterinary School of Alfort, France. [4]Division of Small Animal Internal Medicine, Department of Clinical Veterinary Medicine, University of Bern, Switzerland. [5]INSERM U652, Institut des Cordeliers, Paris, France.

Hypertrophic cardiomyopathy (HCM) is a primary myocardial disease characterized by increased cardiac mass associated with a hypertrophied, non-dilated left ventricle. Diagnosis of overt feline HCM is currently based on 2D and M-mode conventional echocardiography showing the presence of diffuse or segmental left ventricular hypertrophy. Preclinical diagnosis of the disease before occurrence of myocardial hypertrophy could prompt earlier medical treatment to prevent the development of heart failure and thromboembolism, and could also help to identify affected cats with a view to excluding them from breeding programs. Tissue Doppler Imaging (TDI) is a recent sensitive ultrasound technique capable of quantifying segmental myocardial motions. The objectives of this study were 1) to quantify radial and longitudinal left ventricular free wall (LVFW) velocities in cats with hypertrophic feline muscular dystrophy (HFMD) during the preclinical phase of the disease using TDI and 2) to determine if TDI could identify myocardial dysfunction in HFMD animals irrespective of the presence of LVFW hypertrophy. HFMD is an X-linked inherited feline neuromuscular disorder related to a mutation of the dystrophin gene, which also affects the myocardium (concentric LV hypertrophy).

For this purpose 22 healthy controls and 7 cats belonging to an HFMD family (2 affected adult males, 2 heterozygous adult females, one 2.5 month-old affected male kitten and 2 phenotypically normal female kittens from the same litter) were used. All cats were examined with both conventional echocardiography and 2D color TDI.

No LVFW hypertrophy was detected on the 2 carriers or on the affected kitten using conventional echocardiography and histological examination, respectively. The LVFW was also normal for one affected male and at the upper limit of normal for the second. However, LVFW dysfunction, including decrease in systolic and early diastolic velocities, presence of longitudinal post-systolic contractions and increase in late diastolic velocities, was detected on all affected and carrier HFMD cats using TDI.

TDI consistently detects LVFW dysfunction in HFMD cats despite the absence of myocardial hypertrophy. TDI appears therefore more sensitive than conventional echocardiography in detecting regional myocardial abnormalities.

ABSTRACT #80

EVALUATION OF RELATIONSHIPS BETWEEN DOPPLER-DERIVED AORTIC EJECTION VELOCITY AND VENTRICULAR PERFORMANCE IN HEALTHY ANESTHETIZED DOGS. JA Abbott[1], S Aref[2]. [1]Dept. of Small Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, [2]Dept. of Statistics, College of Sciences, Virginia Tech, Blacksburg, VA.

High aortic ejection velocity is a primary diagnostic criterion for identification of patients with aortic stenosis (AS). However, interpretation of peak aortic ejection velocity (AoVel) is confounded by changes in stroke volume. We sought to explore relationships between AoVel and variables that describe ventricular performance and geometry.

Ten healthy dogs were subject to general anesthesia and then echocardiographic examination before and during graded dobutamine (DB) administration. AoVel was obtained from a subcostal transducer site. End-diastolic and end-systolic left ventricular dimensions (LVIDd, LVIDs) were obtained by M-mode echocardiography; fractional shortening (%FS) was derived. Aortic root dimension (Ao) was obtained from long-axis images. Heart rate (HR) was calculated from inter-beat intervals of the Doppler spectrogram. Cardiac output (CO) was determined by thermodilution and indexed to body-weight (CI). Stroke volume was derived from CO and HR and indexed to body weight (SVI).

CI increased linearly with lower doses of DB, but was constant for doses exceeding 22.5 mcg/kg/min ($p < 0.001$). There was a significant linear relationship between AoVel and SVI ($P < 0.001$). There was no relationship between AoVel and the ratio of LVIDd to Ao. Variability in AoVel was best explained by a linear model that included heart rate and %FS ($p < 0.001$, $r^2 = 0.93$). An index, AVI, consisting of AV/(0.66 HR + 2.5%FS) was independent of CI ($p = 0.67$). Over the range of SVI evaluated, SVI had a small positive effect (slope = 0.08, $p = 0.03$) on AVI.

Clinical evaluation of echocardiographic indices that relate AoVel to systolic ventricular performance may be justified.

ABSTRACT #81

STRAIN AND STRAIN RATE IMAGING - NEW ULTRASOUND-BASED PARAMETERS FOR QUANTIFICATION OF REGIONAL MYOCARDIAL FUNCTION. G Wess, TB Wagner, M Killich, K Hartmann. Clinic for Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

Strain (S) and strain rate (SR) imaging are new echocardiographic imaging methods based on tissue Doppler ultrasound, that allow quantitative assessment of segmental myocardial contractility. Ultrasonic S and SR imaging has been developed to overcome problems with tissue velocity imaging, which is influence by overall heart motion, cardiac rotation, and contraction in adjacent segments. Strain is the fractional change from the original dimension and includes both lengthening, or expansion (positive strain) and shortening, or compression (negative strain). Strain rate measures the rate of deformation of a tissue segment and is derived from myocardial velocity gradient between two points. It is measured in s^{-1}. The aim of this study was to describe the regional longitudinal function of the left ventricle (LV) by S and SR analysis in healthy cats.

We studied 109 healthy cats (64 domestic shorthair cats and 45 pure breed cats, mean age 4.4 ± 4.0 years, weight 4.0 ± 1.2 kg). All cats had a normal clinical examination, blood pressure, ECG, normal 2D and Doppler echocardiographic examination. Five cardiac cycles were acquired in color Doppler imaging mode for each wall and stored for off-line analysis using a GE-Vivid 7 and its EchoPac analysis software. Regional peak S, and SR in systole (SR-S), early (SR-E) and late diastole (SR-A), was measured within the basal, mid and apical segments of the interventricular septum, and the LV free wall (FW) using a left apical 4-chamber view. The region of interest was placed in the center of each segment and had a size of 3 × 3 mm. Movement of the myocardial wall was corrected using a semi-automatic tracking method. Temporal filter settings were set to 40 ms.

Mean values ± the standard deviations for each segment are shown in the table. The correlations of SR and S with age, breed and weight were weak, not consistently detected across several myocardial segments and practically irrelevant. Strain and systolic SR values were significantly higher in each of the segments of the septum, compared to the corresponding segments in the LVFW. Differences between segments within the same wall were small and statically significant in only a few segments. These normal values of peak myocardial SR and S can be used as reference values in future investigations of left ventricular function.

	Unit	S-Basal	S-Mid	S-Apical	FW-Basal	FW-Mid	FW Apical
SR S	(1/s)	-2.9 ± 0.8	-2.9 ± 0.6	-2.9 ± 1.0	-2.0 ± 0.9	-2.0 ± 0.8	-1.8 ± 0.7
SR E	(1/s)	3.5 ± 1.5	3.9 ± 1.6^{+}	3.6 ± 1.3	3.4 ± 1.7	3.8 ± 1.7^{+}	3.4 ± 1.4
SR A	(1/s)	2.7 ± 1.1	2.6 ± 1.6	2.1 ± 1.1	2.2 ± 1.0	2.0 ± 0.8	1.4 ± 0.8^{+}
Strain	(%)	-21.6 ± 4.4	-22.5 ± 4.9	-18.7 ± 5.1^{+}	-17.0 ± 4.9	-18.0 ± 4.9	-14.8 ± 4.3^{-}

$^{+}$ Stat sign difference ($P<0.05$) to the segments in the same wall

ABSTRACT #82

USE OF REAL TIME THREE DIMENSIONAL ECHOCARDIOGRAPHY IN THE CHARACTERIZATION OF CONGENITAL AND ACQUIRED CARDIAC DISEASE. Cole SC, Oyama MA, Sleeper MM. Section of Cardiology, Department of Clinical Studies, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Two-dimensional (2D) echocardiography is used extensively in veterinary patients for non-invasive evaluation of cardiac structure and function. Real-time three-dimensional echocardiography (RT3DE) is a recently developed technology that allows the heart to be viewed in three dimensions, and unlike other 3D methods, does not require cumbersome image acquisition protocols or delays due to post-examination reconstruction of image data.

The feasibility of incorporating RT3DE into a routine echocardiographic examination was evaluated over 6 months. RT3DE was utilized in conjunction with standard 2D echocardiography in the evaluation of select patients with both congenital and acquired cardiac disease. Conditions evaluated by RT3DE included tricuspid valve dysplasia, aortic stenosis, pulmonic stenosis, ventricular septal defect, chronic valvular disease, dilated cardiomyopathy, pericardial effusion/cardiac masses, and patients with intracardiac devices. RT3DE images were obtained with a Philips Sonos 7500 cardiac ultrasound system with a 2–4 MHz matrix probe containing approximately 3000 individual elements. 2D images were obtained with either 2–4 MHz or 5–12 MHz probes. After acquisition, both 2D and RT3DE images were transferred to a dedicated workstation for offline analysis.

RT3DE generated satisfactory images in the majority of patients in which it was utilized. Problems with image acquisition generally occurred as a result of patient motion or small patient size not compatible with the low frequency and relatively large footprint of the matrix probe. RT3DE images provided complementary, and in some cases, unique information not appreciable from the 2D examination. We demonstrate that RT3DE is easily incorporated into a routine cardiac study and permits the investigator to obtain unique views of cardiac anatomy that can be readily manipulated and adapted to provide information not possible with standard 2D methods. Based on our findings, applications for RT3DE in veterinary patients are numerous, and include diagnosis of structural and functional abnormalities, non-invasive assessment of cardiac mass and volume, and guidance during interventional procedures.

ABSTRACT #83

PREDICTIVE VALUE OF NATRIURETIC PEPTIDE AND CARDIAC TROPONIN-I TESTING IN DOGS WITH MODERATE TO SEVERE CONGENITAL SUBAORTIC STENOSIS. MA Oyama and DD Sisson. Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL.

Subaortic stenosis (SAS) is a devastating congenital heart disease, leading to death in the majority of dogs that are severely afflicted; however, the ability to predict outcome in any given individual is difficult using current diagnostic tools (i.e., echocardiography, ECG, radiography). Previous investigation in dogs with heart disease demonstrated that plasma atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are elevated in correlation with severity of disease. Furthermore, in dogs with SAS, cardiac troponin-I (cTnI) is elevated, suggesting the presence of underlying myocardial injury. In theory, as myocardial injury increases, so does the risk for an adverse outcome, namely arrhythmias or heart failure. Thus, we hypothesized that plasma concentration of ANP, BNP, and cTnI would predict clinical outcome in dogs with moderate to severe SAS.

Twelve dogs with SAS were recruited into a prospective study. Dogs with an echocardiographic diagnosis of congential SAS and a left ventricular to aortic pressure gradient >50 mmHg were eligible. Baseline ANP, BNP, and cTnI evaluation was performed. Dogs then performed a submaximal exercise protocol consisting of 5 minutes of continuous jogging at a velocity of 4–5 miles/hr. Blood collection for natriuretic peptides and cTnI assay was repeated 5 and 60 minutes after cessation of exercise, respectively. Dogs were followed for an average of 12 months (range = 1–33) during which time 4 dogs died (1 sudden death, 3 CHF, mean time until death = 7 months, range = 1–16). Mean post-exercise BNP concentration was significantly higher in dogs that died as compared to dogs still alive at the time of data analysis (mean ± SEM; died = 49.6 ± 30.0 ng/ml, vs. alive = 4.42 ± 36.8 ng/ml; $p < 0.001$). Left ventricular to aortic pressure gradient was significantly higher in dogs that died (dead = 133 ± 9 mmHg vs. alive = 104 ± 16 mmHg; $p < 0.05$). Despite a wide range of absolute values, subgroup analysis indicated that exercise caused a significant elevation in post- vs. pre-exercise BNP in the 4 dogs that died (died-BNP change = +373 ± 174%, $p < 0.001$ vs. alive-BNP change = −1.06 ± 9.60%, $p > 0.05$). Post-exercise, the absolute and percentage change in ANP was higher in dogs that died, but did not achieve statistical significance (died-post exercise ANP = 1.39 ± 0.26 nmol/l vs. alive post-exercise ANP = 0.584 ± 0.076 nmol/l, $p > 0.05$; died-ANP change = 89.5 ± 55.1% vs. alive-ANP change = 13.1 ± 7.9%, $p > 0.05$). Baseline cTnI tended to be higher in dogs that died (died-baseline cTnI = 0.12 ± 0.05 ng/ml vs. alive-baseline = 0.07 ± 0.03 ng/ml; $p > 0.05$). Post-exercise BNP concentration predicts clinical outcome in dogs with moderate to severe SAS. Study limitations included the small patient population and brevity of follow-up.

ABSTRACT #84

ELEVATED SERUM CARDIAC TROPONIN I LEVELS IN BOXER DOGS WITH ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY. Baumwart RD, João Orvalho*, Meurs KM** The Ohio State University, Columbus, Ohio,*University of California Davis, Davis California ** Washington State University, Pullman, Washington.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) has been described in Boxer dogs. We hypothesized that ARVC Boxers would have elevated cardiac troponin I (cTnI) given the histopathological findings of myocytolysis, myofiber degeneration, and myocyte atrophy seen with ARVC.

Three groups of dogs were studied, ARVC (n = 10), control Boxer (CB) (n = 10), and control non-Boxer (CNB) (n = 10). We defined ARVC by ≥1000 ventricular premature complexes (VPC)/24 hours on ambulatory electrocardiogram (AECG) and normal left ventricle (LV) echocardiographic parameters fractional shortening and LV internal dimension. Group CB was defined by the presence of ≤5 VPCs/24 hours and normal echocardiogram. Group CNB was defined by normal echocardiogram. Serum was collected and stored at −80°C for batch analysis on the Unicel DXI with a minimum detection level of 0.01 ng/ml. One way ANOVA was performed

to detect differences in age, weight, echocardiographic parameters, and cTnI concentrations. Spearman's correlation was performed for VPCs/24 hours on AECG and cTnI for the Boxer populations. Significance was $\alpha \leq 0.05$.

The VPC/24 hours (median (range)) was: ARVC group 2375 (1157–24057) and CB group 2 (0–5). The cTnI concentrations (mean (+/− standard deviation)) for the three groups: ARVC 0.142 ng/ml (+/− 0.05), CB 0.079 ng/ml (+/−0.03), and CNB 0.023 ng/ml (+/− 0.01). A significant difference for serum cTnI ($p < 0.001$) was identified for the three groups. A significant correlation ($r = 0.78$) was identified for cTnI and number of VPCs/24 hours ($p < 0.0001$). We hypothesize the elevation of cTnI in ARVC Boxers is likely due to chronic, low level, and persistent myocardial damage.

ABSTRACT #85

STANDARD POODLE NEONATAL ENCEPHALOPATHY LOCUS MAPS TO CANINE CHROMOSOME 36. DP O'Brien[1], X Chen[1], GS Johnson[1], S Khan[1], T Awano[1], RD Schnable[2], and JF Taylor[2]. [1]University of Missouri, College of Veterinary Medicine, Columbia MO. [2]University of Missouri, College of Agriculture, Food, and Natural Resources, Columbia MO.

Neonatal Encephalopathy (NE) is a fatal encephalopathy of Standard Poodles characterized by developmental delay, progressive ataxia and intractable seizures. Affected pups die or are euthanized before weaning. The condition segregated in the families as an autosomal recessive trait. We report mapping the NE locus to *Canis familiaris* autosome (CFA) 36.

DNA samples from 18 affected Standard Poodle puppies and 77 close family members were collected and stored at −80°C. A subset of 55 family members were genotyped with a panel of multiplexed microsatellite markers developed by Clark *et al.* (Genomics 84(3):550–4,2004). Linkage analysis was performed as previously described (O'Brien *et al.* J Hered 96(7):727–34,2005) and revealed a maximum two-point LOD score of 2.2 suggesting possible linkage to markers on CFA36. We genotyped the entire Poodle family with the five CFA36 markers in the Clark panel plus four additional CFA36 microsatellite markers. This produced a maximum two-point LOD score of 3.6, confirming that CFA36 contains the causative mutation. Recombinations between the markers and the mutant locus narrowed the target region to an 8.7 Mb segment of CFA36 between markers REN179H15 and REN252E18.

ABSTRACT #86

CNS HYPOMYELINATION IN A LITTER OF RAT TERRIERS WITH GOITROUS CRETINISM AND A MUTATION IN THE THYROID PEROXIDASE GENE. R. Pettigrew[1], J. Fyfe[2], G. Brittany[2], D. Lipsitz[3], A. deLahunta[4], B. A. Summers[4], G.D. Shelton[1]. Department of Pathology, University of California, San Diego, La Jolla,CA[1]; Laboratory of Comparative Medical Genetics and Department of Microbiology & Molecular Genetics, College of Veterinary Medicine, Michigan State University, East Lansing, MI[2]; Veterinary Specialty Hospital, San Diego, CA[3]; Department of Biomedical Sciences, Cornell University, Ithaca, NY[4].

Arrested physical and mental development was identified in 3 of a litter of 5 Rat Terrier puppies evaluated at 9 weeks of age. Bilaterally firm symmetrical masses were palpated in the region of the thyroid glands. Spinal radiographs revealed delayed epiphyseal maturation when compared to the unaffected puppies. Low total levothyroxine and free levothyroxine (FT4 by equilibrium dialysis) and markedly elevated thyroid stimulating hormone supported the diagnosis of hypothyroidism. At necropsy, the thyroid gland was grossly enlarged and histopathology confirmed a hyperplastic goiter. In the CNS, myelin deficiency limited to the cerebrum, was grossly apparent. A regional distribution of myelin depletion was confirmed histologically affecting the tracts of the corpus callosum, the pyramids, the corona radiata, the longitudinal fibers of the pons, and also the lateral funiculi of the spinal cord. Myelin reduction was paralleled by axon reduction indicating that myelin depletion was a consequence of reduced axonal formation. A nonsense mutation in the thyroid peroxidase gene was identified in the affected puppies. Both clinically normal parents and one clinically normal littermate were heterozygous for this mutation, and thus carriers. The data confirmed inheritance as a simple autosomal recessive trait. The same mutation was previously described in the Toy Fox Terrier breed. Given the historical and ongoing practice of using Toy Fox Terrier breeding stock in some Rat Terrier breeding programs to obtain a smaller stature, it is highly likely that this mutation crossed into the Rat Terrier breed from the Toy Fox Terrier fairly recently.

ABSTRACT #87

CHARACTERIZATION AND MODE OF INHERITANCE OF AN EPISODIC DYSKINESIA IN THE CHINOOK DOG. RA Packer[1], DP O'Brien[1], JR Coates[1], GS Johnson[2]. [1]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO; [2]Department of Veterinary Pathobiology, University of Missouri, Columbia, MO.

Episodic dyskinesias (EDs) are episodes of abnormal movement or tone distinguished from seizures by the character of the episode and a lack of seizure activity on ictal EEG. We report a familial ED in Chinook dogs.

Pedigrees and medical histories were reviewed for 289 Chinooks. A family of 173 dogs was used for analysis. Episodes were classified as seizures, ED, or unknown based on the owner's description or videotapes, and segregation analysis performed.

ED was identified in 32 dogs and characterized by inability to stand or ambulate, head tremor, and involuntary flexion of one or multiple limbs, without loss of consciousness or autonomic signs. Episode duration was variable, ranging from seconds to hours. Interictal EEGs recorded on two dogs with ED were normal. Four dogs had generalized tonic-clonic seizures, 3 had ED and generalized tonic-clonic seizures, and 10 had episodes of undetermined type.

ED segregation was consistent with an autosomal recessive trait. There was no sex predilection, and affected individuals were born to unaffected parents. In litters where status of littermates was known, 23.7% of offspring were affected (23 out of 97 individuals). Linkage analyses are currently underway.

This movement disorder is prevalent in the Chinook breed. The episodes are most likely an ED of basal ganglia origin, but atypical seizures and muscle membrane disorders remain possible etiologies. The generalized seizures may be a variant phenotype of the same mutation that results in the ED, or the two syndromes may be independent.

ABSTRACT #88

INHERITED ENCEPHALOMYELOPATHY AND POLYNEUROPATHY IN TWO BOXER LITTERMATES. DA Geiger[1], SJ Schatzberg[1], K Cutter-Schatzberg[1], NJ Sharp[1], RC Riis[2], A deLahunta[2]. [1]Canada West Veterinary Specialists (CWVS), Vancouver, BC, Canada. [2]Cornell University College of Veterinary Medicine, Ithaca, NY.

Two six-month-old Boxer littermates (one male and one female) were referred independently to CWVS for evaluation of progressive pelvic limb paresis and ataxia, inspiratory stridor, and visual deficits. Physical exam of both dogs confirmed inspiratory stridor, suggestive of laryngeal paralysis. Ocular exam disclosed microophthalmia, bilateral cataracts, and multifocal linear retinal folds. Neurological exam revealed upper motor neuron paresis and general proprioceptive quality ataxia in the pelvic limbs. Postural reactions were delayed in the pelvic limbs and equivocal in the forelimbs. Cranial nerve exam was normal. Neuroanatomic localization was considered multifocal (CNS and PNS) based on the thoracolumbar (+/− cervical) myelopathy and the high index of suspicion for laryngeal paralysis. A similar clinical phenotype has been described in young Rottweilers, previously referred to as neuronal vacuolation and axonal degeneration. The purpose of this report was to investigate the underlying neuropathology in the Boxer dogs and to compare it to the previously described Rottweiler disorder.

Investigative methods included gross post-mortem and histopathologic examination of both dogs. Neuropathological results included neuronal vacuolation of brainstem nuclei, diffuse axonal loss in the spinal cord, denervation atrophy of the dorsal cricoarytenoid muscles, microophthalmia, congenital cataracts, and retinal dysplasia. We conclude that the neuropathological and ocular lesions identified in the Boxer puppies are nearly identical to those of the condition previously described in Rottweilers. We suggest that this novel Boxer phenotype (along with the Rottweiler disorder) is best referred to as a combined encephalomyelopathy and polyneuropathy.

ABSTRACT #89

NF-2 GENE EXPRESSION IN CANINE MENINGIOMAS. M Campbell, PJ Dickinson, B Roberts, CM Leutenegger, RA LeCouteur. University of California, Davis, School of Veterinary Medicine, Davis, CA.

Meningiomas are reported to be one of the most common brain tumors in dogs, accounting for up to 50% of all reported cases, and carry a poor long term prognosis. Development of novel targeted therapies in human medicine has been based on characterization of these tumors at the molecular level. Of particular interest is the identification of decreased neurofibromatosis type 2 (NF-2) gene function in both familial (neurofibromatosis) and up to 60% of sporadically occurring meningiomas. The NF2 gene is located on human

chromosome 22q12 and encodes a 595 amino acid protein termed merlin or schwanomin. Studies in human meningiomas have found significantly lower merlin levels in specific tumors, particularly in transitional and fibroblastic subtypes compared to meningothelial subtypes. Targeted therapies for meningiomas based on either restoration of NF2 gene function or intervention in NF2 associated pathways will require the identification of subtype specific alterations in both human and canine meningiomas.

The aims of this study were to determine the level of NF-2 gene expression in normal canine brain tissue and in differing subtypes and grades of spontaneous canine meningiomas, and to determine whether there was a correlation with tumor type or grade.

Canine specific NF2 cDNA sequence was obtained by PCR using primers derived from human NF-2 sequence and canine normal liver and brain cDNA. Expression of NF-2 transcripts was determined in 29 archival, frozen meningioma samples by semiquantitative real time TaqMan® RT-PCR using both 5′ (exon 2) and 3′ (exon 12) primers. Transcription levels were calculated relative to normal meninges (arachnoid), cerebrum and grey matter (caudate). Protein expression was assessed in a subset of samples (14) using western blotting.

Canine NF-2, 5′ and 3′ cDNA product was amplified from both normal brain and liver, and tumor samples. and were found to have 94.2% and 93.6%, and 92.8% and 90.1% identity to human and mouse NF-2 sequence respectively. No significant decrease or increase in NF-2 transcription was seen relative to normal tissues looking at all tumor samples as a group. No specific pattern of transcript expression was seen relative to tumor grade or subtype. Several of the tumor samples had decreased expression of NF-2 protein based on western blotting, however no clear association was found with tumor grade or subtype.

Based on this preliminary study, NF-2 is expressed in both normal brain, and spontaneous meningiomas of varying grade and subtype in dogs. Although some tumors appear to have decreased expression at the protein level, there does not appear to be the distinct pattern of expression typically seen in human meningiomas. Analysis of additional samples will be necessary to further characterize the prevalence and importance of NF-2 gene expression in canine spontaneous meningiomas.

ABSTRACT #90

RETROSPECTIVE EVALUATION OF TETANUS IN DOGS: 38 CASES. JM Burkitt, BK Sturges, KE Jandrey. University of California, Davis, CA.

The purpose of this study is to report the clinical course and outcome of tetanus in dogs. Records from 1987–2005 were reviewed for signalment; wound characteristics; presenting clinical signs; time and severity of worst clinical signs; time to wound management, antibiotic therapy, and antitoxin administration; and 28-day survival.

Thirty-eight dogs were included. The most common initial clinical signs were ocular (18) and facial (11) abnormalities. The first day of the most severe clinical signs ranged from Day 0–14 (median Day 4). Nineteen of 38 dogs progressed to recumbency with severe muscle spasms; 14 had abnormalities of heart rate, blood pressure, and/or rectal temperature consistent with autonomic derangement. Seven dogs, six with autonomic derangement, died or were euthanized for complications of tetanus.

There was a statistically significant association between younger age and development of more severe clinical signs ($P = 0.04$). Dogs with surgical wounds tended to have a more severe clinical course than those with external wounds ($P = 0.09$), and a significant inverse relationship between development of severe clinical signs and survival ($P = 0.04$) was found. There was no statistical association between earlier wound management, antibiotic administration, or antitoxin administration and either progression of signs or survival.

We conclude that younger dogs with tetanus may be more likely to develop more severe clinical signs. Furthermore, dogs have a good prognosis for survival unless autonomic derangement occurs.

ABSTRACT #91

CLINICOPATHOLOGICAL FEATURES OF CHOROID PLEXUS TUMORS IN DOGS: 44 CASES. DR Westworth, PJ Dickinson, BK Sturges, KM Vernau, MF Knipe, RA LeCouteur. UC Davis School of Veterinary Medicine, Davis, CA.

Choroid plexus tumors are common neuroepithelial brain tumors in dogs. Two major subtypes are recognized, choroid plexus papilloma (CPP) and choroid plexus carcinoma (CPC). Several criteria have been proposed to distinguish CPP and CPC, however at present there is no uniformity in their usage. Advances in surgical, radiological and novel targeted approaches to treatment are likely to result in significant improvement in prognosis, however appropriate management will require specific tumor categorization. Even with stereotactic CT-guided or surgical biopsy, categorization may be difficult due to the small amount of tissue obtained. The purpose of this retrospective study was to determine the clinicopathological features of CPP/CPC that may aid in accurate tumor classification when combined with biopsy results.

Hospital records were reviewed for dogs with a histological diagnosis of CPP/CPC following necropsy. Information collected included signalment, history, tumor location, advanced imaging characteristics and cerebrospinal fluid (CSF) analysis. The definition of CPC was based on presence of local or distant metastasis, local invasion of neuropil, nuclear atypia, anaplasia and high mitotic index.

Data from 44 dogs were analyzed. Eighteen tumors were classified as CPP and 26 tumors as CPC. Similar to previously reported cases, the mean age at presentation was 6–7 years with no apparent breed predilection or overrepresentation of brachycephalic dogs. A predominance of male animals has been reported, however this was not apparent in either CPPs (9 female, 9 male) or CPCs (11 female, 15 male). The majority of tumors originated in the 4th ventricle, as previously reported, however no CPPs were found involving the lateral ventricles. A significant ($p < 0.01$) relationship was found between tumor subtype and location either within the lateral ventricle or 3rd/4th ventricles. Eighteen MR and 9 CT studies were reviewed. No significant differences were apparent between CPP and CPC with advanced imaging other than the presence of metastatic lesions. The majority of tumors were well demarcated, iso/hyper intense on T1 weighted MR images and hyperintense on T2 weighted MR images. All tumors had strong contrast enhancement either heterogeneously or homogenously. All tumors enhanced on CT images following intravenous contrast administration. CSF analysis was outside reference ranges in all cases, with the most striking finding being an elevation of total protein (mean 103 mg/dl, range 27–366 mg/dl). A significant ($p < .01$) relationship was found between tumor subtype and an elevated total protein of >100 mg/dl (CPP 1/8; CPC 8/11).

Results of this study suggest that tumor location, presence of metastatic lesions on advanced imaging studies and CSF analysis may aid in the presumptive classification of canine choroid plexus tumors antemortem.

ABSTRACT #92

SPINAL CORD DISEASE IN DOGS WITH NEOPLASIA ARISING FROM THE CENTRAL NERVOUS SYSTEM. SA Petersen, BK Sturges, KM Vernau, MF Knipe, PJ Dickinson, RJ Higgins, RA LeCouteur. University of California at Davis, Veterinary Medical Teaching Hospital, Davis, CA.

Spinal cord (SC) neoplasia is a common cause of pain and neurological dysfunction in dogs. It is important to be able to differentiate different types of spinal cord tumors from each other and from other causes of SC disease since prognosis and treatment can vary considerably. The purpose of this study was to determine the types and locations of primary CNS tumor types affecting the spinal cord of dogs, evaluate appearance of tumors on advanced imaging studies, and evaluate efficacy of treatment and average survival times following diagnosis.

Hospital records were reviewed for animals with a histological diagnosis of primary spinal cord neoplasia. Information was correlated relating to signalment, history, neuroanatomic localization, diagnostic imaging, treatment and survival time.

Sixty-seven cases were identified: 39 meningiomas (60%), 10 choroid plexus tumors (15%), 4 astrocytomas (6%), 4 ependymomas (6%), 2 oligodendrogliomas (3%), 2 nephroblastomas (3%), 2 primitive neuroectodermal tumors (3%), 2 undifferentiated gliomas (3%), 1 epithelial cell tumor (1%), and one undifferentiated spinal cord tumor (1%).

The majority of spinal meningiomas were located in the cervical region (29/39). The average age at diagnosis was 9 years (range 5–14 years). Meningiomas were easily apparent as uniformly enhancing contrast enhancing masses on MR imaging. In some cases, myelography was more effective in determining the intradural location of the tumors than MRI. Cytoreductive surgery, with and without adjunctive radiation, was done in 26 dogs. Five dogs were euthanized intra-operatively; 3 dogs did not improve or were worse neurologically following surgery and 18 dogs improved neurologically. The average survival time for dogs that were not euthanized intra-operatively was greater than a year. All untreated dogs were euthanized, with an average survival time of 15 days after diagnosis.

All choroid plexus tumors had metastasized from the brain, however in four cases, the dogs presented for neurologic signs that localized clinically to the spinal cord. Of these, one localized to cervical region and 3 to the TL region of the spinal cord. The average age at the time of diagnosis was 7 years and all dogs were euthanized within 3 weeks of diagnosis (average 5 days).

The average age at diagnosis of the remaining 18 intramedullary tumors was 4 years (range 3 months to 12 years). The majority of tumors were found in the T3-L3 region (11/18). All but one dog was euthanized within 6 weeks of diagnosis (average 5 days). The one exception, a dog with nephroblastoma, survived 5 years following surgical removal of the tumor and radiation therapy before euthanasia due to a radiation induced sarcoma.

Based on this study, the most common primary CNS tumor type affecting the canine spinal cord is the meningioma. Prognosis for this tumor type is favorable with cytoreductive surgery with or without radiation therapy. It is not known whether surgical excision, and/or radiation therapy are indicated for other CNS spinal cord tumors in dogs.

ABSTRACT #93

CEREBROSPINAL FLUID GLUTAMATE LEVELS IN DOGS WITH INTRACRANIAL NEOPLASIA. SR Platt[1], D Marlin[2], N Smith[2], D Flack[3], A De Stefani[1], L Wieczorek[1], V Adams[3]. [1]Centre for Small Animal Studies, [2]Centre for Equine Studies, [3]Centre for Preventative Medicine, The Animal Health Trust, Newmarket, Suffolk, UK.

Cerebrospinal fluid (CSF) glutamate levels may reflect central nervous system (CNS) glutamate-mediated excitotoxicity and may be involved with the pathogenesis of CNS neoplasia and associated seizure activity. Although CSF glutamate levels have been evaluated in dogs with epilepsy and spinal disease, the alterations of this excitatory neurotransmitter in the presence of cerebral neoplasia have not been documented. The objective of this study was to compare glutamate levels in the CSF of dogs with cerebral neoplasia to those with a normal central nervous system.

Twnety-three dogs with intracranial neoplasia (Meningiomas - group Ia; intra-axial tumours - group Ib) and 21 dogs with no evidence of intracranial disease, based upon magnetic resonance imaging (MRI) and CSF analysis (group II), were included in the study. Histological tumor type and the presence of clinical seizure activity were recorded for group I dogs. All dogs had a CSF tap performed at the cisterna magna under general anesthesia after an MRI; 0.3 ml of CSF from each dog was stored in fluoride oxalate containers and randomly assigned to -20 or $-80^{\circ}C$ freezers until analysis. CSF samples (100 ul) were deproteinised with methanol (100 ul) and centrifuged at 14,000 g for 2 minutes. Glutamate analysis was performed using high performance liquid chromatography with fluorescence detection. Derivatised amino acids were resolved by binary gradient elution. Glutamate levels were compared using non-parametric Kruskal-Wallis one-way analysis of variance with post-hoc comparisons of mean ranks and Wilcoxon rank sum tests. Significance was set at $P \leq 0.05$.

Comparison of the glutamate levels indicated that there was a difference in the three groups of dogs ($P = 0.001$). Median glutamate levels were significantly higher in dogs with brain tumors (4.77 μmol/L) compared to the control dogs (3.05 μmol/L). There was no difference in median glutamate levels between dogs with meningiomas (5.65 μmol/L, $n = 11$) and dogs with other brain tumors (4.73 μmol/L, $n = 12$); group Ib tumors included gliomas and metastatic disease. There was no difference in glutamate levels with seizure activity ($P = 0.4$). There was also no effect of freezer storage temperature on results ($P = 0.7$).

The results of this study suggest that glutamate-mediated excitotoxicity may be involved in the pathogenesis of CNS neoplasia in dogs but may not be involved in the pathogenesis of associated seizure activity. Glutamate elevation is a potential future treatment target for dogs with CNS neoplasia.

ABSTRACT #94

COMPUTER-ASSISTED MRI ANALYSIS OF THE CEREBELLUM IN AMERICAN STAFFORDSHIRE TERRIERS WITH CEREBELLAR CORTICAL DEGENERATION. D. Henke, T. Flegel, P. Böttcher. Klinik für Kleintiere, Universität Leipzig, Leipzig, Germany.

The diagnosis of cerebllar cortical degeneration in American Staffordshire terriers is largely based on excluding other lesions and on subjective recognition of abnormalities regarding cerebellar size and CSF content on brain MRI images. MRI interpretation may vary depending on the observer especially in cases with mild changes or in the initial phase of the disease. Therefore, we tried to establish a more objective method of MRI analysis to diagnose or rule out cerebellar cortical degeneration in individual patients with a high degree of certainty.

Cerebellar cortical degeneration ultimately results in deepening of foliae and decrease in total cerebellar size. The result is additional space within and around the cerebellum filled with cerebrospinal fluid (CSF). Computer assisted MRI analysis was used to measure relative cerebellar size, amount of CSF around the cerebellum, and amount of CSF within the cerebellum.

Neurologically normal American Staffordshire terriers and such with typical clinical signs of cerebellar cortical degeneration ($n = 11$ each) were included in this prospective study. Other underlying pathologies were ruled out performing brain MRI studies, CSF analysis, CBC, blood biochemistry, fT4 and TSH measurements as well as follow up examinations.

Brain MRI scans were performed using a 0.5 Tesla superconducting magnet. T2 weighted sagittal images were obtained in 256×256 matrix with a slice thickness of 3 mm. On midline images, cerebellum with and without surrounding CSF, and the entire brain were manually extracted. The following parameters were measured: 1. relative cerebellar size, 2. cerebellum surrounding CSF space, 3. cerebellar CSF index. The CSF index was calculated from a grey scale histogram of the cerebellum based on automatic histogram analysis.

The results of relative cerebellar size and the cerebellum surrounding CSF space were different between groups of neurologically normal dogs and such with cerebellar cortical degeneration using a non-parametric test. Cerebellar CSF index did not show any difference between both groups. In addition, cerebellar size and the cerebellum surrounding CSF space were able to differentiate in an individual patient between normal and cerebellar cortical degeneration. Both tests are characterized by the following statistical parameters: 1. relative cerebellar size: sensitivity 99%, specificity 88% at a cut-off of 14% 2. cerebellum surrounding CSF space: sensitivity 87%; specificity 99% at a cut-off of 13.5%.

Relative cerebellar size and cerebellum surrounding CSF space may be used to differentiate between a normal American Staffordshire terrier and such with cerebellar cortical degeneration.

ABSTRACT #95

MORPHOLOGY OF THE CAUDAL FOSSA IN CAVALIER KING CHARLES SPANIELS. S Cerda-Gonzalez[1], NJ Olby[1], TP Pease[1], S McCullough[2], N Massoud[2], R Broadstone[2] [1]North Carolina State University, Raleigh, NC; [2]IAMS Pet Imaging Center, Raleigh, NC.

Chiari-like malformations and syringohydromyelia (SHM) make up a disease complex recognized in Cavalier King Charles Spaniels (CKCS) with a world-wide distribution, complex mode of inheritance and serious health implications. Abnormalities in caudal fossa morphology are considered major contributors to the development of Chiari-like malformations in this breed. Recognizing that little information exists on the range of caudal fossa morphologies in CKCS and on the relationship of specific anatomical components to clinically evident disease, the aim of this study was to evaluate the caudal fossa morphology in a group of normal and affected CKCS.

MRI images were obtained in 11 clinically affected and 48 unaffected CKCS, and in 5 control dogs of different breeds using a Siemens AG 1.5 T MRI. Ages ranged from 1 to 5 years. Neurological abnormalities were clinically graded between 0 and 5. Dogs were anesthetized and sagittal T2-weighted image sequences of the brain and cervical spine, along with T2 3-dimensional SPACE sagittal images of the same area were obtained. Parameters assessed included height of the foramen magnum, presence of occipital dysplasia and of SHM, degree of cerebellar herniation and of cerebellar indentation and hydrocephalus. Any other abnormalities were also noted. The volumes of the caudal fossa and the forebrain were measured and the volume of the caudal fossa was expressed as a percentage of total brain volume. Each of these parameters was correlated with neurological grade and presence of SHM using ANOVA (p values of <0.05 considered significant).

Fifty-one of the CKCS were classified as morphologically abnormal; 22 of these had SHM. Thirteen dogs with SHM did not have clinical signs; 2 dogs with clinical signs did not have SHM. Observed morphologic abnormalities included mild to marked cerebellar herniation and occipital dysplasia (50/59), medullary kinking (39/59), cerebellar crowding and indentation (55/59) and a dorsal compressive lesion at the level of the first and second cervical vertebral junction (12/59). The dorsal compressive lesion lay immediately cranial to the syrinx in several cases. Clinically affected dogs were more likely to have SHM than unaffected dogs, and the ratio of the caudal fossa volume to the total brain volume was significantly smaller in affected dogs. In contrast, the presence of SHM was not significantly associated with the relative size of the caudal fossa. No single abnormality was predictive of the presence of SHM or of clinical status.

In conclusion, the incidence of caudal fossa and cervical spinal abnormalities is high in CKCS showing clinical signs of Chiari malformation and in unaffected CKCS. The pathogenesis of this disease appears multifactorial rather than due to a single malformation. Follow up of these cases over the next three years will enable us to establish whether these abnormalities predict future development of clinical signs.

ABSTRACT #96

CHARACTERISTICS OF CEREBROSPINAL FLUID FLOW IN CAVALIER KING CHARLES SPANIELS. S Cerda-Gonzalez[1], NJ Olby[1], TP Pease[1], S McCullough[2], N Massoud[2] R Broad-stone[2]. [1]North Carolina State University, Raleigh, NC; [2]IAMS Pet Imaging Center, Raleigh, NC.

Changes in CSF flow are thought to cause syringohydromyelia (SHM) associated with Chiari-like malformations. Anatomical changes in the caudal fossa restrict CSF passage and result in high velocity jets and turbulence, causing syrinx formation. In humans, Phase Velocity Cine Magnetic Resonance Imaging (PVC MRI) has become the standard technique for evaluating CSF flow patterns and velocities in patients with Chiari malformations. PVC MRI has been used to measure CSF flow in Cavalier King Charles Spaniels (CKCS) with SHM[1], but information available is limited. This study evaluated CSF flow in a group of affected and unaffected CKCS.

Fifty nine CKCS and 5 control dogs were evaluated. Neurological deficits were graded from 0–5. Routine T2-weighted and 3-dimensional T2-weighted SPACE sagittal image sequences of the brain were obtained using a Siemens 1.5 T MRI to note the presence of caudal fossa abnormalities. PVC MRI was used, along with Flow Quant Syngo, Argus Viewer and Argus Flow software, to evaluate patterns and peak velocities of CSF flow just caudal to the foramen and at the level of the C2–C3 intervertebral disc space. The effect of head position on CSF flow was assessed. Flow characteristics were correlated with clinical status and morphological abnormalities.

CSF flow measurements were successfully obtained from all dogs. Peak CSF flow velocities in dogs were lower than those reported in humans and were affected by head position. Positioning the head to simulate a normal standing position increased CSF flow in the dorsal subarachnoid space (SAS) and allowed more consistent measurement within this area. CSF flow was obvious within syrinxes, and in some cases the peak velocity was highest in the syrinx. Turbulent flow was mainly present in dogs with SHM and was detected at the level of the foramen magnum, cervical spinal cord, and within syrinxes. Preliminary statistical analysis did not reveal a difference in peak CSF flow velocities between affected and normal CKCS in the areas analyzed, although a trend towards higher peak CSF velocity was noted within the dorsal SAS at the level of the foramen magnum in affected dogs.

In conclusion, CSF flow patterns and velocities are affected by head position in dogs. Turbulent flow occurs in dogs with SHM and can be found within syrinxes. CSF flow velocity may be higher within the dorsal subarachnoid space of affected dogs although additional studies are needed to determine whether this finding is significant. A longitudinal study of the long-term clinical outcome of the dogs imaged will be conducted. The data will be reanalyzed in the light of these findings.

1. March PA, Abramson CJ, Smith M, et al. CSF flow abnormalities in caudal occipital malformation syndrome (abstr.). J Vet Intern Med 2005;19:418.

ABSTRACT #97

ONE-YEAR MAGNETIC RESONANCE IMAGING FOLLOW-UP OF DOGS WITH CERVICAL SPONDYLOMYELOPATHY. Ronaldo C. da Costa, Joane Parent. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

There is no long-term magnetic resonance imaging (MRI) follow-up of dogs with cervical spondylomyelopathy (CSM) in the veterinary literature. The authors prospectively investigated 12 dogs with CSM repeating a MRI study approximately one year after the initial MRI.

Three out of 12 dogs were treated surgically (ventral slot) and nine were treated conservatively. The first and second MRIs were evaluated for the location of the lesion(s) and spinal cord signal changes.

The mean clinical and MRI follow-up period was 14.5 months (12–18 months). MRI of the surgically treated dogs revealed adequate spinal cord decompression in all 3 dogs, with minimal cord compression seen in one dog. Two out of the 3 surgically treated dogs had new cord lesions not observed on the first MRI; one of these dogs developed 3 new cord compressions. Spinal cord signal changes were seen in 2 dogs before surgery and both dogs developed newer signal changes at the same or adjacent sites. In the conservatively treated dogs, the severity of spinal cord compression remained unchanged in 4 out of 9 dogs, and it worsened in 2 dogs. Three out of 9 dogs appeared to have regression of the spinal cord compression in the sagittal images, but the transverse images revealed cord atrophy. Only one out of 9 dogs developed a new area of spinal cord signal changes.

The findings of this study indicate that ventral slot decompression may hasten the development of newer spinal cord compressions and lesions. The incidence and the rate of development of new lesions were inferior in conservatively treated dogs.

ABSTRACT #98

CERVICAL SPONDYLOMYELOPATHY IN DOGS: COMPARISON OF CONSERVATIVE AND SURGICAL TREATMENTS – 104 CASES. Ronaldo C. da Costa, Joane Parent, David Holmberg, Diana Sinclair, Gabrielle Monteith. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

Cervical spondylomyelopathy (CSM) can be treated conservatively or surgically. Conservative treatment has been considered ineffective, providing only short-term benefits. However, there are no studies investigating the efficacy of conservative treatment or comparing it with surgery.

Medical records were searched for cases of canine CSM. Inclusion criteria included a definitive diagnosis achieved by myelography or MRI, and a minimum follow-up of six months.

Follow-up information was collected using telephone interviews and/or recheck examinations.

One hundred and four dogs met the inclusion criteria and were analyzed. Fifty-four dogs were Dobermans and 50 belonged to other breeds. Among the Dobermans, 23 dogs were treated surgically and 31 dogs conservatively. Among the other breeds, 14 dogs were treated surgically and 36 conservatively. The mean follow-up period was 30 months (6–96 months) for conservatively treated dogs and 32 months (6–156 months) for surgically treated dogs.

In the surgically treated group, 81% of dogs improved, 2% stayed the same, and 17% worsened. Among the conservatively treated dogs, 53.5% of dogs improved, 26.5% of dogs stayed the same, and 20% of dogs worsened. Ordinal logistic regression revealed no difference ($P = 0.10$) between groups.

Lifetest procedure (SAS) was used to analyze the survival period. Conservatively treated dogs (43 dogs) had a mean survival of 46.5 months, and surgically treated dogs (33 dogs) a mean survival of 48.2 months. Log rank test revealed no difference between groups ($P = 0.646$).

The results indicate that surgical treatment offers a higher chance of improvement than conservative therapy (although not significantly different), but it does not provide a longer life expectancy. Conservative treatment improved or stabilized the clinical condition in 80% of patients with a long-term follow-up. These findings indicate that conservative therapy can be effectively used as an alternative for surgery in dogs with CSM.

ABSTRACT #99

USE OF ELECTROHYDRAULIC SHOCK-WAVE LITHOTRIPSY FOR THE FRAGMENTATION OF BLADDER CALCULI: A PILOT STUDY IN DOGS. A Defarges, M Dunn. Faculté de Médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC, Canada.

In veterinary medicine, the treatment of bladder calculi usually requires surgery. Electrohydraulic shock-wave lithotripsy (EHSWL) is currently used in humans to fragment bladder stones. Few reports exist regarding the use of EHSWL in dogs. The objective of this study was to evaluate the efficacy and safety of EHSWL to treat bladder stones in dogs.

This prospective study evaluated dogs referred for bladder stones. Evaluation consisted of: physical examination, biochemistry, haematology, urology, urine bacteriology, and urinary tract ultrasonography. EHSWL was performed by passing an electrohydraulic lithotriptor through an endoscope introduced into the bladder through the urethra. Dogs were rechecked (urinalysis and ultrasonography) 1, 3 and 6 months following the procedure.

Four spayed females and four castrated males, weighing 6–16 kg, were included. The average number of stones per dog was 5. Stone size ranged from 4–17 mm. Calcium oxalate were present in 5 cases, struvite in 1, and silicate in 2. Fragmentation was successful in six dogs with a median procedure length of 106 minutes. In three dogs, a cystotomy was required. In one female weighing 2.5 kg, the urethra was too narrow to pass the scope. In two other dogs, the calcium oxalate stones were large and smooth preventing good probe-stone contact. Two of the six dogs treated successfully by lithotripsy presented hematuria and pollakiuria for five days. One dog had stone recurrence 7 months post lithotripsy.

EHSWL may be a clinically relevant technique for treatment of bladder stones in dogs. A large scale study is currently underway.

ABSTRACT #100

QUALITATIVE CHANGES IN PROTEINURIA CORRELATE WITH CHANGES IN KIDNEY STRUCTURE AND FUNCTION AS X-LINKED HEREDITARY NEPHROPATHY (XLHN) PROGRESSES IN AFFECTED MALE DOGS. Paola Lazaretti,[1] Anne Bahr,[1] Jörg M. Steiner,[1] Clifford E. Kashtan,[2] and George E. Lees.[1] [1]Texas A&M Univ. College Station, TX, and [2]Univ. of Minn., Minneapolis, MN.

Male dogs with XLHN have progressive renal disease that is induced by a genetic defect in type IV collagen, which is a glomerular basement membrane (GBM) component. Although the primary renal lesion is a GBM abnormality, as in other glomerular diseases, progression of XLHN to end-stage chronic renal failure is associated with severe structural damage to the tubulointerstitium, as well as to glomeruli. Seeking to identify potential urinary biomarkers of disease progression, we performed this study to correlate the severity of structural renal changes with qualitative changes in proteinuria, as detected by sodium dodecyl sulphate polyacrilamide gradient gel electrophoresis (SDS-PAGE), throughout the course of XLHN in affected dogs.

A standardized protocol was used to monitor clinical disease progression in 18 male dogs with XLHN; 5 normal males were also studied (controls). Normalized albumin (nUAlb) concentration, urine protein-to-creatinine (UPC) ratio, and serum creatinine (SCr) concentration were determined weekly; glomerular filtration rate (GFR) was evaluated monthly by scintigraphy; and ultrasound-guided needle biopsies were obtained at 2, 4, 6, 9, and 12 months of age. Dogs were euthanized when their SCr $\geq$5.0 mg/dL. Cortical interstitial volume fraction (VvI/C) was determined for each biopsy by point-counting digital images of stained sections. Urine stored frozen at -80°C from each of the weeks when a monthly GFR was performed were subsequently analyzed by SDS-PAGE. All urine samples from each dog were run side-by-side on one gel, which was stained with Comassie blue and digitized. Image analysis software (Bionumetrics® 3.0) was used to identify the protein bands by their molecular weight, and the number of high molecular weight (HMW; $\geq$57 kDa) and low molecular weight (LMW; $<$57 kDa) bands in each urine sample was counted.

In affected dogs, clinical disease progression was divided into 5 sequential stages (0, I, II, III, and IV): 0, normal; I, albuminuria with UPC $<$ 2; II, UPC $\geq$ 2 with SCr $\leq$ 1.3 mg/dL; III, SCr 1.4 to 2.4 mg/dL; and IV, SCr $\geq$ 2.5 mg/dL. In affected dogs compared with controls, VvI/C (mean $\pm$ SEM) was not different in Stages 0 (13.9 $\pm$ 0.7 vs 16.1 $\pm$ 1.2) and I (18.4 $\pm$ 1.8 vs 15.7 $\pm$ 1.7), but was increased modestly in Stage II (21.79 $\pm$ 1.6 vs 14.1 $\pm$ 1.3; $p < 0.05$), and greatly in Stages III (38.9 $\pm$ 2.9 vs 14.9 $\pm$ 2.8; $p < 0.001$) and IV (39.6 $\pm$ 3.2 vs 11.6 $\pm$ 2.0; $p < 0.001$). An increased number of HMW bands/sample (glomerular proteinuria) and an increased number of LMW bands/sample (tubular proteinuria) correlated with VvI/C ($r = 0.563$ and $r = 0.647$, respectively, $p < 0.001$ for both) and clinical disease stage.

We conclude that tubular proteinuria can be a marker of the severity of tubulointersitial damage, which is also associated with severity of azotemia, in dogs with progressive primary glomerular disease.

ABSTRACT #101

PALLIATIVE STENTING FOR MALIGNANT URETHRAL OBSTRUCTIONS IN 13 DOGS. C Weisse[1], A Berent[1], K Todd[1], C Clifford[2], J Solomon[3]. [1]Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA; [2]Red Bank Animal Hospital, Tinton Falls, NJ; [3]Section of Vascular and Interventional Radiology Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA.

Transitional cell and prostatic carcinoma are common malignancies of the canine urethra. Dysuria is described in 40–84% of cases, with nearly 10% developing trigonal/urethral obstruction, and death from local disease in up to 60% of cases. Acute obstruction requires invasive treatment or cystostomy tube placement, often resulting in euthanasia. This study describes a novel minimally-invasive approach to place balloon-expandable (BEMS) and self-expandable metallic stents (SEMS) for the relief of malignant urethral obstructions.

Thirteen dogs with malignant obstructions were included. Under fluoroscopic guidance, guidewires and catheters were used to determine the location, extent and measurements of the malignant urethral obstruction and normal adjacent urethral diameter in order to choose the appropriately sized stent. All stents were placed transurethrally under fluoroscopic guidance and positive contrast cystourethrograms documented luminal patency.

The animals were followed to the time of death. Clinical outcomes were recorded, with urinary incontinence classified into four categories: (1) obstruction, (2) atonic bladder, (3) severe incontinence, and (4) mild incontinence-dribbling immediately before or after urination. Stranguria was evaluated as mild or severe.

Three dogs received BEMS and 10 SEMS. Minor complications were seen in two dogs: temporary obstruction from a clot/urethral edema, and stent dislodgement into the bladder. Post-operative urination was documented in 100%. Post-stenting, 10 (77%) were either continent or mildly incontinent. Two developed severe incontinence and one had an atonic bladder. Eight were considered to have a good-excellent outcome, three fair, and two poor.

Trans-urethral stent placement offers a rapid and effective option and should be considered in dogs with malignant trigonal/urethral obstructions.

ABSTRACT #102

ACCURACY OF URINALYSIS IN PREDICTING THE TYPE OF INFECTING BACTERIA IN URINARY TRACT INFECTION (UTI). J. Barsanti, S. Sanchez, M. Wall, C. Barker. College of Veterinary Medicine, The University of Georgia, Athens, GA.

Examination of urine sediment is a commonly performed component of a urinalysis and is more timely and less expensive than culture. The premise of this study was that by examining urine pH and urine cytology one could predict whether the organism causing UTI was either a rod or a cocci, which either did or did not split urea.

In order to examine this premise, the results of urinalysis were compared to urine culture results in 150 cases of UTI, 141 dogs and 9 cats. All animals were patients of the Veterinary Teaching Hospital. Urinalysis was performed routinely by medical technologists, using standard urine dip sticks and unstained microscopy. Urine cultures were performed routinely by the bacteriology department of the Athens Veterinary Diagnostic Laboratory. Urinalysis and urine culture were performed on the same sample, collected by cystocentesis or catheterization. Cultures were quantitative and qualitative and colony counts had to be $>$100,000/ml for UTI to be diagnosed. Only single organism UTI were included.

Infecting organisms were as follows: E. coli, 81 (54%); Klebsiella, 11; Proteus, 9; Pseudomonas, 6; Enterobacter, 6; Staphylococcus, 14; Enterococcus, 17; Streptococcus, 6. If rods were identified on the urine sediment examination, there was a 92% likelihood that rods would be cultured. If cocci were seen, there was an 80% likelihood that cocci would be cultured. In 15/150 (10%) cases, no bacteria were seen on urine cytology; 10 of these animals were infected with rods (8% of rod infections) and 5 with cocci (12% of coccal infections).

Seventy of the urine samples (47%) were acidic and 58 (39%) were alkaline, with the remaining 20 (15%) being neutral. If the urine was acidic, there was a 93% chance that the organism cultured would be a non-urea splitter and a 61% chance that the organism cultured would be *E. coli*. If the urine was alkaline, there was a 28% chance that the organism cultured would be a Proteus or Staphylococcus sp. If a urea splitter was cultured, the urine was alkaline in 16/23 cases (70%). With non-urea splitters, urine was acidic in 51%, alkaline in 33%, and neutral in 16%.

Other findings were that 61% had $<$10 WBC/hpf, none had gross hematuria, 90% had $<$50 RBC/hpf, 77% had a significant proteinuria (any protein with a specific gravity $<$1.030 or $>$ trace in a specific gravity $>$1.030). The range of urine specific gravity was 1.002 to 1.055 with a mean of 1.023 and a median of 1.022.

Although medical technologists are fairly accurate in determining if bacteria are rods or cocci on urine microscopy, urinalysis is not accurate in otherwise determining the type of organism causing UTI.

ABSTRACT #103

EFFECT OF DIETARY SODIUM ON URINE CHARACTERISTICS IN HEALTHY ADULT CATS. [1]H Xu, [1]DP Laflamme, [2]JW Bartges, [1]GL Long. [1]Nestle Purina PetCare, St. Louis, MO; [2]University of Tennessee, Knoxville, TN.

Increased urine volume and decreased concentration of calculogenic materials are important in managing cats with crystal-associated lower urinary tract diseases. The objective of this study was to measure the impact of different concentrations of dietary sodium on water intake, urine volume, urine specific gravity, mineral excretion, relative supersaturation (RSS) and activity product ratios (APR) of calcium oxalate (CaOX) and magnesium ammonium phosphate (MAP) in cats.

Three diets having identical formulations except for the concentrations of sodium and chloride (0.4, 0.8 and 1.2% sodium, dry basis), were fed to 9 healthy adult cats each using an incomplete cross-over design. Each diet was fed for 2 weeks, and urine was collected during the final 72 hours for determination of specific gravity, volume, pH, mineral and electrolyte excretion, and urinary saturation for struvite and calcium oxalate. Least-squares means analysis of variance was used to detect differences among treatments, with $p < 0.05$ considered significant.

The increase in water intake did not reach statistical significance, however, urine volume (ml/kg/day) increased significantly in cats fed the 1.2% sodium diet. Urine specific gravity also tended to decrease ($p = 0.07$) in cats fed 1.2% sodium. Key urinary measures are shown (table).

	0.4% Na	0.8% Na	1.2% Na	St. error	P value
Water intake (ml/kg/day)	27.7	26.6	32.0	2.6	NS
Urine Volume (ml/kg/day)	14.4	16.7	20.1	1.7	< 0.001
Urine specific gravity	1.056	1.055	1.048	0.030	0.07
Urine pH	6.2	6.2	6.2	0.3	NS
Urine Na (mEq/kg/day)	3.12	3.07	4.19	0.84	NS
Urine Ca (mEq/kg/day)	0.19	0.27	0.23	0.04	NS
Urine RSS, CaOX	4.04	2.97	2.52	0.68	NS
Urine APR, CaOX	6.30	4.76	4.20	1.23	NS
Urine RSS, MAP	0.06	0.06	0.10	0.013	NS
Urine APR, MAP	1.26	1.13	0.79	0.17	NS

Increased sodium intake resulted in greater urine volume, which might be of benefit in the management of many types of lower urinary tract diseases. Increased dietary sodium did not increase calcium excretion in cats, which differs from other species. All 3 diets produced urine that was metastable for CaOX, while urine from cats fed the higher sodium diet also was undersaturated for MAP. Moderately increased dietary sodium may be a viable way to increase urination in cats with lower urinary tract diseases.

ABSTRACT #104

IDENTIFICATION OF THE GENE MUTATION THAT CAUSES AUTOSOMAL RECESSIVE HEREDITARY NEPHROPATHY IN ENGLISH COCKER SPANIELS. Ashley G. Davidson,[1] Rebecca J. Bell,[1] George E. Lees,[1] Clifford E. Kashtan,[2] and Keith E. Murphy.[1] [1]Texas A&M Univ. College Station, TX, and [2]Univ. of Minn., Minneapolis, MN.

An inherited nephropathy that causes juvenile-onset renal failure has been recognized in the English cocker spaniel (ECS) worldwide for more than 50 years. Previous studies have shown that the disorder is a primary glomerular disease that is transmitted in an autosomal recessive fashion and is caused by abnormalities of type IV collagen in the glomerular basement membranes (GBM) of affected dogs. Immunostaining has demonstrated that two specific type IV collagen peptides, α3(IV) and α4(IV), that are present in the GBM of normal dogs are totally absent in the GBM of ECS dogs with autosomal recessive hereditary nephropathy (Lees *et al.*, Kidney Int, 1999). Thus, *COL4A3* and *COL4A4*, the genes encoding the missing proteins, were incriminated as candidate genes for the disorder. The purposes of this study were to identify the underlying causative mutation and to develop a genetic test for reliable identification of carriers of the disease trait.

Diagnosis of the nephropathy was based on electron microscopy and immunostaining of kidneys obtained from ECS with juvenile-onset renal failure. Kidneys and blood for RNA and DNA isolation, respectively, were obtained from affected dogs for genetic analyses. Blood samples were also collected from the parents of affected dogs (*i.e.*, obligate heterozygous carriers) and from available first-order relatives (*i.e.*, parents or siblings of carriers, and siblings of affected dogs). DNA was extracted from such samples for genetic analysis.

The levels of specific mRNA transcripts for each of the 2 candidate genes were assessed by real-time quantitative RT-PCR in kidney from affected dogs. Transcript levels were reduced for *COL4A4*, but not for *COL4A3*. These findings incriminated *COL4A4* as the more likely candidate gene.

Primers were designed for PCR amplification of the coding region and flanking splice sites from genomic DNA for each of the 47 exons of *COL4A4*. The PCR products were sequenced and the sequences from affected dogs were compared with sequences from normal dogs. This process identified a mutation in *COL4A4* for which all affected dogs were homozygous and all obligate carriers were heterozygous.

We conclude that the identified mutation in *COL4A4* is the genetic cause of autosomal recessive hereditary nephropathy in the ECS. With this information, a method for identifying carriers of the mutation from genomic DNA samples (*e.g.*, cheek swabs) can be devised. Use of the genetic test will permit breeders of ECS to (1) avoid production of any additional affected dogs, and (2) eradicate the recessive trait that causes this fatal disease from the breed without having to terminate any desirable family lines.

ABSTRACT #105

PRENATAL PROGRAMMING AND ARTERIAL HYPERTENSION IN MARMOSET MONKEYS. C Schlumbohm[1], C Bramlage[2], B Egner[3], E Fuchs[1]. [1]German Primate Center, Goettingen, Germany; [2]Faculty of Medicine, Georg-August-University, Goettingen, Germany, [3]Dr. Egner Clinical Research and Continued Education, Babenhausen, Germany.

Epidemiological studies in humans have shown that low birth weight is associated with increased prevalence of hypertension, obesity and metabolic syndrome in later life (1). In experimental studies with rodents and sheep it was demonstrated that prenatal stress either from maternal calorie or protein restriction or from maternal treatment with synthetic glucocorticoids induced low birthweight, hypertension and/or obesity. It is hypothesized that in humans and possibly also in other species hypertension and metabolic disorders have their origin in fetal life. It is not clear, however, whether prenatal exposition to glucocorticoids would have adverse effects on body mass or blood pressure also in other species, like primates.

This problem is investigated in common marmoset monkeys, a small (350 to 450 g bodyweight) South American non-human primate species with a high reproduction rate in captivity. Two groups of pregnant common marmosets were treated orally either during early (“eDEX”; 42–48 days post conceptionem(p.c.).; N = 15) or during late pregnancy “lDEX”; 90–96 days p.c.; N = 15) with 5 mg dexamethasone per kg maternal body weight per day. Dexamethasone, mixed with 1.6 ml concentrate feed (Nutrical®, Albrecht, Germany) was taken voluntary. A control group of 15 pregnant marmosets received vehicle only. In male offspring urine and serum samples are collected and blood pressure is measured oscillometrically (MD-Scientific 1500, S+B medVET, Babenhausen, Germany) in intervals. The unit has been prior compared in animals with implanted direct line. Measurements showed high correlations and accuracy. Direct line was discontinued due to a too high mortality rate.

Prenatal DEX had no effect on birth weight of male marmosets (con: 31.0 ± 3.7 g; eDEX:30.2 ± 2.4 g; lDEX:30.6 ± 3.7 g ; mean ± SD) and on body weight at 320 d of age (con: 350 ± 50 g; eDEX:323 ± 40 g; lDEX:354 ± 53 g ; mean ± SD). Systolic blood pressure was significantly higher in lDEX compared to eDEX ($p < 0.05$, one way ANOVA, Holm-Sidak test) but did not differ from controls (con: 148 ± 9; eDEX:140 ± 16; lDEX:159 ± 23 mm Hg ; mean ± SD). No effect of prenatal DEX was observed on diastolic blood pressure (con: 73 ± 13; eDEX:68 ± 11; lDEX:79 ± 17 mm Hg; mean ± SD). Urinary aldosterone was significantly ($p < 0.05$) elevated in lDEX compared to eDEX and to controls (con: 89 ± 14; eDEX:99 ± 20; lDEX:171 ± 28 ng/ml; mean ± SEM).

Unlike in rodents DEX during foetal development had no influence on bodyweight in marmoset offspring. Elevation of blood pressure and urinary aldosterone in lDEX adolescent marmosets supports the hypothesis that prenatal stress may program foetal tissues for hypertension in later life. It is suggested that the RAAS is involved in this mechanism. (1) Edwards LJ et al., 2001, Prenatal undernutrition, glucocorticoids and the programming of adult hypertension. Clin Exp Pharm Phys 28: 938–941. # Funded by the EU (“EUPEAH”), Contract No.:QRLI-CT-2002-02758

ABSTRACT #106

COMPLEMENT PROTEIN C3, CIRCULATING IMMUNE COMPLEXES AND ANTIBODIES AGAINST *BORRELIA BURGDORFERI* IN BERNESE MOUNTAIN DOGS. B Gerber[1], S Eichenberger[1], MM Wittenbrink[2], HI Joller-Jemelka[3], CE Reusch[1]. [1]Clinic for Small Animal Internal Medicine, [2]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Zurich, Switzerland [3]Clinical Immunology, Department of Internal Medicine, University Hospital Zurich, Switzerland.

Glomerular disease due to Lyme borreliosis is thought to be caused by circulating immune complexes. It has been shown that Bernese Mountain Dogs (BMD) had more often antibodies against *Borrelia burgdorferi (B.b.)* compared to dogs of other breeds. For the defense against infectious agents complement plays an important role and at the same time it has been shown that complement factor C3 deficiency was associated with glomerular disease. The aim of this study was to compare complement C3 protein concentrations and circulating immune complexes in serum of healthy BMD with and without antibodies against *B.b.* and to compare the results with healthy dogs from other breeds and with BMD with potential glomerular disease.

Health status of all dogs was assessed by questionnaire filled out by the owner, complete blood count, chemistry panel, urinalysis and urine culture. Antibodies against *B.b.* were determined using an ELISA with a whole cell sonicate as antigen. The results were confirmed using Western blot. C3 was measured by a single radial immunodiffusion test with goat anti-dog C3. Immune complexes were determined using a C3d ELISA (IgG and IgM). Data were compared by non parametric tests

159 healthy BMD, 61 control dogs and 5 BMD with overt proteinuria (UPC 3.09–21.43) were included. In the control group 8 different breeds and 4 mixed breed dogs were represented. Seroprevalence of *B.b.* in BMD was 58% compared to 16% in control dogs. Median complement C3 diffusion ring diameter was 8.2 mm, 7.6 mm and 6.3 mm in BMD, control dogs and BMD with proteinuria respectively. ELISA for IgG immune complexes revealed median ODs of 1.359, 2.000 and 1.107 and those for IgM immune complexes were 1.040, 1.052 and 1.570 in the respective groups. C3 was significantly lower in control dogs with antibodies against *B.b.* compared to those without. There was no such difference in BMD. C3 was significantly higher in healthy BMD compared to control dogs. IgG immune complexes were significantly higher in control dogs with antibodies against *B.b.* compared to those without There was no such difference in BMD. IgG immune complexes were significantly higher in control dogs compared to healthy BMD. IgM immune complexes were higher in control dogs and BMD with antibodies against *B.b.* compared to those without.

These results indicate that in healthy BMD low complement protein C3 concentrations or large amounts of circulating immune complexes as potential trigger of glomerular disease are not present even though antibodies against *B.b.* are more frequent.

ABSTRACT #107

REDEFINING THE REFERENCE INTERVAL FOR PLASMA CREATININE IN DOGS: EFFECT OF AGE, GENDER, BODY WEIGHT, AND BREED. AJ Craig[1], J Seguela[1], Y Queau[1], P Murgier[1], D Concordet[1], LM Fleeman[2], P Mimouni[3], JP Braun[1], C Germain[1], and HP Lefebvre[1]. [1]Department of Clinical Sciences and UMR 181 INRA-ENVT Experimental Physiopathology and Toxicology, National Veterinary School of Toulouse, France. [2]Centre for Companion Animal Health, School of Veterinary Science, the University of Queensland, Brisbane, Australia. [3]Clinique Veterinaire, L'Isle Jourdain, France.

Plasma creatinine (Pl-Cr) is the most frequently assayed plasma constituent. The same reference intervals (RI) for Pl-Cr are used whatever the body weight, gender, breed, and age of the canine patient. We hypothesize that a single RI for the whole canine population could be inadequate.

Pl-Cr was sampled in 317 purebred dogs from 32 breeds (2–37 dogs per breed) after a 12 hour fast. The dogs were healthy based on physical examination and plasma biochemistry panel. The population comprised 254 female, 2 neutered female, and 61 male dogs. Age ranged from 0.5 to 16 years. Dogs were divided into four body weight (BW) categories: Mini: 0–10 kg ($n = 36$); Medium: 11–25 kg ($n = 130$); Maxi: 26–45 k ($n = 127$); and Giant: >45 kg ($n = 24$). Pl-Cr was assayed using an enzymatic method (Vitros 250 analyzer). Results are expressed as mean ± SD. RI corresponds to the mean ± 2SD. The effect of BW, gender, and age was assessed using a general linear model (Systat 10.0). Multiple comparisons (Bonferoni adjustment) of mean Pl-Cr for each BW category were performed. Individual breed and age effects were examined within BW categories, when $n > 17$.

Mean Pl-Cr and RI for the whole population was 0.93 ± 0.24 mg/dL and 0.45–1.40 mg/dL, respectively. There was a significant effect of gender ($P < 0.05$) with males having a higher mean Pl-Cr than females, 1.00 ± 0.25 mg/dL and 0.90 ± 0.24 mg/dL respectively. There was a significant breed effect but the statistical interaction between breed and BW was also significant ($P < 0.05$). Pl-Cr (mg/dL) in each BW category was statistically different with higher Pl-Cr in larger dogs ($P < 0.001$) (Mini: 0.70 ± 0.12, range 0.48–1.02; Medium: 0.85 ± 0.16, range: 0.55–1.24; Maxi: 1.01 ± 0.26, range: 0.60–2.01; and Giant: 1.19 ± 0.24, range: 0.88–1.82). There was a significant age effect but the statistical interaction between breed and age was also significant ($p < 0.05$), indicating that effect of age on Pl-Cr is breed-dependent. For example, there was no age effect for Setters ($n = 37$) and German Shepherds ($n = 33$) ($p > 0.05$), however for Pointers ($n = 37$) and Boxers ($n = 18$), there was a significant age effect ($P < 0.05$); the correlation was negative and positive respectively.

We conclude that the RI of Pl-Cr should be based on BW categories. The upper limit of the RI for our normal dogs equals the cut-off value of IRIS stage 1 (i.e. dogs with early sub-clinical renal dysfunction). According to our results, such a cut-off value could delay early diagnosis of renal dysfunction in Mini dogs and inversely be too low for Maxi and Giant dogs. Our study highlights the need for dynamic testing of glomerular filtration rate in clinical settings. Further investigation is needed to more adequately assess the age and gender effects.

ABSTRACT #108

EVALUATION OF CYSTATIN C AS A MARKER OF GFR IN HYPERTHYROID CATS. RE Jepson, L Slater, S Nash, R Neiger, D Church, J Elliott, HM Syme; Royal Veterinary College, London, UK.

Measurement of glomerular filtration rate (GFR) is considered to be the gold standard for evaluation of renal function. In human patients, serum Cystatin C, a cysteine protease inhibitor, has been proposed as a more sensitive endogenous marker of GFR than serum creatinine concentration, particularly in the detection of early renal disease. The aim of this study was to evaluate the use of Cystatin C as a marker of GFR in the cat and to investigate the effect of treatment of hyperthyroidism on Cystatin C concentrations.

Hyperthyroid cats (T4 > 55 nmol/L) were recruited for I^{131} treatment at the Queen Mother Hospital for Animals, Royal Veterinary College, London. All cats had previously been treated with medical therapy (carbimazole/methimazole) with cessation of treatment at least 9 days prior to presentation. Cats received a full physical examination and plasma biochemistry, urinalysis and total T4 concentrations were evaluated. GFR was measured using an exogenous inulin clearance test. These parameters were all re-evaluated 1 month and 6 months after I^{131} treatment. Cystatin C concentration was measured using a commercially available particle-enhanced turbidimetric immunoassay (DAKO Cystatin C PET Kit) using stored serum samples from each of the three visits. Results are reported as mean ± SD at pre-treatment, 1 and 6 months post-treatment time points. The Pearson's Correlation was used to evaluate the relationship between GFR and the reciprocal of creatinine or Cystatin C concentration. A repeated measures ANOVA was used to evaluate change in GFR, creatinine and Cystatin C concentrations between visits.

Nineteen cats were included in the study. An intra-assay CV was 3.2% and 1.6% for feline samples with mean Cystatin C concentrations of 1.43 ± 0.05 and 2.49 ± 0.04 mg/L respectively ($n = 4$). Dilutional parallelism was observed in two cat samples. A significant correlation was found between GFR and the reciprocal of creatinine concentration ($r^2 = 0.67$, $P < 0.01$). However, no correlation was found between GFR and the reciprocal of Cystatin C concentration ($r^2 = 0.001$, $P = 0.79$). As expected there was a significant decline in GFR (4.2 ± 1.19, 2.7 ± 0.92, 2.33 ± 0.90 ml/Kg/min, $P < 0.01$) and a significant increase in creatinine concentration (104 ± 30.7, 130 ± 42.2, 189 ± 63.1 μmol/L, $P < 0.01$) with treatment for hyperthyroidism. However, there was no significant hange in Cystatin C concentration ($P = 1.00$) with treatment of hyperthyroidism (1.94 ± 0.36, 2.03 ± 0.32, 1.99 ± 0.31 mg/L).

Serum Cystatin C does not appear to be a useful marker of GFR in the cat. The thyroid status and treatment of hyperthyroidism with I^{131} does not appear to affect cystatin C concentrations.

ABSTRACT #109

INTRINSIC ACUTE RENAL FAILURE (ARF) ASSOCIATED WITH NON-STEROIDAL ANTI-INFLAMMATORY DRUG (NSAID) USE IN JUVENILE CATS UNDERGOING ROUTINE DESEXING–16 CASES 1998-2005. M Robson[1], D Chew[2], S van Aalst[1]. [1]Veterinary Specialist Group, Auckland, New Zealand. [2]The Ohio State University, Columbus, OH.

Companion animal veterinarians in New Zealand (NZ) were surveyed to evaluate the hypothesis that ARF is associated with the use of NSAIDS during desexing in some cats. Tolfenamic acid, ketoprofen, carprofen and meloxicam are NSAIDS registered for feline use in NZ. Sixteen cases of apparent intrinsic ARF in cats undergoing desexing and having received an NSAID fulfilled the criteria for inclusion. Ten males and 6 females were castrated/spayed respectively. Age range was 3 months to 8 months; mean 5.7 months. All cats were deemed normal by their owners and veterinarians pre-operatively. No cat underwent pre-operative blood work or urinalysis, or received blood pressure measurement or fluid therapy during anesthesia.

Eleven cats received a single perioperative (during or within 4 hours of surgery) injection of one NSAID. Seven cats received meloxicam and 4 received carprofen. Five of the meloxicam doses were within the recommended range, 1 was 133% of the maximum recommended dose and 1 dose was not recorded. All the carprofen doses were within the recommended range. One cat received injectable meloxicam at the recommended dosage and then 3 days of oral meloxicam (meloxicam is not licensed for oral use in cats in NZ). One cat received injectable meloxicam at 166% of the recommended dosage, this was repeated the day after surgery followed by 1 dose of oral meloxicam. One cat received injectable carprofen perioperatively then a second dose (increased by 20%) 4 days later, no patient weight was recorded. One cat received injectable carprofen perioperatively at the recommended dosage then 2 days later was treated with unknown doses of injectable and oral meloxicam. One cat received injectable ketoprofen perioperatively at the recommended dosage then the same dose was repeated 2 days later.

Clinical signs of illness were reported by owners beginning 1–4 days after desexing, mean 1.8 days. Creatinine concentration at re-presentation ranged from 272–1194 micromol/L (3.08–13.51 mg/dl), mean 771 micromol/L (8.72 mg/dl) and median 776 micromol/L (8.78 mg/dl). Urine specific gravity was measured in 11 of the 16 cats, range was 1.010–1.024, mean 1.016, median 1.015. Fourteen of the cats received some form of fluid treatment, ranging in duration from 1–5 days, mean 3 days. One cat recovered without fluid therapy and 1 was euthanised based on blood results. Azotemia was documented to resolve in 8 cats. Four cats were deemed to be normal clinically but blood work was not done. Azotemia did not resolve in 4 cats which were euthanised.

The incidence of intrinsic ARF in cats undergoing desexing associated with NSAID use is not known but it appears that some cats will develop intrinsic renal injury in this setting especially when fluid support and blood pressure monitoring are not provided. Further study is needed to define the safety of NSAIDs in this setting.

ABSTRACT #110

N-ACETYL-ß-D-GLUCOSAMINIDASE INDEX AS AN EARLY BIOMARKER FOR CHRONIC RENAL INSUFFICIENCY IN CATS WITH HYPERTHYROIDISM. C Lapointe, MC Bélanger, M Dunn, C Bédard, M

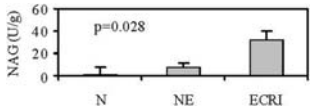
Moreau. Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada.

The diagnostic tools currently used to screen for renal damage in newly diagnosed hyperthyroid cats are either non-sensitive or non-specific (i.e. uremia and microalbuminuria) or impractical (i.e. urinary clearance of inulin). The aim of this study was to determine whether n-acetyl-ß-D-glucosaminidase index (NAG) was a sensitive biomarker for chronic renal insufficiency (CRI) in these cats. NAG is expressed as the ratio of urinary n-acetyl-ß-D-glucosaminidase to urinary creatinine.

Seventeen newly diagnosed hyperthyroid cats and five normal cats (N) were included in the study. All cats were worked up for hyperthyroidism. NAG were measured in N cats and in hyperthyroid cats before the beginning of treatment (baseline NAG) and on each recheck. Hyperthyroid cats were treated with methimazole and reevaluated over a period of three months once they attained euthyroidism. At the end of the study, based on urinary specific gravity and serum creatinine, hyperthyroid cats were divided into two groups: euthyroid cats newly diagnosed with CRI (ECRI) and normal euthyroid cats (NE).

NAG were compared among N, NE and ECRI cats (baseline values). NAG in ECRI cats (32.15 ± 8.03 U/g) was significantly higher than in NE (6.98 ± 3.71 U/g) and N cats (1.36 ± 6.22 U/g) (fig. 1). The treatment of hyperthyroidism did not appear to affect NAG over time in ECRI and NE cats ($p = 0.083$).

In conclusion, NAG may be useful as an early biomarker for CRI in hyperthyroid cats, but further studies are needed.



ABSTRACT #111

INFLUENCE OF HYDROCHLOROTHIAZIDE ON URINARY CALCIUM OXALATE RELATIVE SUPERSATURATION IN HEALTHY ADULT CATS. A Hezel, J Bartges, C Kirk, S Cox, N Geyer, T Moyers, J Hayes, The University of Tennessee, Knoxville, TN.

Thiazide diuretics are used to treat human beings with calcium oxalate urolithiasis, and there is preliminary data of effectiveness in dogs. The purpose of this study was to evaluate daily administration of hydrochlorothiazide (HCZ) to healthy cats on calcium oxalate urinary saturation.

Six healthy, spayed female cats, aged 3–5 years, and weighing 3.8–6.4 kg, were evaluated. Cats received either HCZ suspension (1 mg/kg PO q12hr) or an equivalent volume of a similarly colored placebo suspension in a blinded, cross-over controlled study. Treatments were administered for 2 weeks with a 1 week washout period. Blood ionized calcium concentration and a 24-hour urine sample were collected using a modified litter box at the end of each period. A dry, adult maintenance food (SportMix, Midwestern Pet Foods) was fed to maintain body weight and condition. Twenty-four hour urine samples were mixed, the volume recorded, and pH, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), magnesium (Mg), phosphorous (P), citrate (Cit), oxalate (Ox), and ammonia (Amm) concentrations were determined. Molar concentrations of these analytes were entered into a computer program (EQUIL 89d, University of Florida) for determination of relative supersaturation for calcium oxalate monohydrate (RSScom) and dihydrate (RSScod). Data were analyzed using 2-tailed, paired t-tests; $p \leq 0.05$ was significant.

Body weight did not change between study periods ($p = 0.9$). Significant differences were not found for blood ionized calcium, 24-hour urine pH, or excretion of calcium or citrate. Significant differences were found for (unit/BWkg/24hr; data presented as mean ± standard deviation):

Parameter	Placebo	HCZ	p	Parameter	Placebo	HCZ	p
Volume (ml)	11.8±1.6	19.3±3.8	0.005	Na (mEq)	1.78±0.26	3.22±0.44	0.0003
K (mEq)	1.84±0.19	3.18±0.53	0.002	Mg (mg)	0.42±0.13	2.08±0.43	0.0007
Amm (mM)	17.7±7.6	78.6±37.2	0.02	P (mg)	33.2±4.2	60.1±11.0	0.002
Ox (mg)	2.88±0.59	3.91±0.76	0.04	Cl (mEq)	1.74±0.29	3.17±0.59	0.0005
RSScom	3.48±1.12	1.12±0.70	0.02	RSScod	1.49±0.46	0.48±0.30	0.04

HCZ administration decreased urinary saturation for calcium oxalate and could be useful in managing normocalcemic cats with calcium oxalate urolithiasis. However, it increased excretion of electrolytes, and other compounds, which could result in whole body depletion with long term administration. Long term studies evaluating safety and efficacy in normocalcemic cats with calcium oxalate urolithiasis is warranted.

ABSTRACT #112

THE EFFECT OF HYDROCORTISONE ON URINARY PROTEIN EXCRETION IN DOGS. Schellenberg S[1], Gentilini F[2], Glaus TM[1], Reusch CE[1]. [1]Clinic for Small Animal Internal Medicine, University of Zurich, Switzerland. [2]Departement of Veterinary Clinical Sciences, University of Bologna, Italy.

Proteinuria is commonly encountered in dogs with exogenous or endogenous glucocorticoid excess. Recognized mechanisms of proteinuria include altered glomerular capillary permeability with excess protein filtration, increased tubular protein excretion and decreased tubular resorption. However, to the authors knowledge, it has not been determined whether the effects of hydrocortisone are transient or permanent. The purpose of this study was to evaluate urinary protein excretion in dogs during and after long-term hydrocortisone administration.

Eleven adult Beagle dogs (6m, 5f, 3.5 years) were studied before, during and after administration of hydrocortisone ($n = 5$; I-HAC) (8 mg/kg PO bid for 90 days) or placebo ($n = 6$). Urine protein:creatinine ratio (UP/C), urine albumine:creatinine ratio (UA/C), microalbuminuria (MALB) and sodium dodecyl sulphate-agarose gel electrophoresis (SDS-AGE) were evaluated before (t0), on day 1 (t1), 5 (t2), 28 (t3), 56 (t4), 84 (t5) of treatment and 1 (t6), 5 (t7), 28 (t8), 56 (t9) and 84 (t10) days after withdrawal of hydrocortisone and placebo, respectively.

Before treatment, median UP/C were 0.18 (0.17–0.25) and 0.22 (0.12–0.29) for hydrocortisone and placebo treated dogs, respectively. UP/C increased in the I-HAC group to a maximum of 0.45 (0.18–1.77) at t3 ($p < 0.005$). UA/C were 0.015 (0.009–0.019) and 0.016 (0.005–0.081) in the I-HAC and placebo group, respectively. UA/C increased progressively in the I-HAC group to a maximum of 0.134 (0.033–1.240) on t5 ($p < 0.005$). After discontinuation of hydrocortisone, both UP/C and UA/C progressively decreased to 0.17 (0.12–0.31) and 0.010 (0.005–0.032) by t8, and were not different from values at t0. MALB was already present before treatment in some dogs of the placebo group but not in the I-HAC group. Four dogs of the I-HAC group developed MALB during treatment, which was already resolved at t8. An effect of gender on urinary protein but not albumin excretion was found; UP/C was significantly higher in male dogs before and on each occasion after stopping hydrocortisone administration. SDS-AGE revealed primarily albuminuria in all dogs at different times, with a pronounced increase in the I-HAC group during treatment. Already one month after discontinuation of hydrocortisone SDS-AGE showed no or only weak bands for albumin like at t0. Furthermore, a protein of 25–30 kD was found in urine samples of all male dogs but never of female dogs.

In conclusion, our study shows that long-term glucocorticoid treatment results in a significant but only transient proteinuria that is already resolved within one month of discontinuation of hydrocortisone. Higher UP/C but not UA/C in male dogs is probably due to the 25–30 kD protein found only in male dogs, thought to be the canine prostate specific esterase.

ABSTRACT #113

ESTIMATION OF ACUTE FLUID SHIFTS IN HORSES USING BIOELECTRICAL IMPEDANCE ANALYSIS. C Langdon Fielding[1],; K Gary Magdesian[2]. [1]Loomis Basin Equine Medical Center, Loomis, CA [2]School of Veterinary Medicine, University of California Davis, Davis, CA.

Multi-frequency bioelectrical impedance analysis (MF-BIA) is a non-invasive technology that utilizes an alternating low-frequency electrical current to make predictions about body composition. It has been evaluated in healthy horses, but has not been used during acute fluid shifts. The purpose of this study was to evaluate MF-BIA in detecting fluid fluxes within the extracellular fluid volume (ECFV). We hypothesized that MF-BIA would detect clinically relevant (10–20%) changes in ECFV.

Ten horses were studied in 3 phases: 1) 20 ml/kg isotonic crystalloid expansion during euhydration. 2a; 2b) Furosemide-induced dehydration, followed by 20 ml/kg crystalloid expansion. 3) Blood loss (16 ml/kg), followed by replacement. Mean errors between MF-BIA estimated change and known volume change were compared among the 3 phases using nonparametric ANOVA. Estimated ECFV pre- and post- fluid administration were similarly compared.

The following results were obtained from these phases. Phase 1: MF-BIA detected a statistically significant expansion of the ECFV immediately post and 1-h after infusion. Phase 2a: There was a significant decrease in estimated ECFV at 1 and 4-h post-administration of furosemide. Phase 2b: Dehydrated animals showed a significant increase in ECFV at 2- and 3- h post rehydration. Phase 3: MF-BIA did not identify a significant contraction or expansion of ECFV following blood loss or transfusion. MF-BIA was better at estimating volume changes during dehydration than fluid expansion ($p = 0.03$).

In conclusion, MF-BIA detects changes in ECFV during dehydration and isotonic crystalloid expansion, although better during fluid losses compared to gains. MF-BIA is less useful for changes in blood volume.

ABSTRACT #114

CARDIO PULMONARY EFFECTS OF SMALL VOLUME RESUSCITATION IN ANESTHETIZED ENDOTOXEMIC HORSES. Lucas G. Pantaleon, Martin O. Furr, Harold C. McKenzie II, Lydia Donaldson. Marion duPont Scott Equine Medical Center, Virginia/Maryland Regional College of Veterinary Medicine, VPI & SU, Leesburg VA, USA.

Small volume resuscitation has been advocated in the treatment of endotoxemia in horses. However, its use has not been investigated in an experimental, controlled manner. The objectives of this study were to determine the cardiopulmonary effects of Hypertonic Saline Solution (HSS) plus Hetastarch (HES) as compared to large volume isotonic fluid resuscitation (LVR) during experimental endotoxemia in anesthetized horses.

Eighteen healthy horses were randomly assigned to three groups of six. Anesthesia was maintained with halothane. Endotoxemia was induced by administering 50 mcg/kg of *E coli* endotoxin intravenously. After induction of endotoxic shock the horses were treated over 30 minutes as follows: control group 15 ml/kg of balanced polyionic crystalloid solution, ISO group 60 ml/kg of balanced polyionic crystalloid solution, and HSS-HES group 5 ml/kg of HSS followed by 10 ml/kg of HES.

Hyperdynamic endotoxic shock was created in this model. Cardiac output significantly ($P < 0.05$) increased from baseline in all groups. This increase was more pronounced and lasted longer in the HSS-HES group. Volume overload was demonstrated in the ISO group by a significant ($P < 0.05$) increase in mean central venous pressure. Alterations in ventilation/perfusion were observed in all groups. Although statistically not significant, this appeared less severe in the HSS-HES group.

LVR caused volume overload, while the use of HSS plus HES showed a trend towards less severe cardiopulmonary dysfunction during severe endotoxemia. Small volume resuscitation in endotoxic horses shows promise for its beneficial effects on cardiopulmonary function.

ABSTRACT #115

THE EFFECTS OF AGING ON THE STRUCTURE AND FUNCTION OF THE EQUINE AORTIC VALVE. IM Bowen[1], CM Marr[2], CPD Wheeler-Jones[3], AH Chester[4], J Elliott[3]. [1]School of Veterinary Medicine and Science. University of Nottingham, UK. [2]Rossdale and Partners, Newmarket, UK. [3]Royal Veterinary College, University of London, UK. [4]Imperial College London, University of London, UK.

Aortic valve disease is a common degenerative ageing condition. The mechanisms that result in aortic valve disease are unclear, although recently contractile properties of the valve have been described and suggest a dynamic role in maintaining diastolic competence. It is hypothesized that the valve undergoes functional changes, prior to the onset of structural changes, which alter valve function and play a role in the pathogenesis of valve disease. The aim of this study was to define age related changes in both the structure and dynamic function of the equine aortic valve.

Equine aortic valves, with no evidence of structural valvular pathology were harvested from from 142 horses killed at an abattoir. The horses were assigned four age groups; <5 years ($n = 21$), 6–10 years ($n = 40$), 11–15 years ($n = 46$) and >15 years ($n = 35$). Contractile function was determined *in-vitro* by suspending valve segments between force transducers and assessing the response to a depolarising polyionic solution (118 mmol/l KCl; DKS). Structural changes were determined in a subset of animals ($n = 6$ per group). Collagen content of the valves was estimated by determination of hydroxyproline concentration. The valvular content of the contractile protein α-smooth muscle actin (SMA) was estimated by western blotting of tissue homogenates. Comparisons of contractile responses were made between groups using Kruskal-Wallis and Dunn's multiple comparison tests. Comparisons between hydroxyproline and SMA concentrations were compared between groups using ANOVA with Tukey's multiple comparison tests. Data are expressed as mean values ± SD.

There was a trend for the contractile force to decrease with increasing age ($P = 0.021$). The DKS response was greatest in valves from animals ≤5 years (645 ± 71 mg tension per gram of tissue) and this was significantly greater than the DKS response in valves derived from animals over 15 years of age (428 ± 36 mg per gram; $p < 0.05$). There was a significant correlation between age and tissue hydroxyproline content ($p < 0.001$, $r^2 = 0.52$). The concentration of hydroxyproline in valves from horses ≥15 years (12.8 ± 0.8 mg/gram) was significantly higher than the hydroxyproline content in valves from animals 6–10 years (8.4 ± 0.5 mg/gram) and ±5 years (8.2 ± 0.6 mg/gram; $p < 0.01$). There was no significant difference in the relative concentration of SMA concentration ($p = 0.07$) between the age groups, although there was a trend for SMA to decrease with age such that SMA was approximately 60% higher in animals aged ≤5 years compared to other age groups.

The equine aortic valve develops a reduced maximal contractile function with increasing age which is associated with an increase in collagen content of the valve. Further investigations are warranted to determine whether the increased collagen is a response to reduction in contractile function, or if the increased stiffness of the valve brought about by increase in collagen is responsible for this effect.

ABSTRACT #116

ECHOCARDIOGRAPHIC ESTIMATION OF PULMONARY ARTERIAL PRESSURES IN HORSES WITH CARDIAC DISEASE. MM Durando, J Slack, VB Reef, O Seco-Diaz, G Smith, EK Birks. University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA.

This study was performed in order to determine the usefulness of echocardiography to predict pulmonary artery pressures (PAP) in horses with cardiac disease. Pulmonary hypertension is a complication of various congenital and acquired cardiac diseases, and is an important prognostic indicator. Direct measurement of PAP is an invasive procedure requiring specialized equipment not routinely available to equine practitioners; however most veterinarians have access to ultrasound machines. In dogs and humans, estimation of PAP with echocardiography has been validated, but this has not been done in horses.

Twenty four horses with various cardiac diseases and 12 normal control horses were included in this study. All control horses had normal clinical and echocardiographic examinations. Horses were included if they had a complete 2D, M-mode and Doppler echocardiogram and invasively-measured PAP. Non-standard as well as standard views of the pulmonary artery and aorta were obtained. Pulmonary artery pressures were measured by passing a fluid-filled polyethylene catheter 10 cm distal to the pulmonic valve. Placement of the catheter was confirmed by observing the characteristic pressure waveforms as the catheter was advanced through the chambers. Pressures were zeroed to the point of the shoulder. A mathematical model for predicting pulmonary arterial pressures from ultrasonographic measurements was constructed using multivariate least-squares regression. Significance was set at $P < 0.05$.

Control horses had normal PAP (29.5 ± 1.0 mmHg, mean ± sem). Echocardiographic measurements and myocardial function were within normal limits. In the group with cardiac disease, 19 horses had acquired cardiac disease and 5 had congenital cardiac disease. Five horses had severe cardiac disease with evidence of congestive heart failure. Mean PAP in the horses with cardiac disease was 39.2 ± 2.9 mmHg, with a range from 19.8–82.3 mmHg. In horses with known cardiac disease, a model could be constructed that predicted actual PAP accounting for 79% of the observed variation using 3 non-standard and 2 standard echocardiographic views. When control horses were included in the model, the best prediction of actual PAP was derived from 4 non-standard views, accounting for 76% of the observed variation. If a pressure of 50 mmHg is used as a cutoff for clinically significant pulmonary hypertension than the constructed model provides a sensitivity and specificity of 100%.

This study provides evidence that there is a relationship between echocardiographic measurements of the pulmonary artery and actual PAP. Echocardiography has the potential to be a valuable tool in determining the severity of pulmonary hypertension associated with cardiac disease. However, this study was conducted on a relatively small number of horses, and the validity of this model must be confirmed with larger studies.

ABSTRACT #117

BIOCHEMICAL EVIDENCE OF CARDIAC INJURY IN HORSES WITH CANTHARIDIN TOXICOSIS. TC Holbrook and RJ Panciera. Oklahoma State University, Stillwater, OK.

Cantharidin is a toxic anhydride produced by the male blister beetle. Toxicosis in horses is most commonly associated with ingestion of blister beetle contaminated alfalfa hay. Clinical signs of intoxication may include colic, sweating, ptyalism, tachycardia, tachypnea, pyrexia, synchronous diaphragmatic flutter, dysuria and shock. Clinicopathologic findings common in horses with cantharidiasis include hypocalcemia, hypomagnesemia, hyposthenuria and increased creatine kinase. Postmortem studies have documented myocardial necrosis in some horses with cantharidin toxicosis.

The purpose of this study was to determine if biochemical or electrocardiographic evidence of myocardial injury occurs in horses with cantharidin toxicosis. Horses presented to the veterinary teaching hospital at Oklahoma State University with a history of exposure to blister beetle contaminated hay between April 2003 and January 2005 were included in this prospective study. Heparinized blood samples were obtained at

admission and 24–36 hours later for the quantitation of cardiac troponin-I (cTNI). Plasma was removed and stored at −80C until analysis. cTnI concentration was measured by colorimetric immunoassay (Stratus CS stat fluorometric analyzer). Base Apex ECG tracings were obtained from horses at admission. Paired plasma samples were available from thirteen of fifteen horses included in the study. cTNI concentrations were increased at admission and 24–36 hours later in 46% (six) of the horses in this study. The average cTNI concentration in these six horses was 5.0 ± 2.5 ng/ml at presentation, and 2.6 ± 1.3 ng/ml 24–36 hours later. Seven horses (54%) had cTNI concentrations within normal limits at both sample times (0.016 ± 0.006 ng/ml at presentation and 0.021 ± 0.006 ng/ml 24–36 hours later) No arrhythmias were documented in any of the horses in this study. One horse with increased cTNI was euthanized due to the development of laminitis, all other horses responded to medical therapy. The significance of myocardial injury in horses with cantharidin toxicosis deserves further study.

ABSTRACT #118

EFFECTS OF LONG-TERM LEVOTHYROXINE ADMINISTRATION ON ADIPOSE AND SKELETAL MUSCLE TISSUE GLUCOSE TRANSPORTER GENE EXPRESSION IN MARES. N. Frank[1], M Dhar[1], SB Elliott[1], JS Yuan[2], J Denhart[3]. [1]University of Tennessee College of Veterinary Medicine, Knoxville, TN. [2]University of Tennessee Institute of Agriculture Genomics Hub, Knoxville, TN. [3]Lloyd Incorporated, Shenandoah, IA.

Levothyroxine (L-T4) has been previously administered to horses for the treatment of hypothyroidism despite a lack of evidence to support this diagnosis in the majority of cases. However, many horses with clinical signs previously attributed to hypothyroidism including obesity, regional adiposity, and laminitis appear to respond clinically to L-T4 administration. These anecdotal findings suggest that L-T4 exerts beneficial pharmacological effects and we have previously demonstrated that insulin sensitivity increases in horses treated with L-T4. In this study L-T4 (Thyro-L) was orally administered to 6 adult healthy nonobese mixed breed mares for 12 months at a dosage of 4 teaspoons (48 mg L-T4) once daily to test the hypothesis that L-T4 enhances glucose uptake by increasing the abundance of glucose transporters (GLUTs) within insulin-sensitive tissues. Specifically, we aimed to quantify the expression of GLUT1 and GLUT4 genes within adipose and skeletal muscle tissues collected from each mare at the beginning and end of the 12-month treatment period. Samples of adipose and skeletal muscle tissues were collected from the nuchal crest and semimembranosus muscle, respectively. Tissues were processed to obtain total RNA and GLUT1 and GLUT4 mRNAs were quantified using real-time polymerase chain reaction with primers specific to the respective gene sequences of horses. Administration of L-T4 for 12 months did not significantly alter the expression of GLUT1 in adipose (post/pre ratio = 1.09) and skeletal muscle (ratio = 0.82) tissues, or the expression of GLUT4 in skeletal muscle (ratio = 0.96), but a significant ($P < 0.05$) increase in GLUT4 expression was detected within adipose tissue (ratio = 1.56). A control group was not included in this study, so factors other than L-T4 administration such as time, diet, and season could have influenced results. It should also be noted that the abundance of GLUT4 proteins within cell membranes is regulated by processes that occur following transcription. However, results of this study provide preliminary evidence that L-T4 improves insulin sensitivity in horses by increasing GLUT4 expression within adipose tissues. Further analyses must be performed to determine the effects of L-T4 administration on the abundance and distribution of GLUT1 and GLUT4 proteins within these tissues.

ABSTRACT #119

THE PREVALENCE OF POLYSACCHARIDE STORAGE MYOPATHY IN THE QUARTER HORSE POPULATION. ME McCue, WR Ribeiro, SJ Valberg. University of Minnesota, College of Veterinary Medicine, St. Paul, Minnesota.

Polysaccharide storage myopathy (PSSM) is a common heritable cause of exertional rhabdomyolysis in Quarter Horses (QH). Although 48% of QHs with signs of neuromuscular disease have PSSM, the true prevalence of PSSM in apparently healthy breeding and performance QHs is unknown. The purpose if this study was to determine the prevalence of PSSM in >150 breeding and performance QHs from 6 states. Five primarily breeding ranches and one performance horse ranch enrolled in this study. Two of the breeding ranches had previously produced PSSM horses and those previously diagnosed horses were excluded. Hematoxilin and eosin (H&E), periodic acid Schiff's (PAS) and amylase-PAS stains of percutaneous gluteal muscle biopsy specimens from 164 horses were graded for specific histopathological criteria. The presence of amylase-resistant abnormal polysaccharide was used to definitively diagnose PSSM. Resting serum creatine kinase (CK) activity was assessed in 65 horses.

Of 164 horses sampled, 117 were breeding stock and 47 were performance horses. On the 4 ranches without a recognized history of PSSM, 6.1% (8/132) of horses had PSSM. On these ranches, 4.8% (4/84) of breeding stock, and 8.5% (4/47) of performance horses had PSSM. The prevalence of PSSM on the 2 breeding ranches with a history of producing PSSM horses was 20% (1/5) and 40.7% (11/27). There was no significant difference in gender, age or resting serum CK activity between PSSM affected and unaffected QHs. Mean biopsy grades were significantly higher in PSSM (9.2 ± 2.5) vs unaffected horses (4.0 ± 1.8) Grades for atrophy, necrosis, central nuclei, subsarcolemmal vacuoles, granular glycogen and PAS stain intensity were higher in muscle from PSSM vs unaffected QHs (t test). Subsarcolemmal glycogen, granular glycogen, sarcoplasmic masses and rimmed vacuoles were not significantly associated with a diagnosis of PSSM based on amylase resistant polysaccharide (χ^2).

The results of this study suggest that the prevalence of PSSM in the general population of apparently healthy QHs is approximately 6%. However, an unexpectedly high percentage of horses in certain breeding operations may have subclinical PSSM. Clinical signs may be absent when horses are kept on pasture under management conditions that include low starch diets; extensive pasture turn out and/or consistent exercise. The inadvertent use of animals with subclinical PSSM for breeding may perpetuate this heritable myopathy within the breed. PSSM may become a clinically significant disorder when offspring that inherit PSSM are dispersed and kept under different management conditions.

ABSTRACT #120

HYPERINSULINEMIC EUGLYCEMIC CLAMPING AND INSULIN SENSITIVITY IN BELGIAN DRAFT HORSES WITH POLYSACCHARIDE STORAGE MYOPATHY. AM Firshman[1], JD Baird[2], L Hunt[3], SJ Valberg[3]. [1]College of Veterinary Medicine, Oregon State University, OR, USA. [2]Ontario Veterinary College, [2]University of Guelph, Guelph, ON, Canada, [3]College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA.

Polysaccharide storage myopathy (PSSM) is a glycogen storage disorder seen most commonly in Quarter Horses, Draft Horses and Warmbloods, that is associated with abnormal polysaccharide accumulation in skeletal muscle. Exertional rhabdomyolysis is a consistent feature associated with PSSM in Quarter Horses in addition to enhanced insulin-stimulated glucose excursion from the bloodstream indicating heightened insulin sensitivity. The mechanism relating enhanced glucose uptake and glycogen synthesis in PSSM Quarter Horses with muscle necrosis during exercise is unknown. The prevalence of PSSM in Belgian Draft Horses is >30% and reported clinical signs vary from no signs to exertional rhabdomyolysis, gait abnormalities, progressive muscle atrophy, neuromuscular weakness and poor performance. Due to the differences in their clinical presentation we hypothesized that the pathogenesis of PSSM in Quarter Horses may differ from Belgian Draft Horses. The specific aim of this study was to determine if the heightened insulin sensitivity seen in Quarter horses with PSSM was present in Belgian Draft Horses with PSSM. Five Belgian Draft Horses previously diagnosed with PSSM, based on the presence of amylase resistant abnormal polysaccharide and increased muscle [glycogen] in gluteal muscle biopsy specimens and five normal control Belgian Draft horses without PSSM were used. Each animal underwent a 3-hour hyperinsulinemic euglycemic clamp at which point the investigators were blinded as to the diagnosis of PSSM. Blood collected from one jugular venous catheter was used to determine glucose and insulin concentrations. Insulin diluted in isotonic saline and homologous blood, was infused at 3 mU/min/kg body weight in the alternate jugular vein to maintain a plateau of 200–300 μU/ml above basal concentrations. On site blood glucose concentrations were used to determine the rate of IV glucose infusion necessary to maintain blood glucose at about 100 mg/dl. The first hour was considered an equilibration period. The average blood glucose and insulin concentration throughout the clamp were similar between PSSM and control horses (glucose PSSM 99.0 ± 5.3 mg/dL; controls 95.8 ± 3.5) (insulin PSSM 278.9 ± 52.2 μIU/mL; controls 289.1 ± 29.7). The rate of glucose infusion necessary to maintain euglycemia during the clamp was not significantly different between PSSM (4.4 ± 0.2 mg/kg/min) and controls (4.5 ± 0.1 mg/kg/min) ($P > 0.05$). The results suggest that, unlike the 2-fold higher insulin sensitivity in Quarter Horses with PSSM, Belgian Draft Horses with PSSM do not appear to have enhanced insulin sensitivity compared to breed matched controls. Therefore, the etiopathogenesis of PSSM may differ between Quarter Horses and Belgian Draft Horses.

ABSTRACT #121

GENOMIC AND NON GENOMIC EFFECTS OF DEXAMETHASONE ON EQUINE PERIPHERAL BLOOD NEUTROPHILS. L Lecoq, A Lavoie-Lamoureux, P Vincent, JP Lavoie. Université de Montréal, Saint-Hyacinthe, QC, Canada.

Glucocorticoids exert potent anti-inflammatory actions in a cell-type specific manner and are among the most prescribed drugs for the treatment of equine inflammatory diseases. They are believed to exert their effects primarily through interruption of cytokine-mediated pathways via gene expression (transactivation) or gene repression (transrepression). More recently, the presence of non-genomic pathways has also been described. Currently, little is known concerning the non-genomic effects of dexamethasone (DEX) on neutrophils and the requirement of glucocorticoid receptor (GR) activation for this response. The objective of this study was to evaluate the genomic and non-genomic response of equine neutrophils to glucocorticoids, and the dependency of their receptors in these processes.

The genomic effects of corticosteroids were assessed by studying the IL-8, TNF-α and the Toll-like receptor (TLR)-4mRNA expression using real time RT-PCR. Peripheral blood neutrophils from 6 healthy horses, isolated using ficoll (purity $>$ 96% and viability $>$ 98%), were incubated at 37°C, 5% CO_2 for 6 hours in the presence of 100 ng/ml LPS, and 10^{-6}M DEX alone or combined with the GR inhibitor RU486 (10^{-5}M). The non-genomic effects on neutrophils (oxidative burst) were studied using whole blood from 3 horses incubated in a water-bath with 10^{-6}M DEX (20, 25 and 30 minutes) and 5 µM dicholorofluorescein (DCF), and then stimulated with 5 ng/ml Phorbol-Myristate Acetate in the presence of RU486 (10^{-5}M). The oxidative burst of neutrophils was evaluated using flow cytometry.

DEX significantly down-regulated the LPS-induced IL-8, TNF-α and TLR-4 mRNA expression. Pre-treatment with DEX (25 and 30 minutes) similarly attenuated the PMA-induced oxidative burst of neutrophils. In both studies, the responses observed were attenuated in the presence of RU486.

In conclusion, dexamethasone may attenuate neutrophil inflammatory response through receptor mediated genomic and non-genomic pathways. This finding may contribute to the rapid response (minutes) observed following corticosteroid administration in selected equine inflammatory processes.

ABSTRACT #122

EFFECT OF FENTANYL ON SOMATIC AND VISCERAL NOCICEPTION IN CONSCIOUS HORSES. L. Chris Sanchez[1], Sheilah Robertson[1], Cynthia Cole[1], Lara Maxwell[2]; [1]University of Florida College of Veterinary Medicine, Gainesville, FL and [2]Oklahoma State University, Stillwater, OK.

Transdermal fentanyl is used clinically in horses based upon initial pharmacokinetic data and its anti-nociceptive effect in other species. The aim of this study was to evaluate the effect of fentanyl infusion on visceral and somatic nociception in conscious horses.

Visceral nociception was evaluated using two methods of threshold detection, colorectal distention (CRD) and duodenal distention (DD). Each employed the use of a Mylar® balloon and a computer-controlled barostat for distention. Somatic nociception was assessed via thermal threshold (TT) determination. A probe containing a heater element and adjacent temperature sensor placed on the withers was used for thermal stimulation. Nose-to-ground height was used to assess sedation. Heart and respiratory rates were determined by manual count. All treatments were administered as an IV bolus followed by a continuous rate infusion for a total of two hours. Treatments included four doses of fentanyl (F1–4), saline (negative control), and xylazine (positive control). Six horses were used for the study, and each horse received each treatment. Serum fentanyl concentrations were analyzed by a LC/MS/MS method. All data were analyzed by means of a three-factor ANOVA (SAS Proc Mixed) followed by either a simple t test or a Bonferroni t test for multiple comparisons.

Approximate mean serum fentanyl concentrations (ng/ml) for each treatment were as follows: F1 0.3, F2 1.0, F3 peak of 4, maintenance of 2.5, F4 peak of 10, maintenance of 6. Two horses in the F4 group became agitated and tachycardic during the first 15 minutes of the infusion. No other adverse events were noted.

Fentanyl administration did not result in significant changes in DD or CRD threshold. TT was increased at the 15 minute time point for the F4 group only.

In conclusion, fentanyl did not produce a significant anti-nociceptive effect at the chosen doses, two of which resulted in serum concentrations above those known to produce analgesia in other species.

ABSTRACT #123

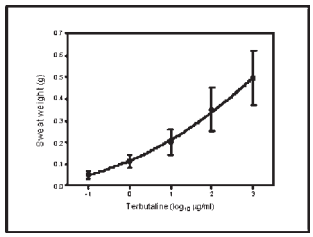
USE OF A QUANTITATIVE INTRADERMAL TERBUTALINE TEST FOR MEASURING SWEAT PRODUCTION IN NORMAL AND ANHIDROTIC HORSES. RJ MacKay, University of Florida, Gainesville, FL.

Anhidrosis or inability to sweat is a common and debilitating condition of horses in hot, humid climates. Sweat response to intradermal injection of a β2-adrenergic agonist has been used as part of the diagnosis. The aims of this work were (1) to quantify the sweat elaborated onto the skin of normal horses injected intradermally with various doses of the β-agonist terbutaline and (2) to use this test to measure sweat responses of horses diagnosed by owners as either free-sweating or anhidrotic.

Seven horses, 5 mares and 2 geldings, aged 7–19 years were used in experiment 1. Each horse was tested monthly for 11 consecutive months. Ambient temperature, relative humidity and dew point were measured at the time of each test. To perform the test, a strip of skin on the lateral side of the neck was clipped and 0.1 ml amounts of either saline of terbutaline sulfate (7 serial 10-fold dilutions in saline, beginning at 1 mg/ml) were injected intradermally. A pre-weighed absorbent pad was taped over each injection site. After 30 minutes, the pad was removed and the weight of absorbed sweat determined by difference. In experiment 2, this quantitative sweat test also was used on horses diagnosed by clients as either free-sweating ($N = 22$) or anhidrotic ($N = 41$). Tests were performed either during December–February (cool) or June–September (warm, humid). Effects of month, season, terbutaline dose, and owner diagnosis were explored by General Linear Model repeated measures analyses. Significance was ascribed to P-values ≤ 0.05.

Net sweat weights (±sd) over the 5 highest terbutaline concentrations (1 mg/ml to 0.1 µg/ml) for all measurements (7 horses × 11 tests each) from experiment 1 are shown in Figure 1. There was significant effect ($P < 0.001$) of terbutaline dose on sweat production, but there was no significant dose×month interaction or month effect. In experiment 2, there were significant effects ($P < 0.001$) of owner classification and terbutaline dose on sweat weight. Sweat weight was not significantly affected by season of testing for either anhidrotic or free-sweating horses. By using ROC analyses, cut-off values for sweat weight could be determined for each terbutaline dose that provided approximately 70% sensitivity and 70% specificity for owner diagnosis of anhidrosis.

The quantitative terbutaline sweat test provided consistent and reproducible data in free-sweating and anhidrotic horses over a wide-range of concentrations. This test should be useful tool in future studies of anhidrotic horses.



ABSTRACT #124

EQUINE ADIPOSE TISSUE IS A SOURCE OF MULTI-POTENT STEM CELLS. TB Meredith, T Sand, RJ Harman, Vet-Stem Inc., Poway CA, USA.

Multi-potent stem cells in adult tissue stores represent a source of undifferentiated cells that possess clinical potential in multiple disciplines of veterinary medicine. Mesenchymal stem cell populations have been found in bone marrow, peripheral blood, muscle, tendon, hepatic, renal, and adipose tissue. Recently, human adipose tissue has been described as a rich source of multi-potent stem cells capable of differentiating into multiple cell lineages including adipocytes, osteoblasts, myoblasts, and chondroblasts in both in

vivo and in vitro models. However, characterization of cells obtained from equine adipose tissue has yet to be described. The purpose of this study was to perform a preliminary characterization of cells isolated from equine adipose tissue in terms of their phenotype and ability to differentiate into multiple cell lineages.

Three unrelated horses were sedated utilizing an alpha-2 antagonist/opiod combination and 20 grams of subcutaneous adipose tissue were recovered from a skin incision dorso-lateral to the tail head. Samples were processed with a protocol based on the use of collagenase digestion of the extra cellular matrix in the tissue, which releases the cells contained therein. The recovered cells were washed, quantified, and assessed for viability using Trypan Blue.

Stromal cells isolated from processed adipose tissue were placed into culture at Cognate Therapeutics, Inc. (Baltimore, MD). The adherent cell population was expanded for up to 3 passages (DMEM, 10% serum, antibiotics/antimycotics). Flow cytometric analysis of cells isolated from adipose tissue from the 3 unrelated horses were found to be positive for CD29, CD44 and CD90, which are markers found on bone marrow stromal cells (mesenchymal stem cells). These samples also expressed variable levels of CD133 and ABCG2, which are both expressed by human adipose derived stem cells.

Two unrelated cell samples were cultured to passage 2 and placed in osteogenic medium (β-glycerophosphate, ascorbic acid-2-phosphate, and dexamethasone) or chondrogenic medium (dexamethasone, ascorbate-phosphate, praline, pyruvate, and TGF-β3). Both cell samples differentiated into osteogenic and chondrogenic cell types as determined by alkaline phosphatase and Von Kossa staining (osteogenic differentiation) and Alcian Blue staining and expression of Collagen Type II (chondrogenic differentiation).

These observations support the existence of cells in equine adipose tissue that express a phenotype consistent with that described in human adipose derived stem cells, and that these cells demonstrate the capacity to differentiate into two distinct cell lineages.

ABSTRACT #125

SINGLE-DOSE PHARMACOKINETICS OF ORAL PIROXICAM IN HORSES. DH Thamm[1], MG Papich[2], DP Lunn[1], YA Elce[2], SB Hussey[1], R Kramer[1]. [1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. [2]North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Nonsurgical therapies for equine cancer are sorely lacking. Pharmacologic inhibition of cyclooxygenase 2 with nonsteroidal antiinflammatory drugs such as piroxicam has been associated with antitumor activity in canine and human tumors, and anecdotes and case reports suggest possible antitumor activity in horses as well. The goal of this study was to determine the plasma pharmacokinetics of piroxicam in clinically normal horses following a single oral dose.

Six clinically normal adult mares from a breeding herd maintained at Colorado State University were utilized in these studies, with Animal Care and Use Committee approval. Reagent-grade piroxicam (Sigma) was administered orally at a dosage of 0.2 mg/kg, in a small amount of grain in order to mimic clinical administration conditions. This dose was chosen based on allometric calculations and anecdotal experience. Serial phlebotomy was obtained following administration, and heparinized plasma was separated and frozen until time of assays. After a 2-week washout period, 3 of the same horses were re-dosed with pharmaceutical grade piroxicam (20 mg capsules) at the same dosage and phlebotomy performed as cited previously. Piroxicam was extracted from plasma samples using solid-phase extraction, and piroxicam concentrations were then measured using reverse-phase HPLC with UV detection.

The dose of piroxicam was well tolerated, with no observed changes in vital signs, stool consistency, food intake, gut sounds, digital pulses or plasma BUN/creatinine. Oral piroxicam (Sigma) at 0.2 mg/kg resulted in a mean C_{max} of 430 ng/mL (range 200–690 ng/mL), mean T_{max} of 0.43 h (range 0.20–0.69 h), mean $T_{1/2}$ of 3.57 h (range 2.71–4.54 h), and mean $AUC_{0\text{-infinity}}$ of 2,720 hr*ng/mL. Chemical reagent-grade piroxicam (Sigma) was bioequivalent to the proprietary capsule formulation based on the parameters assessed.

Based on this data, a 0.2 mg/kg oral dose of piroxicam produce a lower peak concentration (Cmax) in horses compared to peaks reported for cats or dogs (519 and 1350 ng/mL respectively), but the peaks occurred earlier in horses. Elimination in horses was much more rapid than in cats or dogs. These findings are despite a report of antitumor activity at an oral dose of 0.2 mg/kg daily in a horse with squamous cell carcinoma. Studies are ongoing to determine the concentrations of piroxicam necessary to inhibit equine COX-2 activity *in vitro* and to determine whether the 0.2 mg/kg oral dose results in reductions in plasma prostaglandin E2 and thromboxane A2 *in vivo*.

ABSTRACT #126

CYCLOOXYGENASE-2 EXPRESSION IN EQUINE TUMORS. DH Thamm[1], EJ Ehrhart[1], YA Elce[2], JB Charles[1]. [1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO. [2]North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Medical therapies for equine cancer are sorely lacking. Cyclooxygenase-2 (COX-2) is an inducible enzyme expressed in some tumor and stromal tissues, and stimulates the production of prostaglandin E2, which may have growth stimulatory, antiapoptotic, pro-angiogenic and immunosuppressive properties. The pharmacologic inhibition of COX-2 with nonsteroidal antiinflammatory drugs (NSAIDs) has been associated with antitumor activity in various human and canine malignancies, including canine oral squamous cell carcinoma (SCC). The purpose of this study was to assess the expression of COX-2 in a series of archived equine SCC and sarcoids.

COX-2 expression was assessed in formalin-fixed paraffin-embedded tissues from 14 sarcoids and 39 SCC representing various anatomic sites. In 5 SCC cases, paired primary tumors and lymph node or distant metastases were evaluated. Immunohistochemistry was performed using a commercial rabbit polyclonal anti-human antibody (Oxford Biomedical, Oxford, MI) and standard automated immunohistochemical techniques. Equine kidney served as a positive control and substitution of normal rabbit serum for the primary antibody served as a negative control. The percentage of positive COX-2 tumor cells was scored as either 0 = 0%, 1 = 1–5%, 2 = 6–20%, 3 = 21–50%, or 4 = >50% of cells. Additionally, the intensity of the stain uptake was graded as 0 = negative, 1 = weak, 2 = moderate, 3 = strong, and 4 = intense (e.g. equal to or greater than control tissue staining). The percentage score and the intensity grade were then multiplied to give a final immunoreactivity score ranging from 0 to 16. The categories for the final immunoreactivity score were as follows: negative (0), poor (1–3), moderate (4–7), and strong expression (8–16).

COX-2 was expressed in 2 of 14 sarcoids (14%) and 35 of 39 SCC (90%). In the 2 sarcoids expressing COX-2, percentage score was 1 for both and intensity grades were 1 and 2. In SCC, both percentage scores and intensity grades ranged from 0 to 4. Final immunoreactivity scores were negative in 10%, poor in 34%, moderate in 38% and strong in 18%. There were no significant differences in scores or percentage positives between different anatomic sites, but there was a trend toward reduced scores in metastases vs. primary tumors from the same individual.

Based on this information, a majority of equine SCC appear to express COX-2 and thus could be responsive to treatment with NSAIDs. Phase-II evaluation of NSAID therapy for equine SCC is warranted. Techniques are currently being refined to allow assessment of COX-2 immunoreactivity in highly pigmented equine melanomas.

ABSTRACT #127

BORDETELLOSIS IN DOGS: 2000–2005. LM Taylor, LR Johnson, EG Johnson, PH Kass. School of Veterinary Medicine, University of California, Davis.

Appropriate therapy for cough depends on determining the primary cause. This retrospective study examined history, clinical features, radiographic findings, cytologic abnormalities, and microbiologic results to identify discriminating features of *Bordetella* infection in dogs.

Medical records from the University of California, Davis were searched from January 2000 through December 2005 to identify dogs with *Bordetella bronchiseptica* isolated from airway wash fluid. Fifteen cases were identified. For each case, two dogs that had airway sampling performed immediately before and after the affected dog were assigned as matched controls. Clinical data were abstracted for all dogs; radiographs were reviewed in masked fashion. Exact conditional logistic progression was performed to compare potential risk factors between affected and control dogs. Statistical results are reported as P-values, odds ratios (OR), and confidence intervals (CI).

Dogs with bordetellosis were significantly (P: 0.01) younger (mean 4.0 ± 4.2 years) than control dogs (mean 7.1 ± 4.1 years), but the range of affected dogs overlapped. There was a trend towards increased evidence of tracheal sensitivity in dogs with bordetellosis (P: 0.05, OR: 4.6. CI: 1.0–53.1). All dogs with bordetellosis had received some type of antibiotic treatment in the month prior to referral while 20 of 30 (67%) of control dogs had received antibiotics. Only the use of amoxicillin-clavulanic acid was significantly different between the 2 groups; affected dogs were more likely to have received this drug (P: 0.02, OR: 6.2, CI: 1.2–61.3). Prior use of cephalosporins and cough suppressants approached statistical significance (P: 0.06 and P: 0.05), with OR of 5.3 (CI: 0.9–54.7) and 5.3 (CI: 1.0–53.1), respectively. Radiographic findings of interstitial, peribronchial, or alveolar infiltrates did not differ between dogs diagnosed with bordetellosis and those with other causes of cough. While airway wash fluid from both groups of dogs had cytologic evidence of inflammation, dogs with bordetellosis were significantly more like to have bacteria detected cytologically (P: 0.01, OR

11.68, CI 1.5–532.3). This was likely a reflection of positive aerobic cultures in all affected dogs (n = 15) versus only 6 of 30 control dogs. Anaerobic bacteria were found in no dogs with bordetellosis and in 3 of 30 control dogs. This was not statistically significant. *Mycoplasma* species were found in 3 of 30 control dogs and 6 of 15 dogs with bordetellosis. Although this did not reach statistical significance (P: 0.06), it could be clinically significant (OR: 11.68, CI: 0.9–54.7).

No specific clinical or radiographic features distinguished dogs with *Bordetella* infection from dogs with other respiratory diseases prior to isolation of organisms from airway wash samples. This study confirms the need for cytologic and microbiologic assessment of airway samples in dogs with cough to determine appropriate diagnosis and to guide therapy.

ABSTRACT #128

EFFECTS OF CYPROHEPTADINE AND CETIRIZINE ON AIRWAY EOSINOPHILIA IN EXPERIMENTAL FELINE ASTHMA. EK Schooley, CR Reinero; University of Missouri, Columbia, MO.

The use of corticosteroids and bronchodilators has been the mainstay of management of feline asthma. Although generally effective, some cats may develop undesirable side effects or become refractory to this therapy. Therefore, a search for alternative medications is warranted. In a previous study, low dose (2 mg po) cyproheptadine (a serotonin antagonist) showed promise in decreasing airway hyperreactivity in experimental feline asthma. However, at this dose, there was no significant reduction in airway eosinophilia compared with placebo. The pharmacokinetics of cyproheptadine suggests that some cats may require higher doses (up to 8mg po TID) for therapeutic effects. Cetirizine (Zyrtec®) is a 2nd generation selective histamine (H1) antagonist that has been used in humans with allergic disease, including asthma. While cetirizine has been administered anecdotally in allergic cats, its usefulness in feline asthma has not been previously studied. The purpose of this study was to determine the effects of high dose cyproheptadine, and cetirizine on bronchoalveolar lavage fluid (BALF) eosinophil percentages (% BALF eos) in cats with experimentally induced asthma. We hypothesized that if serotonin and histamine are important mediators of the eosinophilic inflammatory response in feline allergic asthma, that a beneficial reduction in % BALF eos should be seen with administration of these drugs.

Nine cats sensitized and challenged with Bermuda grass allergen (BGA) were enrolled in a prospective study that was randomized and blinded, with a crossover design. Drugs were crushed and placed in number 4 gelatin capsules. Cats received alternating 1 week treatments of placebo (flour) BID, cyproheptadine 8 mg po BID, or cetirizine 5 mg po BID. Each treatment period was followed by a 1 week washout period where no drug was administered. Cats were challenged with BGA by aerosol delivery once weekly for 5 minutes for the duration of the study. The aerosol challenge took place 48 hours prior to sample collection. On day 7 of each treatment and washout period, cats were anesthetized for BALF collection. A blind BAL technique was performed by gently passing a 7 Fr polypropylene catheter through the endotracheal tube until resistance was met, and 12 mls of sterile saline was instilled and gently aspirated manually. A 200 nucleated cell count was performed on cytospins of the BALF and % BALF eos was determined. Using univariate repeated measures ANOVA, no significant differences between baseline values of % BALF eos were noted ($p = 0.06$; data not shown), reflecting an adequate washout period. No significant differences were noted between treatment groups with respect to % BALF eos ($p = 0.51$; mean ± SD: placebo 35% ± 19, cyproheptadine 25% ± 17, cetirizine 35% ± 20). In conclusion, administration of cyproheptadine and cetirizine did not decrease airway eosinophilia in experimental feline asthma. This study does not support the use of either drug for the treatment of eosinophilic inflammation in feline asthma.

ABSTRACT #129

FLEXIBLE BRONCHOSCOPY AND BRONCHOALVEOLAR LAVAGE IN THE CAT: PROCEDURE AND OUTCOME (2001–2006). LR Johnson, TL Drazenovich. School of Veterinary Medicine, University of California, Davis.

Bronchoscopy and bronchoalveolar lavage (BAL) are commonly used to determine specific causes of respiratory signs in cats, however a detailed evaluation of technique, complications, and outcome of bronchoscopy and BAL in the cat has not been described.

Feline bronchoscopy procedures were reviewed for the size and type of endoscope used, BAL volumes instilled and recovered, and number and type of complications. Three endoscopes were used for bronchoscopy including Scope 1: a 2.5 mm fiberoptic endoscope, Scope 2: a 3.8 mm videoendoscope, and Scope 3: a 5.0 mm fiberoptic endoscope. Total BAL volume and volume/kg instilled, and percent BAL recovery were compared among scopes using a one-way ANOVA. Data are presented as mean ± SD. Significance was set at $P < 0.05$.

Bronchoscopy was performed in 68 cats using scope 1 (29 cats), scope 2 (17 cats), or scope 3 (13 cats). In 9 procedures, the bronchoscope used was not recorded. 120 BALs were performed in 64 cats via instillation of 5 or 10 ml aliquots. One lavage was performed in 20 cats, 2 lavages in 34 cats, 3 lavages in 8 cats, and 4 lavages in 2 cats. BAL volume instilled (as mls or mls/kg) was significantly lower for scope 2 than scope 3 ($P < 0.05$). When scope 2 was used, a significantly higher percentage of fluid was recovered than when using scope 1 or 3 ($P < 0.05$).

	Scope 1	Scope 2	Scope 3	*P* value
Body weight (kg)	4.45±1.46	5.13±1.32	5.20±1.20	0.1776
Age (yrs)	7.9 ± 4.5	7.4 ± 4.2	7.5 ± 3.9	0.9029
Total mls instilled	15.1 ± 6.1	11.4 ± 4.1	22.8 ± 17.9	0.0118
Total mls/kg	3.32 ± 1.37	2.62 ± 1.81	5.05 ± 2.64	0.0186
Percent volume recovery (%)	51 ± 23	73 ± 23	52 ± 22	0.0004

Overall, 51 of 68 (64%) bronchoscopies were completed without incident. In 7 cats, mild complications were reported and primarily consisted of desaturation to a SpO_2 of <80% (n = 5). The remaining 2 cats had prolonged recovery and were spontaneously breathing but remained intubated for up to 6 hours. Four cats had moderate complications and were placed in an oxygen cage for recovery in the intensive care unit, and 2 cats had severe complications that required intervention, both for pneumothorax. In 1 cat, a fine needle lung aspirate had been performed after bronchoscopy. The second cat developed spontaneous pneumothorax in association with the bronchoscopy procedure or post-procedure ventilation. Four cats were euthanized after the procedure due to failure to alleviate the cause of respiratory distress (n = 1) or poor prognosis for recovery of ventilation (n = 3). Complications occurred in 9/29 procedures with scope 1, 2 of 17 with scope 2, and 3 of 13 with scope 3. The scope was not recorded for 3 events.

Flexible bronchoscopy with collection of multiple BAL aliquots was well tolerated in the majority of cats examined here.

ABSTRACT #130

CLINICAL AND LABORATORY FINDINGS IN 17 CATS WITH CHRONIC BRONCHIAL DISEASE. B Schulz[1], U Müller[1], C Werckenthin[2], S Hecht[3], G Wess[1], R Müller[1], J Hirschberger[1], K Hartmann[1]; [1]Clinic for Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany; [2]Institute for Medical Microbiology, Infectious and Epidemic Diseases, Ludwig-Maximilians-University, Munich, Germany, [3]University of Tennessee, Knoxville, TN, USA.

Considerable research has been performed in the last years investigating pathophysiology and therapeutical aspects in research models of feline asthma, but not much information is available on cats with naturally occurring bronchial disease. Aim of the present study was to investigate historical, clinical, and diagnostic features in cats presented with signs consistent with feline bronchial disease.

Seventeen client-owned cats were prospectively enrolled in the study. Inclusion criteria were history of cough, wheezing and/or episodes of dyspnoea. Excluded were patients with upper respiratory tract disease, neoplasia, cardiovascular, pleural and mediastinal disease. All cats underwent a clinical, laboratory, and cardiologic examination, and thoracic radiographs were taken. Bronchoalveolar lavage (BAL) was performed in all cats and cytological exams and bacterial cultures were investigated.

Median age of the cats was 7 years, median age at onset of clinical signs was 6 years. Chronic cough was the clinical sign most frequently reported by owners (88%). Abnormal breathing pattern or abnormal sounds on auscultation were noted in 94% of cases. Radiographic lung patterns were described as bronchointerstitial in 16 cats (94%), and bronchointerstitial and alveolar in one cat. Cytology of BAL revealed physiologic cell population in 24%, increased numbers of eosinophilic granulocytes in 53%, increased neutrophils in 6%, and signs consistent with mixed neutrophilic/eosinophilic inflammation in 17% of cases, respectively. Bacterial cultures of BAL were negative in 47% of cats. Samples of 2 patients revealed growth of *Mycoplasma* sp.; low numbers of other bacterial species were detected in the remainder of the cases.

The study demonstrated that feline bronchial disease is characterized by chronic cough, abnormal breathing pattern, or pathologic lung sounds. Bronchointerstitial radiographic lung pattern is a common feature of the disease. BAL cell pattern is not pathognomonic for feline bronchial disease and bacterial infection seems uncommon. As in human asthma described, *Mycoplasma* sp. might play a primary or secondary role in feline bronchial disease.

ABSTRACT #131

HEMATOLOGICAL CHANGES IN 42 DOGS NATURALLY INFECTED WITH *ANGIOSTRONGYLUS VASORUM*. Willesen J, Jensen ALJ, Kristensen AT, Koch J. Department of Small Animal Clinical Sciences. Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Canine angiostrongylosis is being diagnosed in increasing numbers worldwide. Untreated the infection is potentially fatal due to severe inflammatory and hemostatic changes. Knowledge of the haematological changes caused by this nematode primarily originates from studies of experimentally infected dogs or from retrospective studies of clinical case series. Most studies have focused on changes present at time of diagnosis. Findings have been inconsistent although eosinophilia and thrombocytopenia often are reported. Few data exists documenting hematological changes during the course of treatment. The objective of the present study was to prospectively investigate the hematological changes occurring during treatment of dogs naturally infected with *A. vasorum*.

54 dogs naturally infected with *A. vasorum* were included in the study. Dogs dually infected with *Crenosoma vulpis* as well as *A. vasorum* were excluded. The dogs were all clinically ill displaying symptoms such as cough or dyspnea, but none of the dogs included presented with overt clinical sign of bleeding. Physical examination, haematological (CBC) and fecal (Baermann) analysis were performed on the day of inclusion and treatment initiation (day 0) and subsequently on day 7 and day 42. Data from dogs with positive Baermann sample on day 42 were excluded (6 dogs), and further six dogs did not have a CBC performed on all three visits. In total data from 42 CBC's was analysed. Mean and 95% CI were calculated for all parameters and paired t-test was performed to compare the initial data on day 0 with results from day 7 and 42.

The means of all hematological parameters before, during and after treatment where within the respective reference intervals. However a significant decrease of total WBC ($p < 0{,}0001$), segmented neutrophil ($p = 0{,}0023$), eosinophil ($p = 0{,}029$), lymphocyte ($p = 0{,}0022$) and monocytes ($p = 0{,}033$) counts were observed when comparing results from day 0 and 7 with day 42. Eosinophilia was present in 9 of 42 dogs on day 0. A slight but non-significant rise in WBC, neutrophils, monocytes and eosinophils were observed from day 0 to 7. No significant changes in PCV, Hb or thrombocytes were demonstrated.

The results presented in this study demonstrates, that although the recorded parameters remains within the reference intervals during the 42 day study period, a natural infection with *A. vasorum* does leads to leukocyte derangements, possibly due to an inflammatory response within the individual patient. These leukocyte derangements, likely attributable to inflammatory response may only be detected when performing serial measurements before and after treatment. The clinical consequence of these findings are that detecting an inflammatory response due to angiostrongylosis in dogs using CBC is not always possible unless serial measurements during and after treatment are performed.

ABSTRACT #132

ATRIAL, SA NODAL, AND AV NODAL ELECTROPHYSIOLOGY IN STANDING HORSES: REFERENCE VALUES AND ELECTROPHYSIOLOGIC EFFECTS OF QUINIDINE AND DILTIAZEM. CC Schwarzwald[1], RL Hamlin[1], JD Bonagura[1], Y Nishijima[1], C Meadows[1], CA Carnes[2]. [1]Veterinary Clinical Sciences, College of Veterinary Medicine and [2]College of Pharmacy, The Ohio State University, Columbus, OH.

The purpose of this study was to describe supraventricular electrophysiology and to characterize the electrophysiologic effects of quinidine and co-administered diltiazem in healthy horses.

Fourteen healthy horses (8–17 years, 468–646 kg) were used in this study.

All studies were conducted in standing, unsedated horses. Arterial blood pressure, surface electrocardiogram, and right atrial electrogram were recorded during sinus rhythm and during programmed electrical stimulation and rapid right atrial pacing. Recordings were made at baseline, after quinidine (10 mg/kg IV over 30 min, n = 7; or 12 mg/kg IV over 5 min, followed by 5 mg/kg/h CRI, n = 7), and following co-administration of diltiazem at repeated doses of 0.125 mg/kg IV over 2 min.

Quinidine significantly prolonged the atrial effective refractory period, shortened the functional refractory period (FRP) of the AV node, and increased the ventricular response rate during rapid atrial pacing. Repeated doses of diltiazem were given until ventricular response rate was not more than 10% above baseline values. Diltiazem increased the FRP, controlled ventricular rate in a frequency-dependent manner, and caused dose-dependent suppression of the sinoatrial node. The cumulative effective dose of diltiazem ranged from 0.125 to 1.125 mg/kg. Quinidine and diltiazem caused a significant, but well tolerated decrease in arterial blood pressure.

Supraventricular electrophysiologic parameters can be determined in standing, unsedated horses by use of standard techniques. Diltiazem, 0.125–1.125 mg/kg IV, is effective and apparently safe for ventricular rate control in this model of supraventricular tachycardia and may be useful for rate control in horses with atrial fibrillation.

ABSTRACT #133

ECHOCARDIOGRAPHIC DETECTION OF ATRIAL MECHANICAL DYSFUNCTION IN HORSES AFTER CONVERSION OF ATRIAL FIBRILLATION TO SINUS RHYTHM. CC Schwarzwald, K Schober, JD Bonagura. College of Veterinary Medicine, The Ohio State University, Columbus, OH.

The purpose of this study was to characterize left atrial (LA) mechanical function by means of two-dimensional echocardiography and pulsed-wave tissue Doppler imaging (TDI) in normal horses and in horses after conversion of atrial fibrillation (AF) to sinus rhythm (SR).

Ten healthy Standardbred controls (3–16 y, 457–586 kg) and 5 Standardbreds treated for AF (2–8 y, 427–498 kg) were included in this study. The duration of AF ranged between 3 days and over 5 months. Four horses had lone AF. One horse was diagnosed with dilated cardiomyopathy based on echocardiographic findings. Four horses were treated medically (quinidine PO) and one horse was treated by means of transvenous electrical cardioversion after medical treatment had failed.

Echocardiography was performed in non-sedated horses using a GE Vivid 7 echocardiograph. Horses with AF were examined 24 and 72 hours after conversion to sinus rhythm. All studies were performed by a single examiner and stored for off-line analysis. All measurements were performed batch-wise and in random order with the examiner blinded to patient information and time-point of examination. Left atrial volume (modified Simpson's), area, and diameter were determined at the onset of active contraction (onset P wave) and at end-diastole (closure of mitral valve) using a right-parasternal long-axis view optimized for the left atrium. Active fractional volume change (LA-FVC), fractional area change (LA-FAC) and fractional shortening (LA-FS) were calculated. The TDI wall motion pattern of the left-atrial free wall was characterized by a consistent biphasic wave. Maximal positive wall motion velocity (v_{max}), time from onset of the P wave to beginning of the positive wave (t_{Am}) and duration of the positive wave (d_{Am}) were measured and t_{Am}/d_{Am} was calculated. Control values were compared to 24 h and 72 h values, respectively, using a Mann-Whitney test. Values were reported as median [min–max]. The level of significance was $p = 0.05$.

LA-FVC, FAC, and FS were 27 [14–34] %, 20 [10–28] %, and 9 [4–13] % in controls, −2 [(−9)–(+9)] %, −4 [(−8)–(+7)] %, and 1 [(−5)–(+1)] % in AF horses 24h after conversion (AF24; $p = 0.003$, 0.003, 0.003), and 0 [(−38)–(+16)] %, 7 [(−22)–(+12)] %, and 2 [(−12)–(+5)] % 72 h after conversion (AF72; $p = 0.004$, 0.012, and 0.006). LA v_{max} was 4.7 [2.5–11.0] cm/s (controls), 1.8 [0.0–3.8] cm/s (AF24; $p = 0.017$), and 2.5 [1.5–3.9] cm/s (AF72; $p = 0.020$). LA t_{Am}/d_{Am} was 1.1 [0.6–1.3] (control), 1.6 [1.5–1.9] (AF24; $p = 0.006$), and 1.4 [1.2–1.9] (AF72; $p = 0.012$). Residual plasma quinidine concentrations were 0.37 [0.16–0.67] µg/mL (AF24) and 0.17 [0–0.26] µg/mL (AF72).

These results demonstrate that LA function in horses can be significantly depressed for at least 3 days after conversion of AF to SR, and may indicate AF-induced functional atrial remodeling or underlying myopathy. Further studies are required to assess the influence of athletic condition, duration of AF, underlying heart disease, and the method of treatment on atrial mechanical dysfunction, the time course of recovery of mechanical function, and its prognostic relevance.

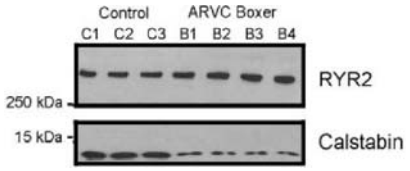
ABSTRACT #134

ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY IN BOXER DOGS IS ASSOCIATED WITH CALSTABIN2 (FKBP12.6) DEFICIENCY. MA Oyama[1], S Reiken[2], KM Meurs[3], AR Marks[2]. [1]Department of Clinical Sciences, University of Pennsylvania, Philadelphia, PA, [2]Columbia University, College of Physicians and Surgeons, New York, NY, [3]Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a common cardiac affliction in the Boxer dog, and is characterized by ventricular arrhythmias and sudden death. In humans, certain forms of ARVC are associated with abnormalities of the cardiac ryanodine receptor (RYR2) and its associated regulatory molecule, calstabin2 (also called FKBP 12.6). In cardiac myocytes, RYR2 is located on the sarcoplasmic reticulum (SR) and

controls release of calcium from SR stores into the cytosol during excitation-contraction coupling. Thus, RYR2 channels are open during systole and closed during diastole. Binding of calstabin2 to the tetrameric RYR2 molecule stabilizes the closed state of RYR2 preventing calcium "leak" during diastole. Loss of calstabin2 binding through decreased calstabin2 transcription or sympathetically-mediated PKA hyperphosphorylation of RYR2 ostensibly causes diastolic "leak" of calcium into the cytosol, which can trigger arrhythmias and reduce efficiency of calcium cycling for myocardial contraction. Based on the critical role of RYR2 and calstabin2 in the development of arrhythmias, we hypothesized that Boxers with ARVC would possess alterations in the RYR2-calstabin2 complex. Left ventricular tissues from 4 Boxers with ARVC were obtained at post-mortem. mRNA levels of RYR2 and calstabin2 genes were determined using a canine oligonucleotide microarray and validated via real-time PCR. Results demonstrated that calstabin2 mRNA in Boxers was significantly down-regulated as compared to healthy controls and Doberman pinschers with dilated cardiomyopathy (Boxer vs. control, 13.3-fold downregulation; Boxer vs. Doberman, 7.0-fold downregulation). Immunoprecipitation and immunoelectrophoresis indicated markedly reduced amounts of calstabin2 in the RYR2 complex of affected Boxers as compared to controls while mRNA levels of RYR2 were unaffected. Our data indicate significant calstabin2 deficiency in Boxers with ARVC. Loss of calstabin2 is a likely cause of the clinical signs associated with ARVC in these dogs. Further work involving potential calstabin2 gene mutations, right ventricular calstabin2 levels, and response to treatment is warranted.

Immunoelectrophoresis of immunoprecipitated RYR2-Calstabin2 complex. Boxers with ARVC demonstrate markedly reduced calstabin2 amounts compared to control.



ABSTRACT #135

COMPARISON OF SOTALOL AND MEXILETINE VERSUS STAND ALONE SOTALOL IN TREATMENT OF BOXER DOGS WITH VENTRICULAR ARRHYTHMIAS. Robert Prošek,[1,2] Amara Estrada,[1] Darcy Adin.[1] [1]University of Florida, Gainesville, FL [2]Veterinary Specialists Incorporated –Animal Heart Centers, Miami, FL.

Ventricular tachyarrhythmias are common in Boxer dogs with the end result of uncontrolled tachyarrhythmias being progression of clinical signs such as syncope or potentially death. The purpose of this study was to evaluate the effect of two antiarrhythmic protocols on number of ventricular premature complexes (VPC), ventricular tachycardia (v-tach) and/or R on T, and syncopal episodes in Boxer dogs with ventricular arrhythmias. Boxers with >500 VPC or grade 4 severity arrhythmia [VPC on the downslope of preceding T wave (R on T), ventricular tachycardia (>3 consecutive VPC) or both] based on a 24-hour Holter recording were treated with either sotalol or sotalol and mexiletine orally for 13 to 17 days. Patients were excluded if they had concurrent diseases but no attempt was made to exclude patients based on cardiac function or cardiac chamber size. Results of pre- and post-treatment Holter monitors were compared in regard to number of VPC/24 hr, ventricular tachycardia, R on T, and syncopal events. Sixteen Boxers were randomized with eight receiving sotalol (1.5–3.1 mg/kg BID) and eight receiving sotalol and mexiletine (1.5–3.0 mg/kg BID and 5–7.5 mg/kg TID). Treatment efficacy was defined as >85% reduction in number of VPC and/or absolute elimination of ventricular tachycardia. $P < 0.05$ was considered significant. One dog receiving standalone sotalol had to be withdrawn due to changes in his background medications. Greater than 85% reduction in number of VPC was noted in 7/8 dogs treated with sotalol and mexiletine, while only 2/7 dogs had 85% reduction of VPC with standalone sotalol ($P = 0.04$). Furthermore, dogs in mexiletine-sotalol group had a 100% reduction (7/7) in v-tach and/or R on T, while the sotalol group had a 33% (2/6) reduction resulting in a significant difference between the two groups ($P = 0.02$). Significant reductions were also noted in maximum and mean HR in dogs treated with mexiletine-sotalol as compared to sotalol alone. Significant changes in syncope were not observed pre- and post-treatment in either treatment group. In conclusion, this study suggests that combination antiarrhythmic therapy with sotalol and mexiletine can be successful in Boxers and warrants further investigation.

ABSTRACT #136

PHYSIOLOGICAL FLOW MURMURS IN CAVALIER KING CHARLES SPANIELS. LH Olsen, R Hjarbaek, HD Pedersen. Department of Basic Animal and Veterinary Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Physiological flow murmurs are common findings in young dogs predisposed to myxomatous mitral valve disease and constitute an important differential diagnosis to soft mitral regurgitation murmurs. Only experienced observers are able to distinguish between these murmurs using auscultation. A better understanding of the physiological background is desired. The aim of the study was to evaluate echocardiographic and hematologic changes in Cavalier King Charles Spaniels (CKCS) with physiological flow murmurs.

12 CKCS (3 males and 9 females; 3.9 ± 1.8 ys (mean ± SD)) with physiological flow murmurs and 11 control dogs (CKCS without physiologic flow murmurs) (4 males and 7 females, 4.9 ± 2.3 ys (mean ± SD)) were included. Cardiac auscultation and echocardiographic examination were performed. Mitral regurgitation assessed by color flow mapping was an exclusion criteria for the study. Measurements of left ventricular dimensions and blood outflow from the heart were made from the video-recorded echocardiograms: left ventricular end-diastolic diameter, left ventricular end-systolic diameter, aortic diameter (valvular, subvalvular and supravalvular), interventricular septum thickness, degree of interventricular septum protrusion into the left ventricular outflow tract during systole, maximal aortic respectively pulmonary flow velocity. Hematocrit and plasma protein concentration were evaluated with regard to blood viscosity.

The CKCS with physiological flow murmurs had an increased maximal outflow rate in aorta ($P < 0.0001$) (1.48 ± 0.16 m/s (mean ± SD)) and increased heart rate during the cardiac examination ($P = 0.002$) (134 ± 24 beats/min (mean ± SD)) compared to control dogs (1.15 ± 0.11 m/s and 106 ± 14 beats/min (mean ± SD)). In addition, a decreased septum thickness was found in CKCS with physiological flow murmurs ($P = 0.03$) (9 mm (9–11) (median and quartiles)) compared to control dogs (10 mm (9–10) (median and quartiles)). No difference in left ventricular diameter, aortic diameter, degree of interventricular septum protrusion, maximal pulmonary flow velocity, hematocrit or plasma protein concentration was found between CKCS with physiological flow murmurs and control dogs. All auscultated physiological flow murmurs were of low to moderate intensity (<grade 4 (out of 6)).

In conclusion, CKCS with physiological flow murmurs have increased maximal aortic flow velocity and have increased heart rate compared to CKCS without physiological flow murmurs. A decreased thickness of the interventricular septum was found in CKCS with physiological flow murmurs, however, no other echocardiographic changes or changes in hematocrit and plasma protein concentration were found.

ABSTRACT #137

THE TRANSCRIPTION OF ENDOTHELIN-1 AND RECEPTORS IN MITRAL VALVES EXPOSED TO STATIC STRETCH. LG Pedersen[1], J Zhao[2], J Yang[2], PD Thomsen[1], H Gregersen[2], JM Hasenkam[3], M Smerup[3], HD Pedersen[4], LH Olsen[1]. [1]Department of Basic Animal and Veterinary Sciences, The Royal Veterinary and Agricultural University, Copenhagen, Denmark [2]Centre of Excellence in Visceral Biomechanics and Pain, Aalborg Hospital, Aalborg, Denmark [3]Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Skejby Sygehus, Denmark [4]Safety Pharmacology, Novo Nordisk A/S, Maaloev, Denmark.

Endothelin-1 (ET-1) appears to play a role in the pathogenesis of myxomatous mitral valve disease (MMVD) but the mechanism by which it acts is poorly understood. Previous studies indicate an involvement of the endothelin system in stretch stimulated pathways of different cell types. As an altered valvular motion is a central theme in MMVD the aim of this study was to investigate if the transcription of ET-1 and ET receptors A and B (ET_A-R and ET_B-R) was influenced by stretch loading in mitral valves leaflets.

One leaflet specimen from each of 10 porcine mitral valves were exposed to a static stretch load of 1.5 N for 3.5 hours in an oxygenated normal Krebs Ringer (NKR) buffer at 37°C together with matching control specimens without weight loading. Weight was loaded in the papillary muscle with one primary chorda connecting to the leaflet. Subsequently, the abundance of ET-1, ET_A-R and ET_B-R mRNA was estimated by real-time PCR in the chordal insertion area of the leaflets and expressed relative to the level of 18S rRNA. Viability studies showed a cell survival of min. 98% after 8 hours in NKR buffer at 37°C.

The analyses showed an increased transcription of ET_B-R in stretch-exposed leaflet segments compared to unstretched segments (median 2.23 (quartiles 1.37 and 2.70) vs. median 1.56 (1.38 and 2.17), $P = 0.03$) whereas the mRNA expression of ET_A-R (0.11 (0.08 and 0.21) vs. 0.12 (0.07 and 0.22), $P = 0.90$) and ET-1 (5.84 (4.22 and 9.69) vs. 4.91 (3.34 and 5.99), $P = 0.51$) remained unchanged.

Thus, stretch increased the transcription of ET_B-R in porcine mitral valve leaflets. It is speculated that ET_B-R plays a protective role in an attempt to retain valvular function by increasing the nitric oxide (NO) production. The finding could lead to a better understanding of the pathogenesis of myxomatous mitral valve disease.

ABSTRACT #138

CLOPIDOGREL (PLAVIX®) AND COLLATERAL VESSEL DEVELOPMENT IN EXPERIMENTAL FELINE AORTIC THROMBOSIS. DF Hogan[1], WR Widmer[1], MP Ward[2]. [1]Purdue University School of Veterinary Medicine, West Lafayette, IN. [2]Texas A&M University College of Veterinary Medicine, College Station, TX.

Cardiogenic embolism is common in cats and associated with high morbidity and mortality. The primary site of infarction is the aortic trifurcation which results in paresis or paralysis. However, experimental feline models have demonstrated that interruption of aortic flow alone will not result in clinical signs due to an extensive collateral circulatory network which allows flow around the site of occlusion. Platelet-released serotonin has been implicated in the loss of this collateral flow and pre-treatment with serotonin antagonists increases collateral circulation in experimental models of feline aortic thrombosis. Clopidogrel exhibits an in vitro vasomodulating effect with reduced vasoconstrictive response to serotonin and other vasoactive substances in femoral and pulmonary arterial ring preparations. Clopidogrel exerts dramatic antiplatelet effects in the cat including reduced serotonin release. The goals of this study were to demonstrate if clopidogrel prevents the loss of collateral flow associated with an *in vivo* experimental feline aortic thrombosis model and if this would result in improved neurologic function.

Ten normal, purpose bred cats were randomly allocated to receive either placebo or clopidogrel (75 mg PO q 24 hrs) in blinded fashion. The model was created by a combination of aortic ligation and coil embolization. Blood flow to the distal limbs was quantified by angiography and nuclear scintigraphy at baseline and 48 hours post-thrombosis. Comparative neurologic and motor scales were used to assess neurologic function at baseline, anesthetic recovery, 24 and 48 hours post-thrombosis. Differences between treatment groups at study entry, end of study and within groups between study entry and end of study were determined.

Clopidogrel resulted in improvement in all parameters however, there were only significant improvements in; 1) comparative neurologic scale score in the left limb at recovery and 24 hrs, right limb and cumulative limb score at 24 hrs and 2) motor scale score at recovery and 48 hrs.

We conclude that clopidogrel appears to exert an in vivo vasomodulating effect in this experimental model of feline aortic thrombosis. The findings from this small pilot study suggest that clopidogrel therapy may result in improved pelvic limb circulation and reduced clinical signs with cardiogenic embolism which could improve morbidity and mortality with this devastating disease. Additionally, this in vivo evidence may impact preventative therapy for human patients at risk for ischemic stroke.

ABSTRACT #139

DETERMINATION OF THE EFFECT OF LOW MOLECULAR WEIGHT HEPARIN ADMINISTRATION ON COAGULATION PARAMETERS IN HEALTHY CATS. C Vargo, S Taylor, A Carr. Western College of Veterinary Medicine, Saskatoon, SK.

Administration of low molecular weight heparins (LMWH) has been recommended as an alternative to unfractioned heparin (UH) in the treatment of cats with feline aortic thromboembolism. LMWH has several advantages over UH including a significant reduction in the potential for therapy-induced hemorrhagic complications. There is, however, very little published data regarding the optimal protocol for LMWH administration in cats. The objectives of the present study were to: 1) determine the effect of LMWH administration according to a clinically recommended protocol on anti-Xa activity in normal cats; 2) determine whether alterations in anti-Xa activity are associated with alterations in prothrombin time (PT), partial thromboplastin time (PTT) or clinical bleeding; and 3) determine the effect of LMWH administration on platelet count and antithrombin III (ATIII) concentrations in cats.

A standard dose of 100 IU/kg of the LMWH, Dalteparin sodium was administered subcutaneously to 8 healthy cats twice daily for 7 days (13 doses). Blood samples were obtained to measure anti-Xa activity at time 0 and 4, 6, 8 and 12 hours after the first dose and at 4, 6, 8 and 12 hours after the last dose. Anti-Xa activity was measured using a validated assay at the Cornell Veterinary Coagulation Laboratory. PT, PTT, ATIII, platelet count, and PCV/TP were determined at time 0 on day 1 and 4 hours after the final dose on day 7. PTT was also measured 4 hours post-dalteparin administration on day 2.

Dalteparin administration according to this protocol resulted in maximal detectable anti-Xa activity at 4 hours in 4/8 cats, returning to baseline by 6 hours. No anti-Xa activity was detected in the remaining 4 cats at any sampling period. PT, PTT, ATIII, platelet count and PCV/TP were unaffected by LMWH administration at this dose. These results suggest that this dose of Dalteparin is inadequate to achieve an anticoagulant effect and further studies are warranted to determine the proper dose and dosing interval.

ABSTRACT #140

EFFECTS OF CLINICALLY RECOMMENDED DOSES OF IV ADENOSINE AND VAGAL MANEUVERS ON HEART RATE AND BLOOD PRESSURE IN CONSCIOUS DOGS. MT Small, MA Booth, Y Fujii, TC DeFrancesco, CE Atkins, NJ Olby, BW Keene. North Carolina State University, Raleigh, NC.

Adenosine and various vagal maneuvers are advocated for diagnosis and therapy of supraventricular tachycardias. This study examined the effects of adenosine and vagal maneuvers on heart rate, PR interval and blood pressure in normal conscious dogs. Six dogs were anesthetized with propofol and instrumented with cephalic, central venous, and pedal arterial catheters. Arterial pressure and a lead II ECG were monitored continuously. The dogs were considered awake for study when consecutive recordings of heart rate and arterial pressure taken every 10 minutes varied by $<10\%$. The order of maneuvers (vagal, adenosine and placebo) was randomized, and the investigators recording and measuring the data were blinded to the maneuvers. Adenosine was administered peripherally and centrally at doses of 6, 3, 1.5, 0.5, 0.25, and 0.125 mg, each followed by a 3 ml flush. Vagal maneuvers included ocular pressure, carotid sinus massage, and gag reflex induction. Simulated ocular pressure served as a sham and 3ml saline injections followed by flush served as placebo. Repeated measures ANOVA and Bonferroni multiple comparison testing were used to identify maneuvers that significantly changed the ECG or arterial pressure. Pre-maneuver parameters remained constant, and no differences were detected over time. Physical vagal maneuvers failed to alter heart rate or arterial pressure significantly. Adenosine significantly reduced arterial pressure before commensurate increases in heart rate occurred. This vasodilatory response to adenosine and the apparent activation of the baroreceptor reflex may be responsible for the lack of clinical utility of adenosine in the diagnosis and treatment of supraventricular tachyarrhythmias in dogs.

ABSTRACT #141

EVALUATION OF THE NORMAL FELINE PANCREAS WITH COMPUTED TOMOGRAPHY. K Mix, MH Fabiani. Gulf Coast Veterinary Specialists, Houston, TX.

Pancreatitis is the most common disease of the feline exocrine pancreas, but antemortem diagnosis of feline pancreatitis remains a diagnostic challenge. In human medicine, computed tomography (CT) of the abdomen is a valuable tool for the diagnosis of pancreatitis. Use of CT for the diagnosis of pancreatitis in veterinary medicine has not been widely employed, and the efficacy of this modality for the diagnosis of feline pancreatitis is unknown. Published data on the use of CT for the assessment of the feline pancreas in health and in disease are conflicting. The purpose of this study was to determine whether the normal feline pancreas can be reliably identified with CT imaging, to identify landmarks for the localization of the normal feline pancreas, and to determine whether the use of oral or intravenous (IV) contrast agents enhanced visualization and assessment of the normal feline pancreas.

Ten staff-owned adult cats, between the ages of four and eleven years were used in this study. Each cat participated in this study for two days. On the first day, each cat received a physical examination, a complete blood cell count (CBC), blood chemistry profile, urinalysis, abdominal radiographs, and abdominal ultrasound. Each cat was judged to be free of any serious health problems and of any disease affecting the pancreas or GI tract. The cats were fasted overnight before the second day of the study. Anesthesia was induced in each patient with either IV propofol or inhaled isoflurane. Each cat was intubated and anesthesia maintained with isoflurane. Cats were positioned in dorsal recumbency and helical CT images were obtained of the cranial abdomen during a breath hold. Three helical CT series were obtained over the same region of the cranial abdomen in each cat. The first series was non-contrast enhanced. The second series was enhanced with iothalamate

sodium 400 mgI/ml IV contrast, at a dose of 400 mgI/pound. The third series was enhanced with IV and oral contrast.

This study identified the pancreas in all ten cats using non-contrast enhanced, IV contrast enhanced, and oral contrast enhanced CT imaging. The body of the normal feline pancreas is ventral to the portal vein. The left limb of the normal feline pancreas is adjacent to the gastric body. The right limb of the normal feline pancreas is adjacent to the duodenum. The normal feline pancreas is homogenous and smoothly marginated. It is hyperattenuating to the spleen and the liver. Identification and assessment of the normal feline pancreas is improved with IV contrast enhancement. The use of oral contrast administration improved visualization of the pancreas in only two cats (20%).

Results of this study demonstrate that the normal feline pancreas may be reliably identified with helical CT imaging. Intravenous contrast enhancement is useful for identification of the feline pancreas. Oral contrast enhancement may also improve the ability to identify and assess the pancreas, but the value of oral contrast was inconsistent in this study. Further studies evaluating the appearance of the diseased feline pancreas are warranted.

ABSTRACT #142

EVALUATION OF ENDOSONOGRAPHY AS A NEW DIAGNOSTIC TOOL IN FELINE PANCREATITIS. A Schweighauser[1], F Gaschen[2], K Allenspach[3], J Steiner[4], L Gaschen[2]. [1]Vetsuisse Faculty, University of Bern, Switzerland. [2]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA. [3] Royal Veterinary College, University of London, GB. [4]Gastrointestinal Laboratory, Texas A&M University, College Station, TX, USA.

Cats with pancreatitis often present with vague and inconsistent clinical signs. Therefore, accurate diagnosis and therapeutic intervention are difficult to achieve. The current gold standard of diagnosis is based on the measurement of feline pancreatic lipase (fPLI) concentration in serum, which may take several days depending on assay availability. Most cases are therefore clinically diagnosed by a combination of clinical symptoms and transabdominal ultrasound (AUS) findings consistent with pancreatitis. The purpose of this study was to evaluate endosonography (EUS) as an additional tool for the diagnosis of feline pancreatitis.

Thirteen healthy cats and 9 cats clinically suspected of having pancreatitis were included in the study. Investigations performed in all cats included serum biochemistry profile, serum amylase and lipase activities, complete blood count, urinalysis, serum fPLI concentration, and AUS. In addition, EUS was performed under general anesthesia and AUS was repeated. The diagnosis of pancreatitis was made on the basis of an increased serum fPLI concentration. The width of the left and right pancreatic limbs as measured by AUS and EUS were compared, echogenicity and homogenicity assessed, as well as subjective comparisons between the two methods in both groups of cats performed.

Of the 13 healthy cats, one was diagnosed with chronic pancreatitis based on fPLI and EUS appearance. In the remaining 12 control cats, no significant differences in sonographic measurements between AUS and EUS were found. However, general visualization of the normal pancreas was superior with EUS. A diagnosis of pancreatitis was confirmed by an elevated serum fPLI concentration in 7 of 9 cats with clinically suspected pancreatitis. Diagnosis of pancreatitis could be made transabdominally in all 7 cats, and no distinct EUS parameter could be identified that altered the diagnosis. However, the pancreatic margins and parenchyma could be resolved better with EUS when compared with AUS in all 7 patients.

In this study, performance of EUS did not alter the diagnosis of 7 cats with pancreatitis when compared to AUS performed by an experienced ultrasonographer. However, EUS may be indicated in obese cats when AUS fails due to higher penetration depth or in cats with a hyperechoic mesentery.

ABSTRACT #143

CONTINUING PANCREATIC INFLAMMATION OR REDUCED EXOCRINE FUNCTION ARE COMMON IN DOGS AFTER ACUTE PANCREATITIS. Jenny G Sinclair[1], Linda M Fleeman[1], Jacquie S Rand[1], David A Williams[2], Jörg M Steiner[3]. [1]Centre for Companion Animal Health, School of Veterinary Science, the University of Queensland, Brisbane, Australia; [2]Department of Clinical Sciences, University of Illinois, Champaign Urbana, IL; [3]GI Laboratory, Texas A&M University, College Station, TX.

Following clinical recovery from acute pancreatitis, dogs might have an increased risk for subclinical pancreatitis. This could cause ongoing destruction of the pancreas and decline in exocrine and/or endocrine function. The incidence of subclinical pancreatitis or exocrine pancreatic insufficiency (EPI) following pancreatitis in dogs is unknown. The aim of this study was to evaluate possible continuing pancreatic inflammation or EPI after acute pancreatitis.

As part of an ongoing, prospective, longitudinal, clinical study, 7 dogs with spontaneous acute pancreatitis were monitored for 6 months. Initial diagnosis was based on rigorous clinical and laboratory criteria in addition to ultrasonographic changes consistent with pancreatitis. All dogs made a complete clinical recovery and were discharged from the hospital within 8 days. No dog showed signs of EPI prior to the study. Serum concentrations of pancreatic lipase immunoreactivity (cPLI), trypsin-like immunoreactivity (cTLI), and C-reactive protein were assayed at 0, 1, 2, 4, and 6 months. Owners kept daily records of any clinical signs consistent with pancreatitis or EPI.

During the initial clinical episode, 5/6 dogs (83%) in which cPLI was measured had an increased cPLI value diagnostic for pancreatitis (>200 µg/L). The other dog had a value above the reference range (2.2–102.1 µg/L) but below the diagnostic cut-off value. During the 6 month follow-up, 2 dogs (28%) had a serum cPLI concentration >200 µg/L on at least one occasion. Neither dog showed clinical signs consistent with pancreatitis. At initial presentation, serum cTLI concentration was within the reference range (5–35 µg/L) for all 7 dogs. By 2 months following recovery 2/7 dogs (28%) had decreased cTLI concentrations of 3.0 and 2.6 µg/L. All subsequent cTLI values for these 2 dogs were consistent with EPI (<2.5 µg/L), but clinical signs were not observed in either dog. The dog that initially had a serum cPLI concentration above the reference range, but below the diagnostic cut-off value, was 1 of the 2 dogs that subsequently developed a very low serum cTLI. This dog also had serum cPLI values consistently below the reference range (<2.2 µg/L) following clinical recovery. The other dog with serum cTLI <2.5 µg/L also had a cPLI value below the reference range on one occasion. Serum C-reactive protein concentration was increased (>7.6 mg/L) in 6/7 dogs (86%) at initial diagnosis, but not subsequently.

In summary, more than half the dogs (4/7, 57%) had evidence of either continuing pancreatic inflammation or reduced functional mass of acinar cells following clinical recovery from acute pancreatitis. We conclude that continuing, subclinical, pancreatic inflammation is common in dogs that have apparently recovered from acute pancreatitis.

ABSTRACT #144

ASSOCIATION BETWEEN SERUM TRIGLYCERIDE AND CANINE PANCREATIC LIPASE IMMUNOREACTIVITY (cPLI) CONCENTRATIONS IN MINIATURE SCHNAUZERS. PG Xenoulis, JS Suchodolski, CG Ruaux, ES Swim, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Miniature Schnauzers are generally considered to have a high incidence of both idiopathic hypertriglyceridemia and pancreatitis. In human beings hypertriglyceridemia is a possible cause of pancreatitis, and such a relationship has also been suggested for dogs. However, studies about a cause and effect relationship of hypertriglyceridemia and pancreatitis in dogs are lacking. In recent studies, serum canine pancreatic lipase immunoreactivity (cPLI) was reported to be both highly sensitive and specific for the diagnosis of pancreatitis. The aim of this study was to investigate a possible association between serum triglyceride (TG) and cPLI concentrations in Miniature Schnauzers.

Serum cPLI and TG concentrations were measured in serum samples from 195 Miniature Schnauzers submitted to the Gastrointestinal Laboratory for analysis of gastrointestinal function. Samples were then grouped according to serum cPLI concentration (greater or lower than 200 µg/L, the current cut-off value for the diagnosis of pancreatitis). The association between serum TG and cPLI concentrations was analyzed by calculation of correlation coefficient, receiver operator characteristic (ROC), likelihood ratio, and odds ratio (GraphPad Prism 4.0).

Fifty of 195 dogs (25.6%) had a serum cPLI concentration of 200 µg/L or more and 145 of 195 dogs (74.4%) had a serum cPLI concentration below this value. Also, 115 dogs (59.0%) had a TG concentration above 108 mg/dl (reference range 26–108 mg/dl) and 80 dogs (41.0%) had a TG concentration below this value. There was a significant positive correlation between serum TG and serum cPLI concentrations (Spearman $r = 0.321$; $p < 0.0001$). Calculations of likelihood ratio for elevated cPLI suggested a cut-off TG concentration of 902.5 mg/dl (likelihood ratio: 3.95). The odds ratio for an elevated serum cPLI concentration with a TG concentration of 902.5 mg/dl or more was 4.7 ($p = 0.012$; 95% confidence interval: 1.42 to 15.58). Twelve dogs had a TG concentration of 902.5 mg/dl or more and 7 of those dogs (58%) had a cPLI above 200 µg/L, while of the 183 dogs with TG concentration below 902.5 mg/dl, 42 (23%) had a PLI above 200 µg/L ($p = 0.012$). Finally, medical records were available for 11 of the twelve dogs with

a TG concentration of 902.5 mg/dl or more, and all (100%) had clinical signs compatible with pancreatitis and/or a history of chronic pancreatitis.

In conclusion, there was a significantly higher likelihood for Miniature Schnauzers with TG $\geq$ 902.5 mg/dl to have a serum cPLI concentration above 200 µg/L compared to Miniature Schnauzers with TG $<$ 902.5 mg/dl. These findings suggest that hypertriglyceridemia, when severe, is a risk factor for pancreatitis in Miniature Schnauzers.

ABSTRACT #145

FALSE POSITIVE RESULTS OF MEASUREMENT OF FECAL ELASTASE CONCENTRATION FOR THE DIAGNOSIS OF EXOCRINE PANCREATIC INSUFFICIENCY IN DOGS. JM Steiner[1] and N Pantchev[2]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Vet Med Labor GmbH, Ludwigsburg, Germany.

Recently a new test for the diagnosis of exocrine pancreatic insufficiency (EPI), fecal elastase concentration, has been developed and validated for both humans and dogs. However, in both species false positive test results have been reported. The goal of this study was to identify the rate of false positive test results for fecal elastase in a group of dogs with severely decreased fecal elastase concentrations.

Fecal samples from 31 dogs that had been submitted because of a clinical suspicion of EPI and had shown a subnormal fecal elastase concentration ($\leq$40 mg/g feces; Fecal Elastase 1®, ScheBo Tech) were selected for this study. Samples were divided into 2 groups, those with severely decreased fecal elastase concentrations ($\leq$10 mg/g feces; considered diagnostic for EPI) and those with fecal elastase concentrations in the equivocal range ($>$10 but $\leq$40 mg/g feces). Serum trypsin-like immunoreactivity concentration (cTLI; reference range 5.0 to 35.0 µg/L) was measured in serum samples from all 31 dogs and a serum cTLI concentration of $\leq$2.5 µg/L was considered diagnostic for EPI, while a serum cTLI concentration $<$5.0 µg/L but $>$2.5 µg/L was considered to be in the equivocal range.

Of the 31 dogs with decreased fecal elastase concentrations, 26 had severely decreased concentrations and 5 had concentrations in the equivocal range. Of the 26 dogs with severely decreased fecal elastase concentrations, 20 (76.9%) had serum cTLI concentrations diagnostic of EPI, while 6 (23.1%) had serum cTLI concentrations within or above the reference range (values: 6.4, 8.0, 9.5, 10.9, 42.3, and 66.4 µg/L). Of the 5 dogs with fecal elastase concentrations in the equivocal range one dog had a serum cTLI concentration in the equivocal range (value: 3.2 µg/L). The other 4 had serum cTLI concentrations within the reference range (values: 7.7, 8.2, 8.3, and 8.8 µg/L).

In conclusion, fecal elastase concentration is associated with a high rate (23.1%) of false positive test results when serum cTLI concentration is used as the gold standard. Given the high cost of treatment of EPI, especially in large breed dogs and the associated rate of euthanasia in dogs diagnosed with this disease, a false positive rate of 23.1% is of concern. Therefore, a diagnosis of EPI should be confirmed by measurement of serum cTLI concentration in dogs with a decreased fecal elastase concentration. The cause of decreased fecal elastase concentrations in dogs that do not have EPI remains to be determined.

ABSTRACT #146

ACTIVATION OF NUCLEAR FACTOR-KAPPA B IN DOGS WITH CHRONIC ENTEROPATHIES. N Luckschander[1], M Welle[2], J Hall[3], U Forster[2], N Wenzlow[2], P Hermann[2], D Dobbelaere[2], F Gaschen[4]. [1]Department of Clinical Veterinary Medicine and [2]Department of Veterinary Pathology, University of Bern, Switzerland. [3]Department of Biomedical Sciences, Oregon State University, Corvallis, OR. [4]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

Homeostasis in the intestinal microenvironment between the immune system and luminal antigens from bacterial flora or food appears disrupted in canine chronic enteropathies (CE). Increased numbers of CD4 T cells are present in the duodenal mucosa of dogs with CE. Associated proinflammatory cytokines likely play a role in the pathogenesis of these disorders. Many of the proinflammatory cytokine genes have associated nuclear factor-kappaB (NF-κB) binding sites, which allow NF-κB to regulate gene transcription. The purpose of this study was to investigate 1) the occurrence of NF-κB activation during mucosal inflammation *in situ*, 2) the mucosal distribution pattern of cells producing activated NF-κB within treatment groups, and 3) the effect of specific therapy on NF-κB expression.

NF-κB activation was investigated in duodenal mucosal biopsy specimens of 27 dogs with CE (13 dogs with food responsive disease [FRD], 14 dogs with inflammatory bowel disease [IBD]), and 13 healthy control animals. Twenty-one dogs with CE (10 FRD, 11 IBD) were available for repeat evaluation following therapy. NF-κB activation was detected using a mouse monoclonal antibody, which selectively binds to the nuclear localization sequence of activated NF-κB. To identify macrophages in the lamina propria, biopsies were stained using the MAC 387 antibody, which recognizes calprotectin. Using a square eyepiece reticule and standard light microscopy at 500x magnification, double stained cells for NF-κB and MAC 387 in the lamina propria were counted and expression of activated NF-κB in epithelial cells was analyzed semiquantitatively.

Our study revealed that significantly greater numbers of NF-κB positive macrophages are present in dogs with CE compared to control dogs ($p < 0.01$). Activation of NF-κB was significantly up-regulated in epithelial cells of FRD dogs compared to IBD dogs ($p = 0.03$). After therapy, the number of NF-κB producing macrophages and of up-regulated epithelial cells were significantly decreased ($p < 0.01$, respectively) in dogs with CE, suggesting down-regulation following successful therapy. In conclusion, activation of NF-κB appears to play a pivotal role in the pathophysiology of canine CE, especially in dogs with FRD.

ABSTRACT #147

DETECTION OF THE GENE ENCODING *CLOSTRIDIUM PERFRINGENS* ENTEROTOXIN IN THE SMALL INTESTINE OF HEALTHY DOGS AND DOGS WITH DIARRHEA. PG Xenoulis[1], JM Steiner[1], L Granly[2], T Egelund[2], JS Suchodolski[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX. [2]Karlslunde Dyreklinik, Karlslunde, Denmark.

Clostridium perfringens is a normal constituent of the intestinal microflora in dogs, but has also been associated with diarrheal disease in this species. The pathophysiology of *Clostridium perfringens* associated diarrhea has not been determined in dogs, although *Clostridium perfringens* enterotoxin (CPE) has most commonly been suspected to cause diarrhea. However, previous studies were unable to establish a cause and effect relationship between the presence of this toxin and/or the gene encoding CPE (CPE gene) in feces and diarrhea. Recent evidence suggest that CPE might be associated with small, rather than large, intestinal disease. Thus, the aim of this study was to identify and compare the prevalence of the CPE gene in the small intestine between healthy dogs and dogs with diarrhea.

Intestinal content was collected from the small intestine of 27 healthy dogs from multiple sources that were euthanized for unrelated projects, and 23 dogs with diarrhea that underwent endoscopy as part of their diagnostic work up. Bacterial DNA was purified by phenol:chloroform:iso-amylalcohol extraction. The presence of *Clostridium perfringens* was detected by polymerase chain reaction (PCR) amplification using specific primers targeting the genes encoding *Clostridium perfringens* 16S ribosomal RNA (16S rDNA) or *Clostridium perfringens* alpha toxin. The CPE gene was also amplified by PCR using specific primers. Polymerase chain reaction amplicons were separated by electrophoresis in 2% agarose gel. The association between the presence of the CPE gene and diarrhea was evaluated by Fisher's exact test.

Clostridium perfringens was identified in the small intestine of 19 (70.4%) of the 27 healthy dogs and 13 (56.5%) of the 23 dogs with diarrhea. The frequency of *Clostridium perfringens* identification was not statistically different between the two groups of dogs ($p = 0.309$). The CPE gene was detected in 0 (0%) of the 27 healthy dogs, and 4 (17.4%) of the 23 dogs with diarrhea. This difference was statistically significant ($p = 0.038$).

In this study, there was a significant association between the presence of the CPE gene in the small intestine and diarrhea. It is important to note that the CPE gene was detected only in the small intestine of dogs with diarrhea, but in none of the healthy dogs. These findings suggest that the presence of *Clostridium perfringens* strains harboring the CPE gene in the small intestine may play a role in the pathogenesis of *Clostridium perfringens* associated diarrhea.

ABSTRACT #148

CLINICAL FEATURES OF FOODBORNE AFLATOXIN HEPATOTOXICITY IN 23 DOGS. D Dereszynski[1], S Center[1], A Hadden[1], J Randolph[1], M Brooks[2], K Palyada[3], S McDonough[3], J Messick[2], K Bischoff[2], H Erb[2], S Gluckman[4], S Sanders[4]. Departments of Clinical Sciences[1], Population Medicine & Diagnostic Sciences[2], and Biomedical Sciences[3], College of Veterinary Medicine, Cornell University, Ithaca, NY and Mendon Village Animal Hospital[4], Mendon, NY.

During late 2005, a serious aflatoxin contamination was recognized in a manufactured pet food causing illness and death in numerous dogs. After

product recall, regional veterinarians identified many overtly affected dogs. Seventeen dogs were referred to our Teaching Hospital (11 underwent necropsy). We report on these 17 dogs and an additional 6 deceased dogs from other veterinary hospitals. Breed distribution included Labrador Retrievers (n = 8), Cocker Spaniels (5), Golden Retrievers (2), one each Standard Poodle, Lakeland Terrier, Shetland Sheepdog, Border Collie, Scottish Terrier, and Irish Setter, and 2 dogs of mixed breeding from 13 separate households; 13 dogs were from breeding kennels. There were 3 intact males, 4 neutered males, 10 intact females, and 6 neutered females with age ranging from 0.8 to 10.5 yrs and weight from 8 to 37 kg. Clinical signs in order of onset included: anorexia (22), lethargy (20), vomiting (20), polydipsia and polyuria (17), hematemesis, melena, or hematochezia (14), jaundice (17), bruising (12), abdominal distention (modified transudate) (8), hemorrhagic body cavity effusions (11), peripheral edema (13), and hypotension (7). Seven dogs became terminally encephalopathic; 2 were hypoglycemic. Unanticipated death occurred in 5 dogs before aflatoxicosis was suspected. Dogs were grouped according to survival; data is expressed as median values. Nonparametric methods detected differences in age, weight, and clinicopathologic features. All $\alpha = 0.05$, two tailed P values.

Dogs that died (n = 17) were significantly younger (2.8 vs 4.5 years), had lower total protein (5.0 vs 6.6 gm/dL) and higher total bilirubin (5.1 vs 0.6 mg/dL) concentrations, and higher AST (121 vs 60 U/L) and ALP (171 vs 123 U/L) activities; all $P < 0.05$. Coagulation tests predicting mortality included a longer APTT (31.5 vs 16 secs) and PT (30.8 vs 18 secs), lower concentration of fibrinogen (34 vs 196 mg/dL), and lower activities of AT (17 vs 51%), Protein C (18.5 vs 50%), and Factor VII (33.5 vs 85.5%); all $P < 0.05$. Decreased Protein C (<70%) and AT (<65%) activities were found in all 17 dogs in which they were measured regardless of survival. Hypocholesterolemia (<150 mg/dL) was a consistent finding in these dogs, whereas only modest increases in serum liver enzyme activities were realized. We conclude that hypocholesterolemia and reduced Protein C and AT activity have clinical utility in dogs as biomarkers of hepatic injury caused by aflatoxin ingestion. Presumably, aflatoxin inhibition of hepatic protein synthesis is the common cause of these abnormalities. The terminal nature of aflatoxicosis in severely affected dogs could not be offset by aggressive interventional therapy including blood components, antioxidants, l-carnitine, silibinin, and vitamin K_1.

ABSTRACT #149

DOUBLE-BLIND, PLACEBO-CONTROLLED TREATMENT WITH D-PENICILLAMINE AGAINST HEPATIC COPPER ACCUMULATION IN LABRADOR RETRIEVERS. G Hoffmann[1], PG Jones[2], TSGAM van den Ingh[3], P Bode[4], J Rothuizen[1]. [1]Department of Clinical Sciences of Companion Animals, [3]Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Netherlands, [2]Waltham Center for Pet Nutrition, Leics, UK, and [4]Interfaculty Reactor Institute, Delft University of Technology, Netherlands.

D-Penicillamine is recommended for the treatment of copper toxicosis in Bedlington terriers, and for preclinical copper accumulation in children with Wilson's disease[1]. The aim of our study was to investigate whether D-penicillamine is an effective drug to decrease hepatic copper concentrations in Labrador retrievers.

We investigated 33 Labradors with hepatic copper accumulation above normal range (above 400 mg/kg dry weight liver). The dogs were related to former patients with copper associated chronic hepatitis. Treatment consisted of D-penicillamine (15 mg/kg PO BID, group-1) or a placebo (group-2) in a double-blinded trial. Hepatic copper concentrations were measured before and after treatment over 3 months. All dogs were evaluated clinically, and by histologic examination of liver biopsies.

Adverse effects observed by the owners included vomiting (5 dogs of group-1, 3 dogs of group-2), anorexia (3 dogs of each group), and diarrhea (one dog of group-1). Adverse effects were severe in 3 dogs of group-1, and required a change in the medication protocol, and exclusion from the study.

The mean hepatic copper concentration of dogs in group-1 was 1228 mg/kg dry weight (dw) (range 416–2440), and 1080 mg/kg dw in group-2 (range 531–2559). There was no difference in hepatic copper concentrations between group-1 and group-2 before treatment (p = 0.39). After treatment with D-penicillamine copper concentrations had decreased significantly (mean 720 mg/kg, range 60–2100, p = 0.041). This was different from the placebo group, where no difference was found (p = 0.16).

Results of this study support that D-penicillamine is an effective drug to decrease hepatic copper concentrations in Labrador retrievers.

[1] American Association for the study of liver diseases, practice guideline on the management of Wilson's disease: https://www.aasld.org/eweb/docs/wilson_withcorrection.pdf

ABSTRACT #150

VACUOLAR HEPATOPATHY IN DOGS: 336 CASES (1993–2005). LM Sepesy[1], SA Center[1], JF Randolph[1], KL Warner[1], HN Erb[2]. Departments of Clinical Sciences[1] and Population Medicine & Diagnostic Sciences[2], College of Veterinary Medicine, Cornell University, Ithaca, NY.

Vacuolar hepatopathy (VH) due to cytosolic glycogen distention of hepatocytes is common in dogs exposed to excess glucocorticoids. However, VH also occurs in dogs lacking glucocorticoid treatment or hyperadrenocortism (HAC). We retrospectively studied a canine population with VH to investigate its relationship with iatrogenic or endogenous glucocorticoid excess or other illnesses. Using a computerized database, a series of search strings, and histologic review of liver sections, we identified 336 VH cases. Liver sections were morphologically graded (numerical) defining acinar zone and severity of VH (moderate = 26–50%; severe => 51% vacuolated cells). Records were reviewed for glucocorticoid treatments, tests of adrenal function, and routine clinicopathologic data. Dogs were stratified into groups based on: 1) underlying disorder, 2) glucocorticoid exposure (VHG+, VHG−), 3) VH zonal distribution, and 4) VH severity. Non-parametric methods detected differences in clinicopathologic features between groups. Two by two tables detected differences in: 1) gender and reproductive status between dogs with VH and a control hospital population ('93, '97, '01); 2) histologic groups and VHG status. All $\alpha = 0.05$.

Group disorders were: neoplastic (n = 94), acquired hepatobiliary (43), adrenal (40), neurologic (38), immune-mediated (34), gastrointestinal (31), portal vascular anomalies (13), renal (12), infectious (6), cardiac (5), diabetes mellitus (3), and miscellaneous (17; <3 dogs each). There were 186 VHG+ and 150 VHG− dogs. Even though more VHG+ dogs had severe VH ($P < 0.0001$), glucocorticoids had no influence on zonal involvement. Although VHG+ dogs had higher median alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities than VHG− dogs ($P \leq 0.05$), broad range overlap prohibited predictive utility of these enzymes for determining glucocorticoid exposure. Of 226 dogs with high ALP, 45% were VHG−. Compared to the control population, VH dogs were older (9 vs 6 yrs), more likely to be intact males (19% vs 14%) or neutered females (49% vs 41%) and less likely to be neutered males (25% vs 34%); all $P < 0.05$. Prominent morphologic VH features included a diffuse distribution involving zones 2 and/or 3.

We conclude that nearly 50% of dogs with moderate to severe VH and nearly 50% of dogs with VH associated with increased ALP lack overt glucocorticoid exposure. Neoplasia and congenital or acquired hepatobiliary disorders were common primary conditions. Thus, VH often signals the presence of a primary disease process and does not reliably implicate glucocorticoid treatment or HAC as the underlying cause. These findings support an association between VH, high ALP activity, and illness-invoked physiologic stress. Dogs with biopsy confirmed VH should be evaluated for chronic exposure to steroidogenic hormones, adrenal disorders, or underlying neoplasia as important first steps. Furthermore, finding cytologic evidence of VH on fine needle aspiration may misleadingly suggest VH in some dogs with other types of liver disease.

ABSTRACT #151

CHRONIC INFLAMMATORY HEPATIC DISEASE IN LABRADOR RETRIEVERS: CLINICAL PRESENTATION AND PROGNOSTIC FACTORS. J Shih, J Keating, L Freeman, CRL Webster, Cummings School of Veterinary Medicine at Tufts University.

A predisposition for chronic inflammatory hepatic disease is thought to exist in Labrador retrievers, but has not been described in the literature. The aim of this study was to describe the clinical presentation, determine outcome and identify prognostic factors in these dogs. Medical records of 24 Labrador retrievers with a histopathological diagnosis of chronic inflammatory hepatic disease were reviewed and information was collected on history, clinical signs, results of clinicopathologic testing and diagnostic imaging and outcome. As necessary, owners or referring veterinarians were contacted for additional information on outcome. Hepatic biopsies were reviewed and scored for disease activity (degree of degeneration and inflammation on H&E), fibrosis (Trichome stain) and copper accumulation (Rhodanine stain). A clinical score dependent on the presence of clinical signs and biochemical parameters linked to hepatic synthetic function was generated for each dog, with a range of 0–13, with 13 being the most severe.

The median age at diagnosis was 9.3 yrs (range 3.9 to 14.0 yrs), with no sex predilection. No dogs were on known hepatotoxic drugs. Clinical signs were nonspecific and included inappetance, vomiting, lethargy and weight loss. All dogs had increases in one or more serum hepatobiliary enzyme and 70% of the dogs had increases in 3 or more enzymes. Hyperbilirubinemia, prolongation of PT or PTT or hypoalbuminemia were seen in 45%, 37% and 21% of the dogs, respectively. All dog tested for leptosporosis (9) or ANA (3) titers were negative. Non-specific ultrasonographic abnormalities of the liver were present in 87% of dogs. The median clinical score was 2.9 (range, 0–8).

The mean histopathology activity and fibrosis scores were 3.5 +/− 1.6 and 2.5 +/− 1.2, respectively. Rhodanine positive copper staining was present in 11/17 biopsy specimens with a mean score of 1.8 +/− 1.0. Copper was present in 2 more zones in most dogs. Median survival was 374 days (range, 1–2645 days). A prolonged PT ($p = 0.01$) and an abnormal neutrophil count ($p = 0.009$) were associated with survival less than 2 months. The clinical score correlated with survival time ($r = -0.87$, $p = 0.02$) and histopathological staging ($r = 0.49$, $p = 0.04$). Dogs with anorexia ($p = 0.05$), serum hypoglobulinemia ($p = 0.05$) or a prolonged PT ($p = 0.03$) had a shorter survival time than those without these abnormalities.

In summary, a progressive hepatopathy exists in Labrador retrievers marked by chronic inflammation with varying degrees of fibrosis and copper accumulation on hepatic biopsy. A clinical scoring system which reflects the presence of clinical signs and hepatic synthetic function is associated with survival time and histopathological staging by hepatic biopsy and may be useful with further validation as a noninvasive method to predict prognosis. Although most dogs had hepatic copper accumulation, whether this was the cause or result of the inflammatory disease could not be determine

ABSTRACT #152

PERCUTANEOUS TRANSVENOUS COIL EMBOLIZATION (PTCE) OF CANINE INTRAHEPATIC PORTOSYSTEMIC SHUNTS: EXPERIENCE IN 33 DOGS. C Weisse[1], JA Solomon[2], A Berent[1], K Todd[1], C Cope[2] [1]Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA; [2]Section of Vascular and Interventional Radiology Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, PA.

The purpose of this study was to prospectively evaluate the use of percutaneous transvenous coil embolization (PTCE) of canine intrahepatic portosystemic shunts (IHPSS).

Thirty-three dogs with congenital IHPSS received 37 PTCE procedures, and 32 were available for follow-up. Shunts were typically accessed by a percutaneous jugular approach and contrast venography was performed to delineate the shunt anatomy. Partial shunt attenuation was achieved with the use of metallic stents placed within the vena cava and thrombogenic coils placed within the shunt while monitoring portal blood pressure. Major peri-operative complications (1/37;3%) included temporary portal hypertension in one dog, and minor peri-operative complications (4/37;11%) included coil migration due to improper stent placement in two dogs, jugular bleeding in one dog, and seizure in one dog. Peri-operative mortality (3/37;8%) resulted from suspected portal hypertension in one dog, seizures and aspiration pneumonia with euthanasia in one dog, and duodenal ulcer hemorrhage/perforation with euthanasia in one dog. Overall mortality (10/32;31%) was secondary to GI disease in four of ten dogs.

Fifteen dogs (47%) are clinically normal without medication, seven dogs (22%) have not been weaned off medication yet, and the remaining ten dogs (31%) died or were euthanized. Eight dogs (8/33;24%) have had melena, three of which occurred prior to IHPSS PTCE.

Intrahepatic shunt PTCE results in lower peri-operative morbidity and mortality rates and similar success rates when compared with previously reported open surgical procedures. Gastrointestinal ulceration was a common finding among this population of dogs and lifelong gastroprotectant medications are now recommended by the authors.

ABSTRACT #153

SUSTAINED SEVERE HYPOGLYCEMIA DURING SURGERY AS A GENESIS OF GLOBAL BRAIN DAMAGE IN POST LIGATION SEIZURE OF CONGENITAL PORTOSYSTEMIC SHUNTS DOGS. S Torisu, M Washizu, D Hasegawa, H Orima. Nippon Veterinary and Animal Science University, Tokyo, Japan.

Eight among 161 canine congenital portosystemic shunts (CPSS) cases underwent surgical attenuation of shunts experienced post ligation seizure (PLS) within 72 hours after surgery. Two among 8 cases had brain MRI examination before surgery and during PLS episode. Two among 8 cases had brain MRI examination only during PLS episode. Brain MRI during PLS episode revealed severe global cerebral ischemia (GCI) in each case. MRI of GCI can be obtained by hypotension, hypoxia and/or hypoglycemia. During surgery, blood pressure and oxygen saturation were continuously monitored; however, blood glucose monitoring was not continuous. Sustained severe hypoglycemia was suspected as a genesis of global brain damage.

Retrospectively, hypoglycemia (<60 mg/dl) and seizure relation was evaluated: higher incidence of PLS (7/8) were in the hypoglycemic group. The new study of CPSS dogs is utilized to measure blood glucose every 30 minutes during surgery in 8 dogs: severe hypoglycemia (<30 mg/dl) was observed in 2 dogs following temporal shunt ligation. Artificial pancreas (AP) was utilized to monitor the glucose uptake of the liver continuously during surgery in 12 CPSS dogs. Marked sudden increase in glucose uptake was observed in 3 dogs following temporal shunt ligation.

We suspected the genesis of PLS is due to global brain damage from sustained severe hypoglycemia during surgery. Especially, the hypoglycemia develops during temporal ligation or total ligation since the portal venous blood flow increases to liver dramatically and glucose uptake also increases. Severe hypoglycemic period might sustain for a certain critical period time until glucose was reinfused. Global brain damage due to sustained hypoglycemia may result in fatal severe seizure activity with 72 hours after surgery.

ABSTRACT #154

COPPER-ASSOCIATED CHRONIC HEPATITIS IN LABRADOR RETRIEVERS: 15 CLINICAL PATIENTS AND THEIR FAMILY. G.Hoffmann, T.S.G.A.M.van den.Ingh, P. Bode*, J.Rothuizen. Department of Clinical Sciences of Companion Animals, University of Utrecht, Netherlands, *Interfaculty Reactor Institute, University of Delft, Netherlands.

Chronic hepatitis is a histological diagnosis, characterized by the presence of fibrosis, inflammation and hepatocellular apoptosis and necrosis. Cirrhosis can result as the end-stage of the disease (''Liver Diseases and Pathology Standardisation Research Group''). The term chronic hepatitis is used irrespective of the cause of the disease, which is usually unknown, although some cases have been associated with infections, anticonvulsant drugs, and copper accumulation. Inherited copper toxicosis is a well described disease in the Bedlington Terrier, where a genetic mutation causes accumulation of copper in hepatocytes, resulting in chronic hepatitis. Further hepatic copper storage seems to be breed-associated in the West Highland White terrier, skye terrier, Doberman and Dalmatian.

This study presents clinical, laboratory, and pathologic examination results of 15 Labrador retriever patients with probable copper-associated chronic hepatitis. The patient group consisted of 11 female and four male Labrador retrievers, all of which were registered at the breed club. The average age was 7 years at clinical presentation (range 2,5–10,5 years). All dogs were presented for gastrointestinal signs, including anorexia in all, and vomiting in 8 of 15 dogs. Chronic hepatitis and copper accumulation were diagnosed based on histologic examination of liver biopsies in all patients. The inflammatory infiltrate was lymphocytic, and co-localized with copper accumulation in zone 3, in all biopsies, with variable amounts of other celltypes (plasma cells, neutrophiles, and monocytes). A relative increase in ALT activity far above a relative increase in AP activity, as well as the hepatic localization of copper in zone 3 suggested a primary copper storage disease rather than primary cholestatic liver disease.

Eight family members of two patients were examined for subclinical disease. The mean hepatic copper concentration measured in this group of 8 clinically healthy siblings and offspring was 1317 μg/g dry weight liver (range 402–2576 μg/g). This was different from examination results of a healthy control group of six unrelated Labradors, which revealed a mean hepatic copper concentration of 233 μg/g dry weight liver (range 120–304 μg/g).

Our objective was to describe the clinicopathologic findings in 15 Labrador retrievers with suspected copper-associated chronic hepatitis and to reveal if a genetic basis of the disease is likely by examination of family members from these patients. Our findings support a genetic defect to underlie copper-associated chronic hepatitis in the Labrador retriever.

ABSTRACT #155

PHARMACOKINETICS OF PENCICLOVIR FOLLOWING ORAL ADMINISTRATION OF FAMCICLOVIR TO CATS. S.M. Thomasy[1], D.J. Maggs[2], N.K. Moulin[2], S.D. Stanley[1]. [1]KL Maddy Equine Analytical Chemistry Laboratory, [2]Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA.

Feline herpesvirus-1 (FHV-1) is a major cause of respiratory and ocular disease in cats. Famciclovir is an oral prodrug of penciclovir, a nucleoside deoxyguanosine analogue with potent antiviral activity against FHV-1. This study investigated the pharmacokinetics of penciclovir following single and multiple dose oral administration of famciclovir (62.5 mg) to 8 healthy, intact female cats (mean ± SD weight 4.3 · 1.1 kg and age 2.5 ± 1.5 yrs). A balanced crossover design was used to assign treatments which were separated by a minimum of 10 days.

Treatment 1 consisted of a single oral dose of famciclovir. Treatment 2 consisted of a multiple dose trial of famciclovir given orally every 8 h (n = 4) or 12 h (n = 4) for 3 days. In treatment 1, venous blood was sampled at fixed time points for 12 h after famciclovir administration. In treatment 2, trough blood samples were obtained immediately prior to famciclovir administration. Following administration of the last dose of famciclovir, blood samples were collected at the same post-treatment time points as treatment 1. Plasma was stored at −20°C until analysis of penciclovir using liquid chromatography/mass spectrometry. Penciclovir concentration-time data were assessed using non-compartmental analysis and a one-compartmental model with first-order absorption (±lag time) and first-order elimination. To determine whether penciclovir accumulated with multiple doses of oral famciclovir, linear regression of the plasma trough samples was performed. A 2 sample *t* test was used to assess whether dosing interval had a significant ($P < 0.05$) effect on penciclovir pharmacokinetic variables. Data are presented as mean ± SD.

Following a single dose of famciclovir, the maximum penciclovir concentration (C_{max}) of 0.33 ± 0.12 μg/ml occurred at 4.6 ± 1.8 h, and the elimination half-life was 3.1 ± 0.9 h. Dosing famciclovir every 8 h versus 12 h increased the minimum observed steady state plasma penciclovir concentration during a dosing interval from 0.06 ± 0.03 to 0.18 ± 0.07 μg/ml and the dose normalized (15 mg/kg) area under the plasma concentration-time curve from 2.4 ± 1.3 to 5.5 ± 1.0 μg × h/ml. During either dosing interval, the slopes of the penciclovir trough concentration versus time plots were not significantly different from zero, indicating that penciclovir did not accumulate. However, penciclovir C_{max} of 0.34 ± 0.18 or 0.68 ± 0.29 μg/ml following multiple doses of famciclovir every 12 h or 8 h, respectively was notably less than the *in vitro* inhibitory concentration 50 (IC_{50}) for FHV-1 (3.5 μg/ml)

Presumably due to poor absorption or metabolism of famciclovir, cats will likely require a higher dose of famciclovir than in other species examined to achieve target plasma penciclovir concentrations.

ABSTRACT #156

CETIRIZINE (ZYRTEC®) PHARMACOKINETICS IN HEALTHY CATS. MG Papich,[1] E Schooley,[2] CR Reinero[2], [1]North Carolina State University, Raleigh, NC, and [2]University of Missouri, Columbia, MO.

Cats commonly develop allergies involving the GI tract, skin, and the respiratory system. Although glucocorticoids are effective therapy, high-dose or long-term administration is undesirable. Additionally, cats with diabetes mellitus, infectious disease, certain types of heart disease, etc. may not tolerate glucocorticoids. In humans, second generation antihistamines such as cetirizine (Zyrtec®) have been used for allergic disease because they lack the side effects associated with older antihistamines. Although cetirizine has been used anecdotally in cats, there are currently no data on its pharmacokinetics. The objective of this study was to determine the pharmacokinetics of cetirizine in cats after a single oral dose. We hypothesized that a dose of 5 mg per cat (approximately 1 mg/kg) of cetirizine administered orally once daily would produce plasma concentrations in a range reported to be effective for humans (0.0039–0.027 mcg/mL).

Nine healthy, client owned cats were used. Heparinized blood (2 mL) was collected at baseline, and at 0.5, 1, 2, 4, 6, 8, 10 and 24 hours after oral administration of 5 mg of cetirizine (dose range, 0.59–1.36 mg/kg). No adverse drug effects were observed in any cat. The plasma was separated by centrifugation within 1 hour of collection and samples were frozen (−20°C) until analysis. A reverse-phase HPLC assay was developed by fortifying feline plasma obtained from blood donors with a pure analytical reference standard. The plasma concentrations were analyzed with a compartmental pharmacokinetic model, using first-order input and one-compartment distribution. The mean terminal half-life was 10.7 (+/− 4.1) hrs and mean peak plasma concentrations were 3.8 (+/− 1.5) mcg/mL. The volume of distribution and clearance (both corrected for absorption) were 0.256 (+/− 0.09) L/kg, and 0.295 (+/− 0.086) mL/kg/min, respectively, suggesting a small volume of distribution and low clearance. Mean plasma concentrations were maintained above 0.85 mcg/mL for 24 hours. These plasma concentrations are above the level reported to produce therapeutic benefits in humans. These results indicate that with a single dose of approximately 1 mg/kg orally in cats, cetirizine was well-tolerated and produced high plasma concentrations compared to what has been reported in humans. The half-life is long enough to maintain plasma concentrations with once-daily dosing. Determining the optimum plasma concentration and dose will be necessary before clinical use can be initiated.

ABSTRACT #157

DOSE NOT RATIO OF (N-6) AND (N-3) FATTY ACIDS IS RESPONSIBLE FOR CHANGES IN THE PLASMA FATTY ACID PROFILE OF NORMAL DOGS. JA Hall[1], RA Picton[1], MM Skinner[1], DE Jewell[2], RC Wander[3]. [1]Department of Biomedical Sciences, Oregon State University, Corvallis, OR. [2]Science and Technology Center, Hill's Pet Nutrition Inc, Topeka, KS. [3]Department of Nutrition, The University of North Carolina, Greensboro, NC.

The beneficial effects of adding dietary (n-3) polyunsaturated fatty acids (PUFA) to dog foods have been reported. However, it is not established whether this results from the absolute dose of (n-3) fatty acids (FA) fed or the ratio of (n-6) to (n-3) FA in the food. The purpose of this study was to determine whether it is the dose of (n-3) FA consumed (g/kg body wt/d) or the relative ratio of (n-6) to (n-3) FA in the food that determines plasma FA composition in dogs.

Thirty-two healthy female, geriatric Beagles (7 to 10 y) were fed foods containing (n-6) to (n-3) FA ratios of either 40 : 1 or 1 : 1 for 12 or 36 weeks. In another study, Beagles were fed foods with the same 1 : 1 ratio of (n-6) to (n-3) FA and the same energy % fat by weight, but increasing concentrations of (n-6) and (n-3) FA. Plasma FA were measured after completing the feeding studies.

Dogs consuming a higher dose of (n-3) and (n-6) FA (ratio 1 : 1), had significantly (P = 0.03) higher plasma concentrations of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and total (n-3) FA compared to dogs consuming the lowest dose of (n-3) and (n-6) FA (ratio 1 : 1). Furthermore, constancy of the dose of (n-3) FA fed over long periods of time was necessary to maintain plasma levels of total (n-3) FA, EPA, DHA, and PUFA. These results indicate that for dogs consuming diets with a low (n-6) to (n-3) FA ratio it was the absolute dose of (n-3) FA fed, not the relative ratio of (n-6) to (n-3) FA, that determined plasma (n-3) FA concentrations.

ABSTRACT #158

DOCOSAPENTAENOIC ACID (22:5n-3) IS NOT A SUBSTRATE FOR LECITHIN: CHOLESTEROL ACYL TRANSFERASE IN N-3 FATTY ACID FED DOGS. J.E. Bauer[1], A.L. Spencer[1], M.K. Waldron[2]. [1]Companion Animal Nutrition Lab., College of Veterinary Medicine, Texas A&M University, College Station, TX, [2]Nestle-Purina PetCare, St. Louis, MO, U.S.A.

We have previously shown that docosahexaenoic acid (DHA) is important for puppy neurologic development and retinal function. Substrates for DHA synthesis include docosapentaenoic acid (DPA) and alpha-linolenic acid (ALA); however net conversion rates from ALA are low in canine and other species. We have previously found that DPA accumulates in plasma total phospholipid fractions (PL) when ALA is fed but DHA does not. However, this accumulated DPA is not present in plasma cholesteryl ester fractions. This observation suggests that DPA is not a substrate for the high density lipoprotein-bound plasma enzyme, lecithin:cholesterol acyl transferase (LCAT). This enzyme is responsible for the first step in the return of cholesterol and fatty acids to the liver after esterification of tissue cholesterol with long chain fatty acids; a phenomenon known as reverse cholesterol transport. The present study was undertaken to determine whether DPA specifically accumulates in phosphatidyl choline (PC) and CE fractions of dogs fed diets containing either linseed oil or fish oil n-3 fatty acids. The presence of DPA in PC but not CE would indicate that this fatty acid is not a substrate for LCAT under these conditions. It would also provide a physiologic mechanism to conserve limited amounts of DPA for its forward conversion to DHA, (e.g. in neurologic tissues), rather than its return to the liver. Thirty adult mixed breed dogs were divided into 3 groups (n = 10 each). A low fat basal diet was supplemented with either linseed oil (LSO), Menhaden fish oil (MHO), or beef tallow (BTO) with suitable amounts of linoleic acid for 28 days at a dosage of 18 g oil per 100 g basal diet. Blood was collected into EDTA tubes, plasma total lipids were extracted with chloroform:methanol (2 : 1, v/v), PC and CE were fractionated via thin-layer chromatography, and methyl ester derivatives were quantified by capillary gas chromatography. Fatty acid compositions were analyzed using one way ANOVA. The PC fraction of the LSO diet group contained significantly increased amounts of eicosapentaenoic acid (EPA) but not DPA or DHA vs. the BTO control group. This finding suggests that DPA accumulation in total PL when LSO is fed occurs in a phospholipid subfraction other than PC. Neither DPA nor DHA were found in the CE fractions of LSO fed dogs. By contrast, significantly increased amounts of PC- EPA, DPA, and DHA were found in the MHO group. However, DPA did not accumulate in CE fractions of this group although a slight DHA increase was seen. These data confirm that, when diets high in fish oil are fed, DPA is present in plasma PC but not in plasma CE. Thus, transfer of DPA from PC to CE mediated by plasma LCAT does not occur and DPA is likely not a suitable substrate for the enzyme. This mechanism helps conserve DPA for its potential role as substrate for DHA synthesis in neurologic tissues.

ABSTRACT #159

RELATIONSHIP BETWEEN PLASMA AMINO ACIDS, C-REACTIVE PROTEIN, ILLNESS SEVERITY AND OUTCOME IN CRITICALLY ILL DOGS. DL Chan[1], EA Rozanski[2], LM Freeman.[2] [1]Royal Veterinary College, North Mymms, Hertfordshire, UK. [2]Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA.

The aims of this study were to determine and relate plasma amino acids and serum C-reactive protein (CRP) concentration to illness severity and outcome in critically ill dogs. Dogs diagnosed with acute pancreatitis, trauma, sepsis or septic peritonitis were eligible for the study. Healthy dogs were used as controls. A fasting blood sample was collected from each dog within 12 hrs of enrollment. Plasma was analyzed for 24 different amino acids using an automated analyzer (Model 7300, Beckman Instruments, Palo Alto, CA) and serum CRP was assayed using a commercially available ELISA kit (Tri-Delta Phase™ Canine CRP Assay, Tridelta Diagnostics Inc, Morris Plains, NJ). Physiological parameters were used to calculate a Survival Prediction Index 2 score (SPI2) as a measure of illness severity (King LG, et al, 2001).

Forty eight critically ill and 24 healthy dogs were evaluated. The distribution of dogs among disease groups was as follow: sepsis (n = 14), septic peritonitis (n = 9), acute pancreatitis (n = 14), and severe trauma (n = 11). Median age of critically ill dogs was 7.3 yrs (range 1.3–15.9 yrs) and was not significantly different from controls [5.5 yrs (2.0–15.0 yrs), P = 0.09] or among disease groups (P = 0.20). Median SPI2 score for this population of critically ill dogs was 0.62 (0.23–0.91). Dogs with sepsis had significantly lower median SPI2 scores when compared with dogs with severe trauma [0.52 (0.36–0.88) vs. 0.72 (0.54–0.87), respectively, P = 0.04]. Overall mortality rate was 41.6% (20/48). Median CRP for critically ill dogs was 57.7 μg/mL (2.6–98.4 μg/mL) and was significantly higher than controls [3.2 μg/mL (2.5–23.1 μg/mL), P < 0.001] but not different among disease groups, P = 0.37, or between survivors and non-survivors (P = 0.29). No correlation between CRP and SPI2 was detected, r = 0.17, P = 0.25.

Compared to controls, critically ill dogs had significantly lower concentrations of serine (P = 0.001), glycine (P < 0.001), alanine (P = 0.001), citrulline (P < 0.001), methionine (P < 0.001), arginine (P < 0.001), and proline (P < 0.001).Compared to survivors, non-survivors had significantly lower concentrations of serine (P = 0.04), valine (P = 0.04), isoleucine (P = 0.01), leucine (P = 0.04), and arginine (P = 0.02). Serum CRP concentrations were negatively correlated with plasma serine, glutamic acid, glycine, alanine, citrulline, methionine, lysine, arginine, and proline.

Serum CRP is markedly elevated in critically ill dogs, but not discernible among different diseases or between survivors and non-survivors. In this population of dogs, significant depletion of particular amino acids was identified. Of these, serine and arginine may be particularly important as they were also significantly lower in non-surviving than in surviving critically ill dogs. Future studies evaluating the potential role for particular amino acid supplementation in the treatment of certain diseases in dogs are warranted.

ABSTRACT #160

INHIBITION OF PRO-INFLAMMATORY CYTOKINE AND COX-2 EXPRESSION IN CHONDROCYTES AND MONOCYTES BY AVOCADO SOYBEAN UNSAPONIFIABLES (ASU). TK Al-Talib[1]; RY Au[1]; PV Phan[1]; AY Au[1]; CG Frondoza Ph.D.[1,2] [1]Nutramax Laboratories, Inc. Edgewood, Maryland, [2]Johns Hopkins University, Department of Orthopaedic Surgery, Baltimore, Maryland.

The cytokines TNF-α, and IL-1β, and the enzyme cyclooxygenase-2 (COX-2), are known as the principal mediators in chronic inflammatory disorders. COX-2 is the critical enzyme involved in inflammation by regulating the production of prostaglandin (PG) E_2. Non-steroidal anti-inflammatory drugs (NSAIDs) are used extensively to suppress inflammation and alleviate pain, particularly in osteoarthritis, by inhibiting cytokine and PG synthesis. More recently, alternative approaches to the management of pain and inflammation have provided encouraging clinical results. Among these are extracts from Avocado Soybean Unsaponifiables (ASU). However, little is known about the effect of ASU on cellular targets. The present study tested the hypothesis that ASU inhibits gene expression of COX-2, TNF-α and IL-1β in chondrocytes and monocytes. The surrogate monocyte-macrophage-like THP-1 cells were used.

Articular chondrocytes (5×10^5/well) from the metacarpal joints of mature Holsteins and human THP-1 monocyte-like cells (5×10^5/well) were pre-incubated with: (i) ASU (Nutramax Laboratories Inc.), (25 μg/ml) or (ii) control media alone for 72 and 24 hrs respectively. Cells were re-incubated with control media alone or 20 ng/ml of lipopolysaccharides (LPS) for: (a) 1 hr to determine gene expression by reverse transcription-polymerase chain reaction (RT-PCR) analysis and (b) 24 hrs to measure secreted PGE_2 levels by immunoassay. Primers specific for bovine and human COX-2, TNF-α, IL-1β and GAPDH as the housekeeping gene were used. The gels containing ethidium bromide were electrophoresed to visualize the DNA bands under UV light. Three to five separate runs were performed. Multiple comparisons by one-way ANOVA (Tukey post-hoc analysis) were performed using the SigmaStat statistical program where $p < 0.05$ was considered statistically significant. ASU reduced baseline expression of COX-2, TNF-α and IL-1β in non-activated bovine chondrocytes. Moreover, ASU blocked the activation of these mediators in cells induced by LPS. Blockage of COX-2 expression led to significant reduction of secreted PGE_2 by 93 ± 1% ($P < 0.01$). Similarly, pre-incubation of THP-1 cells with ASU for 24 hrs followed by activation with LPS for 1 hr profoundly blocked the expression of TNF-α and IL-1β transcripts compared to control cells activated with LPS alone.

The present study demonstrates for the first time that ASU dramatically suppresses the expression of TNF-α and IL-1β in chondrocytes and monocytes, while confirming the reduction of COX-2 transcripts in chondrocytes. This observation supports the positive clinical findings that ASU ameliorates pain and inflammation. Our study supports the proposed utility of ASU in the management of painful conditions, exemplified by osteoarthritis.

ABSTRACT #161

CANINE DIETARY IRON RECOMMENDATIONS ARE INSUFFICIENT TO CORRECT IRON DEFICIENCY IN GROWING DOGS. C Kirk, M Fry, G Daniel, J Bartges, The University of Tennessee, Knoxville, TN.

Iron deficiency in dogs results from disorders of chronic blood loss (e.g. gastrointestinal disease or blood-sucking parasites) or low dietary intake (e.g. milk-fed puppies). Signs of deficiency in dogs include anemia, lethargy, and poor growth rates. AAFCO recommendation for adequate dietary intake in dogs is 80 mg/kg dry matter (dm). Conventional hematologic indices used to identify iron deficiency are not reliably predictive of iron status. The objective of this study was to evaluate reticulocyte indices with changing iron status caused by dietary iron depletion and repletion.

Seven mixed breed dogs (4 male/4 female), at 16 weeks of age, were studied. Food and water was provided free-choice throughout the study. All dogs were fed a commercial puppy chow (Purina High Energy Puppy Food, St. Louis, MO) for a 12 d baseline period. During iron-depletion, all dogs were fed an iron-free purified diet (Fe 0 mg/kg dm) that was otherwise complete and balanced. Dogs were considered to have iron deficiency (ID) when hemoglobin concentrations decreased to ≤80% of baseline values. Upon diagnosis of ID, all dogs were fed an iron-replete purified diet (Fe 90 mg/kg dm; revised NRC iron recommendations for growing dogs) until iron deficiency resolved (hemoglobin concentration ≥ 95% of baseline). Parenteral iron (iron dextran, 20 mg/kg) was given to 4 dogs not responding to dietary iron repletion and 3 dogs immediately upon development of ID. Values from baseline and subsequent days were compared directly using a paired t-test. The overall performance of conventional and reticulocyte hematologic indices (presented elsewhere) was evaluated by receiver operator characteristic (ROC) analysis.

In all dogs, hemoglobin concentration decreased to ≤90% of baseline values by d 35 and to ≤80% of baseline values in 6 of 7 dogs at d 60 (median time). By days 41, 35, 41, and 13 of dietary repletion, the 4 dogs fed the repletion food alone had negligible increases in hematologic indices indicating a lack of dietary iron repletion. Following parenteral iron supplementation, indices of iron deficiency resolved within 14 days in 5/7 dogs and within 19 days in all 7 dogs.

In conclusion, the revised NRC recommendation for dietary iron (Fe 90 mg/kg dm) is not adequate to replete iron status in iron deficient growing dogs. Dogs with evidence of iron deficiency should receive supplemental iron treatment (parenteral or oral) to adequately replete iron stores, even when the underlying cause of iron deficiency has been resolved.

ABSTRACT #162

ARTERIAL HYPOXEMIA IN EXERCISING THOROUGHBRED HORSES IS NOT AFFECTED BY PRE-EXERCISE BRONCHODILATOR ADMINISTRATION. T. E. Goetz, M. Manohar, A. S. Hassan. College of Veterinary Medicine, University of Illinois, Urbana, IL.

Pulmonary injury evoked airway inflammatory/mast cell release of chemical mediators in human subjects has been proposed to contribute to exercise-induced arterial hypoxemia (*Am J Respir Crit Care Med* 155(3):1090–1094, 1997). Since airway inflammatory mediators are known to cause constriction of small airways thereby causing maldistribution of alveolar ventilation, the present study examined whether administration of a potent β_2 adrenergic bronchodilator would affect the development and/or severity of arterial hypoxemia in Thoroughbred horses performing strenuous exertion.

Two sets of experiments, namely, control (saline/placebo) and β_2 adrenergic bronchodilator (clenbuterol HCl administered at 1.0 μg/kg, IV, 10 minutes pre-exercise) studies were carried out on 7 healthy, sound, exercise-trained Thoroughbred horses in random order, 7 days apart. In both treatments, arterial and mixed-venous blood-gas/pH measurements were made at rest before and after saline/clenbuterol HCl administration, and during maximal exercise performed at 14 m/s on a 3.5% uphill grade for 120 s.

Galloping at this workload elicited maximal heart rate and induced exercise-induced pulmonary hemorrhage in all horses in both treatments, thereby indicating that capillary stress failure related pulmonary injury had occurred. In both treatments, arterial hypoxemia (O_2 tension: rest = 102.5 ± 2.2 mm Hg versus maximal exercise = 73.8 ± 2.5 mm Hg), desaturation of hemoglobin (hemoglobin-O_2 saturation: rest = 98 ± 1.6% versus maximal exercise = 85.4 ± 1.8%), hypercapnia (CO_2 tension: rest = 43.2 ± 2.1 mm Hg versus maximal exercise = 54.9 ± 2.4 mm Hg), and acidosis (pH: rest = 7.420 ± 0.032 versus maximal exercise = 7.095 ± 0.035) of a similar magnitude developed during maximal exertion, and statistically significant differences between the control and bronchodilator studies could not be demonstrated.

The failure of pretreatment with a potent β_2 adrenergic bronchodilator to significantly modify exercise-induced arterial hypoxemia argues against a major role for pulmonary injury related airway inflammatory mediator(s) induced bronchoconstriction in bringing about arterial hypoxemia in galloping Thoroughbred horses.

ABSTRACT #163

THE RATIO OF URINE DEOXYPYRIDINOLINE TO PYRIDINOLINE IDENTIFIES HORSES WITH HYPERELASTOSIS CUTIS (A.K.A. HEREDITARY EQUINE REGIONAL DERMAL ASTHENIA OR HERDA). C Swiderski[1], M Pasquali[2,3], L Schwarz[3], C Boyle, R Read, R Hopper, P Ryan, A Rashmir-Raven[1]; [1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS. [2]Department of Pathology, University of Utah, Salt Lake City, UT; [3] ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

Hyperelastosis Cutis (a.k.a Hereditary Equine Regional Dermal Asthenia or HERDA), an inherited disorder of increasing prevalence in Quarter Horses, is characterized by areas of fragile, hyperextensible skin. Normally innocuous contact, such as saddling, leads to subcutaneous hematomas and wounds that heal poorly. Affected horses generally cannot be ridden; most are humanely destroyed. Pedigree analysis supports an autosomal recessive mode of inheritance and has identified affected bloodlines. HERDA exhibits many clinical similarities with a collection of human hereditary collagen disorders, the Ehlers-Danlos Syndrome (EDS). Elevated urine ratios of the collagen pyridinium crosslinks deoxypyridinoline (DPD) and pyridinoline (PYD) are used to diagnose EDS Type VIA (collagen lysyl hydroxylase deficiency). Our study was a blinded comparison of DPD:PYD ratios in the urine of 39 clinically normal horses and 19 horses previously diagnosed with HERDA. Total DPD and PYD in urine were determined by high pressure liquid chromatography. The ratio of DPD:PYD was significantly higher in horses known to have HERDA than in healthy controls ($p < 0.0001$) using a two-tailed independent samples *t* test for unequal variances. The mean ratio difference between groups was 2.48, with a 95% confidence interval of (2.30, 2.67). Pyridinium crosslinks are the intermolecular bonds of collagen. DPD and PYD are derived from two and three hydroxylysine residues, respectively, with PYD being the predominant Type I collagen crosslink in normal individuals. These data suggest that abnormal hydroxylation of collagen lysine residues may be significant in the pathogenesis of HERDA.

ABSTRACT #164

STIMULATED SECRETION OF MATRIX METALLOPROTEINASES FROM EQUINE KERATINOCYTES BY DIFFERENT STRAINS OF *STREPTOCOCCUS BOVIS*. P.J. Johnson, A.M. Cogswell, J.R. Middleton, J.M. Kreeger, N.T. Messer, D.A. Wilson and VK Ganjam. University of Missouri, College of Veterinary Medicine, Columbia, MO.

It has been hypothesized that overproduction of *Streptococcus bovis* exotoxins may contribute to risk of laminitis in horses that have ingested large quantities of grain. Absorption of exotoxins into the circulation may activate host matrix metalloproteinases (MMP) in the hoof lamellar interface and contribute to the pathogenesis of laminitis. As a preliminary step to better understanding why only some horses (75%) develop laminitis when treated by carbohydrate overload, we hypothesized that the large intestine of laminitis resistant horses may be colonized by non-toxigenic strains of *S. bovis*.

Streptococcus bovis was isolated using highly selective membrane-bovis agar from the large intestine of horses after carbohydrate overload. Isolation of *S. bovis* was confirmed using a commercial biochemical typing system. Six distinct strains of *S. bovis* were identified using pulsed-field gel electrophoresis. *Streptococcus bovis* colonies were subsequently cultured in DMEM (1,000 bacteria/ml) at 37°C and 5 ml of supernatant was removed after 12 and 24 hours. Supernatants were centrifuged, filtered (0.2 μm), and stored in aliquots at −80°C.

Keratinocyte monolayers, confirmed by cytokeratin IHC, were prepared in DMEM in 24-well plates from skin biopsy specimens acquired from 3 healthy horses. Keratinocyte monolayers were treated with serial dilutions of bacterial supernatants for 12 and 24 h. Keratinocyte conditioned medium was tested for gelatinase activity by zymography.

Untreated keratinocytes primarily secreted pro-MMP-9 with small amounts of active MMP-9, pro-MMP-2, and active MMP-2. Treatment of keratinocyte monolayers with bacterial culture supernatant from each of the 6 bacterial strains stimulated the secretion of active and pro-forms of both MMP-9 and MMP-2. Stimulated MMP-9 secretion was significantly greater for the 24-hr incubation than for the 12-hr incubation whereas secretion of MMP-2 was greater for the 12-hr incubation than for the 24-hr incubation. There were no differences between the 6 strains of *S. bovis*.

These results suggest that distinct strains of *S. bovis* are similar with respect to exotoxin production and that equine susceptibility to carbohydrate-induced laminitis is not likely attributable to the presence or absence of different strains of this bacterial species.

ABSTRACT #165

TAHITIAN NONI® EQUINE ESSENTIALS™: A NOVEL ANTI-INFLAMMATORY AND A COX-2 INHIBITOR WHICH REGULATES LPS-INDUCED INFLAMMATORY MEDIATOR EXPRESSION IN EQUINE NEONATAL MONOCYTES. J Xu[1], AC McSloy[1], BK Anderson[1], RG Godbee[2], SF Peek[1], BJ Darien[1]. [1]University of Wisconsin, School of Veterinary Medicine, Madison, WI. [2]University of Nevada, Reno, NV.

TAHITIAN NONI™® Juice is a well recognized natural herbal product made from the *Morinda citrifolia* tree, native to Polynesia. *Morinda citrifolia* (NONI) reportedly has a broad range of therapeutic effects including antibacterial, antiviral, analgesic, anti-inflammatory, and immune enhancing effects. It has established antioxidant and selective COX-2 inhibitory activity in human inflammatory cells. Our hypothesis was that *TAHITIAN* NONI™ Equine Essentials™ would modulate endotoxin (lipopolysaccharide, LPS)-induced inflammatory responses in equine foal monocytes by regulating cyclo-oxygenase-2 (COX-2) expression, as well as expression of other inflammatory cytokines, specifically TNF-α, IL-1β, IL-8, and IL-6.

Neonatal foals were enrolled in the study after adequate passive transfer (IgG > 800 mg/dl), was confirmed by a SNAP IgG test at 24 hrs of age. Subsequently, experimental foals (n = 2) received 60 ml *TAHITIAN* NONI™ Equine Essentials™ orally twice daily for 60 days. The 2 remaining foals served as aged matched controls.At days 10 and 60 blood was taken from which peripheral monocytes were isolated. Monocytes from each foal were divided into an untreated control group and a group that was stimulated with LPS for 2 hours at 1000 ng/ml. Quantitative PCR analysis of COX-2, TNF-α, IL-1β, IL-8, IL-6 mRNA expression was determined, expressed as mean relative fold (x), change and the values obtained from control and experimental, foals compared.

At day 10, *TAHITIAN* NONI™ Equine Essentials™ treated foals had a dramatic fold reduction in COX-2, TNF-α, IL-1β, IL-8 & IL-6 expression in LPS-stimulated monocytes of 23×, 10×, 15×, 30× and 35×, respectively, when compared to age-matched controls.

Although less dramatic than day 10 results, a similar pattern was observed at day 60. *TAHITIAN* NONI™ Equine Essentials™ treated foals had a reduction in COX-2, TNF-α, IL-1β, IL-8 & IL-6 expression in LPS-stimulated monocytes of 9×, 180×, 8.5×, 22× and 35×, respectively, when compared to age-matched controls. The relatively small numbers of subjects precluded further statistical analysis and we realize this to be a limitation of this study.

Monocytes isolated from foals receiving *TAHITIAN* NONI™ Equine Essentials™ had markedly decreased COX-2, TNF-α, IL-1β, IL-8, and IL-6 mRNA expression following LPS stimulation when compared to control foals. Reduction in expression of these pro-inflammatory mediators, most notably at 10 days of age, suggests that *TAHITIAN* NONI™ Equine Essentials™ may be a promising novel anti-inflammatory therapy, warranting further consideration of its use clinically.

ABSTRACT #166

CLINICAL EVALUATION OF A SERUM IgE (Fc EPSILON RECEPTOR) TEST FOR EQUINE INSECT HYPERSENSITIVITY. M Lendau, J Pringle, Swedish University of Agricultural Sciences, Uppsala, Sweden.

The purpose of the study was to investigate whether IgE measurements in equine serum can be used as a diagnostic test for insect hypersensitivity and for identifying allergens in horses.

In a blinded study the IgE response to specific insect allergens (*Culicoides, Stomoxis, Simulium, Culex and Tabanus.*) was measured during insect season in 11 horses with a clinical diagnosis of insect related cutaneous hypersensitivity and 11 clinically normal control horses from the same stable environment. The horses in the study were of different breed, sex and ages. Blood samples were analysed with a commercially available ELISA test, HESKA™, which by adding the Fc epsilon receptor should reduce the number of false positives. The test is a positive/negative with a cut-off value of 150 units, and thus statistical comparison was not performed.

Of the 11 hypersensitive horses all tested positive for IgE to *Culicoides*, whereas eight of the control group were also positive for IgE to this allergen. However, the IgE values were similar or substantially higher for the hypersensitive horses when compared to their matched controls (mean 2.25 times higher, range 0.7–3.3 times higher). For the other insect allergens both groups had similar positive results to allergen specific IgE. A high test value for the allergic horse tended to correspond with a high value for its' control.

Horses that do not suffer from insect hypersensitivity were thus shown to have an IgE production during insect season.

The study showed that it is possible with this method to detect high IgE levels in serum as a response to specific allergens. However, as many of the stable-matched control horses also had insect specific IgE levels deemed as positive, this test does not appear suitable for use diagnostically for insect allergies.

ABSTRACT #167

NON-INVASIVE ESTIMATION OF PULMONARY ARTERIAL PRESSURES IN HORSES WITH RECURRENT AIRWAY OBSTRUCTION. J Slack[1], MM Durando[1], DM Ainsworth[2], VB Reef[1], SA Jesty[2], G Smith[1] and EK Birks[1]. [1]University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA. [2]Cornell University, College of Veterinary Medicine, Ithaca, NY.

Significant pulmonary hypertension is known to occur secondary to recurrent airway obstruction (RAO) in horses. How this relates to disease severity or long-term prognosis is not known. In part, this may be due to the difficulty and/or invasive nature of monitoring pulmonary artery pressures (PAP) in a routine clinical setting. Various echocardiographic parameters can be used in humans and dogs with respiratory disease to non-invasively predict PAP, but these parameters have not been investigated in the horse. The goal of this study was to correlate echocardiographic measurements with invasively-measured PAP in horses with RAO.

Thirteen RAO-susceptible and 7 control horses were evaluated on two separate occasions. At the time of the first examination, all horses had been pastured for 3 months. Horses were examined a second time after 3 weeks of stall confinement and feeding of dusty hay. Induction of disease was confirmed and disease severity determined by clinical examination, esophageal pressures and BALF analysis. A complete 2-D, M-mode and Doppler echocardiagraphic examination was performed and non-standard, as well as standard measurements of the pulmonary artery and aorta were obtained. PA pressures (PAP) were measured by passing a fluid-filled polyethylene catheter to 10 cm beyond the pulmonic valve. Catheter placement was confirmed by observing characteristic pressure wave-forms during passage. A mathematical model for predicting pulmonary arterial pressures from ultrasonographic measurements was constructed using multivariate least-squares regression. This model accounted for 95% of the variation in observed PAP.

Mean esophageal pressures increased in RAO-susceptible horses after induction, confirming disease induction (11.2 $\pm$ 2.6 cm H_2O pre vs. 32.2 $\pm$ 3.5 cm H_2O post, mean $\pm$ sem, $p < 0.0001$). Mean PAP increased significantly in RAO-susceptible horses after induction (31.2 $\pm$ 1.7 mmHg vs. 47.9 $\pm$ 5.4 mmHg, mean $\pm$ sem, $p = 0.007$). There were no significant changes in any parameters after induction in controls. A mathematical model could be constructed incorporating 1 standard and 3 non-standard echocardiographic measurements of the PA and aorta that adequately predicted actual PAP ($R^2 = 0.95$).

This study confirmed that horses with RAO may develop pulmonary hypertension. In addition, echocardiographic dimensions were related to PAP, and may be a useful means of monitoring disease progression or treatment. It is important to remember that this study was conducted on a limited number of horses, and further studies are needed to confirm the validity of this model.

ABSTRACT #168

EFFECTS OF INTRAVENOUSLY ADMINISTRATED OMEPRAZOLE ON GASTRIC JUICE PH AND GASTRIC ULCER SCORES IN ADULT HORSES. F. Andrews[1]; N. Frank[1]; C. Sommardahl[1]; B. Buchanan[1]; S. Elliott[1]; V. Allen[2]. [1]University of Tennessee, Knoxville, TN; [2]Premier Pharmacy Labs, Inc., Weeki Wachee, FL.

Gastric ulcer disease is a serious health problem in adult performance horses. The purpose of this study was to evaluate the efficacy of omeprazole powder in sterile water administered intravenously on gastric juice pH in adult horses with naturally occurring gastric ulcers. Omeprazole (0.5 mg/kg, IV) was administered once daily for 5 days to 6 adult horses with endoscopically confirmed gastric ulcers. Gastric juice was aspirated through the biopsy channel of an endoscope and pH measured before and 1 hour after administration of omeprazole on day 1, and then before and after administration of omeprazole on day 5. Gastric ulcer scores were recorded on day 1 before administration of omeprazole and on day 5, 23 hours after the 4th daily dose. Gastric juice pH and ulcer scores were compared between the times using an ANOVA. When compared with the pre-injection value (2.01 $\pm$ 0.17), mean $\pm$ SEM gastric juice pH was significantly ($P < 0.05$) higher when measured one hour after administration of the initial dose (4.35 $\pm$ 0.94), and before (5.27 $\pm$ 0.71) and 1 h after (7.00 $\pm$ 0.10) administration of omeprazole on day 5. Non-glandular gastric ulcer number score significantly decreased from 3.2 $\pm$ 0.08 to 2.0 $\pm$ 1.1, but non-glandular gastric ulcer severity score remained the same. Few glandular ulcers were seen in the study and scores did not change. Because of its potent and long duration of action on gastric juice pH, this intravenous formulation of omeprazole shows promise for treatment of Equine Gastric Ulcer Syndrome (EGUS) in horses with dysphagia, gastric reflux, or other conditions that restrict oral intake of omeprazole paste.[a] Furthermore, measurement of gastric juice pH can be used to determine whether an appropriate dosage of omeprazole has been selected. [a]GastroGard™ Paste, Merial Limited, Duluth, GA.

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ABSTRACT #175

EFFECTS OF KETAMINE INFUSION ON HEMODYNAMIC AND IMMUNOLOGIC VARIABLES IN A CANINE MODEL OF ENDOTOXEMIA. AE DeClue, ES Lechner, JR Dodam, LJ Rubin, X Feng, LA Cohn; University of Missouri, College of Veterinary Medicine, Columbia, MO.

Ketamine is recommended as an anesthetic agent in septicemic patients because of its unique cardiostimulatory effects. Additionally, ketamine attenuates proinflammatory cytokine production and cardiovascular derangement, and decreases mortality in endotoxemic rodent models. We hypothesized that ketamine would ameliorate the hemodynamic and immunologic alterations associated with endotoxemia in dogs. Nine mongrel dogs were assigned to either ketamine or placebo (saline) treatment groups. A cephalic catheter was placed and either ketamine (0.5 mg/kg, IV bolus + 0.12 mg/kg/h, IV) or saline (0.25 ml/kg, IV + 0.5 ml/h, IV) was administered for 2.5 h according to treatment group. At 30 min, LPS (1 mcg/kg, IV) was administered. Temperature, heart rate (HR), systolic arterial blood pressure (SAP) and CBC were evaluated at time 0, 1 h, 1.5 h, 2 h, 2.5 h and 4 h. Serum TNF-α activity was measured at time 0, 1 h and 2 h. The treatment groups were reversed and the study repeated after a 10 day rest period. All dogs developed neutropenia and increased serum TNF-α activity but alterations in temperature, HR and SAP were variable after LPS administration. A significant difference was observed in development of either hypothermia or pyrexia with ketamine (1/9) compared to placebo (6/9). No significant difference was noted in HR, SAP, CBC or TNF-α activity between treatments. This study indicates that ketamine may ameliorate the deleterious effects of LPS on thermoregulation, but not HR, SAP, CBC or TNF-α activity in this model of endotoxemia.

ABSTRACT #176

USE OF CONVENTIONAL AND REAL-TIME PCR TO DETERMINE THE EPIDEMIOLOGY OF HEMOPLASMOSIS IN ANEMIC AND NON-ANEMIC DOGS. JE Sykes, NL Drazenovich, LM Ball, CM Leutenegger. University of California, Davis, California.

The goals of this study were 1) to compare the results of a conventional PCR assay to those of real-time PCR assays for *Mycoplasma haemocanis* (*Mhc*), '*Candidatus* Mycoplasma haematoparvum' (*Mhp*) and '*Candidatus* Mycoplasma haemominutum' (*Mhm*) when applied to blood samples collected from dogs presenting to the University of California, Davis Veterinary Medical Teaching Hospital (VMTH); 2) to use the results to

determine the prevalence of these infections in dogs, and whether any correlation exists between infection and clinical presentation; and 3) to determine whether other hemoplasmas might exist in North American dogs.

Blood samples were obtained over a 1-year period from 113 anemic dogs (HCT < 36%) and 108 non-anemic dogs presenting to the VMTH. For each dog, the results of the CBC collected at the time of sampling were recorded. The electronic database of the VMTH was searched for each patient, and the signalment, environment, medication history, and clinical diagnosis relating to the visit at which PCR testing was performed were recorded. Samples were subjected to conventional PCR as described previously (Jensen *et al* 2001), as well as real-time PCR for *Mhm*, *Mhf*, and *Mhp*. In samples testing positive using real-time PCR, hemoplasma copy number was determined using the standard curve method with normalization to canine GAPDH.

The sensitivity and specificity of real-time PCR compared with conventional PCR were 100% and 40%, respectively. Nine dogs (4%) tested positive for hemoplasma DNA using both conventional PCR and real-time PCR. Hemoplasma copy numbers in these dogs ranged from 1.8 to 2.3×10^4 (mean 3162)/10^6 nucleated cells. Two were positive for *Mhc*, 3 for *Mhm*, 3 for *Mhp* and 1 for both *Mhm* and *Mhp*. Positive results using conventional PCR were associated with lymphoid neoplasia and chemotherapy. A total of 127 dogs (57%) tested positive using real-time PCR only. Hemoplasma copy numbers in these dogs were low, from 0.2 to 5.1/10^6 nucleated cells. Six dogs were positive for *Mhc*, 80 for *Mhm*, 10 for *Mhp*, 15 for both *Mhp* and *Mhm*, 14 for both *Mhm* and *Mhc*, 1 for both *Mhp* and *Mhc*, and 1 for all three species. Organism loads in excess of 1.8/10^6 nucleated cells were significantly associated with lymphoid tumors, chemotherapy, and/or splenectomy when compared with dogs testing negative. However, no association with these variables was noted when all dogs testing positive were considered. Mean total white cell count, neutrophil count and monocyte count were lower in dogs testing positive than dogs testing negative, regardless of copy number. No apparent association between breed, age, gender, or anemia and positive test results was found.

In conclusion, pet dogs may be commonly infected with at least three hemoplasma species at low levels. Organism loads may be increased by chemotherapy and/or splenectomy. Additional studies are ongoing to determine whether hemoplasmas may play a causative role in some cases of canine lymphoma, because almost all dogs with lymphoma in this study were receiving chemotherapy.

ABSTRACT #177

USE OF CONVENTIONAL AND REAL-TIME PCR TO DETERMINE THE EPIDEMIOLOGY OF HEMOPLASMOSIS IN ANEMIC AND NON-ANEMIC CATS. JE Sykes, NL Drazenovich, LM Ball, CM Leutenegger. University of California, Davis, California.

The goals of this study were 1) to compare the results of a conventional PCR (cPCR) assay to those of real-time PCR assays for *Mycoplasma haemofelis* (*Mhf*), '*Candidatus* Mycoplasma haematoparvum' (*Mhp*) and '*Candidatus* Mycoplasma haemominutum' (*Mhm*) when applied to blood samples collected from cats presenting to the University of California, Davis Veterinary Medical Teaching Hospital (VMTH); 2) to use the results to determine the prevalence of these infections in cats, and whether any correlation exists between infection and clinical presentation; and 3) to determine whether other hemoplasmas might exist in North American cats.

Blood samples were obtained over a 1-year period from 135 anemic cats (HCT < 26%) and 128 non-anemic cats presenting to the VMTH. For each cat, the results of the CBC collected at the time of sampling were recorded. The VMTH electronic database was searched for each patient, and the signalment, environment, medication history, and clinical diagnosis relating to the visit at which PCR testing was performed were recorded. Samples were subjected to cPCR as described previously (Jensen *et al* 2001), as well as real-time PCR for *Mhm*, *Mhf*, and *Mhp*. In samples testing positive using real-time PCR, hemoplasma copy number was determined using the standard curve method with normalization to feline GAPDH. When the results of cPCR and real-time PCR were discordant, both assays were repeated. Results were considered positive if samples tested positive repeatedly using one or both assays.

Forty-seven cats (18%) tested positive for hemoplasma DNA. The sensitivity and specificity of real-time PCR compared with cPCR was 76% and 95%, respectively; with re-testing, this increased to 87% and 99.5%, respectively. Sequencing of four cPCR products from samples testing negative using real-time PCR revealed the presence of two *Mhp*-like organisms, one *Mhm*-like organism, and one organism most closely related to a novel hemoplasma reported recently in Switzerland (*Mnov*). Forty-four cats tested positive for small hemoplasmas (*Mhm* or the *Mhp*-like organism, but not *Mhp*), and three cats tested positive for large hemoplasmas (*Mhf* or *Mnov*). Co-infections were not identified. Copy numbers of hemoplasma DNA ranged from 588 to 2.4×10^7 (mean 2.4×10^6)/10^6 nucleated cells. Surprisingly, mean Hct in cats testing positive for small hemoplasmas was higher than that in cats testing negative. Risk factors for small hemoplasma infection included nasal squamous cell carcinoma, FIV infection, outdoor access, and older age.

In conclusion, cats in the United States are infected with at least 4 hemoplasmas: an *Mhp*-like organism, *Mhm*, *Mhf*, and the novel Swiss organism. The association between nasal squamous cell carcinoma and FIV infection may reflect outdoor roaming status of infected cats. Conventional PCR was more sensitive than real-time PCR because of sequence variation between infecting organisms. The degree of bacteremia in infected cats was high, and cPCR was uncommonly negative in samples testing positive using real-time PCR.

ABSTRACT #178

PREVALENCE OF *CYTAUXZOON FELIS* DNA IN BLOOD OF CATS WITH SUSPECTED HEMOPLASMOSIS. MD Haber[1], AM Ishak[2], MD Tucker[1], HS Marr[1], MR Lappin[2], AJ Birkenheuer[1]. [1]North Carolina State University, Raleigh, NC. [2]Colorado State University, Fort Collins, CO.

Anemia in cats is associated with many infectious agents; the hemoplasmas, *Mycoplasma haemofelis* (Mhf) and '*Candidatus* M. haemominutum' (Mhm) are amongst the most common. However, in many cases of suspected hemoplasmosis, PCR assay results are negative. *Cytauxzoon felis* is a protozoan primarily reported in domestic cats from the south-central and southeastern United States. To our knowledge, there have been no studies prospectively evaluating clinically ill cats for *C. felis* infections. The purpose of this study was to determine the prevalence of *C. felis* DNA in blood of a select group of clinically ill cats collected from around the United States.

Blood samples from 37 clinically ill cats suspected by the referring veterinarian to be infected by Mhf or Mhm were selected for study based on sample availability. The samples had previously been assayed for DNA of Mhf, Mhm, *Ehrlichia* spp., *Bartonella* spp., and *Anaplasma* spp. The DNA had been maintained frozen until thawed and tested using a *C. felis*-specific PCR assay. Additionally, the samples were evaluated for the presence of PCR inhibitors by ensuring that feline DNA could be amplified. Positive controls were *C. felis*-infected whole blood and negative controls were water (no DNA).

The samples were collected between January 2001 and November 2004 from cats that resided in Arkansas (n = 1), California (n = 5), Colorado (n = 15), Connecticut (n = 3), Florida (n = 1), Indiana (n = 1), Maine (n = 2), Massachusetts (n = 1), Michigan (n = 1), North Carolina (n = 1), Oklahoma (n = 1), Rhode Island (n = 1), Washington (n = 2), Wisconsin (n = 1) and Virginia (n = 1). Ages ranged from 4.8 months to 18 years old. DNA of Mhf (2 cats), Mhm (2 cats), or both (4 cats) was amplified from 8 of 37 samples. DNA of *B. clarridgeiae* was amplified from one sample and DNA of *B. henselae* was amplified from one sample with concurrent Mhm DNA. DNA of *C. felis*, *Ehrlichia* spp., or *Anaplasma* spp. was not amplified from any sample. Two samples contained PCR inhibitors.

Failure to amplify *C. felis* DNA from this group of cats suggests the organism was not the cause of the presenting complaint. This may be in part due to the fact that the majority of samples were from cats in geographic areas in which *C. felis* has not been recognized. Further investigations should evaluate larger numbers of anemic cats from geographic areas where *C. felis* is endemic.

ABSTRACT #179

INHIBITORY EFFECT OF R-9-(2-PHOSPHONOMETHOXYPROPYL) ADENINE ON THE REPLICATION OF FELINE IMMUNODEFICIENCY VIRUS *IN VITRO* AND POSSIBLE USE OF ITS ORAL MEDICATION derivative. Sae Tanaka[1], Ryoma Une[1], Saki Goto[1], Fuminori Mizukoshi[2], Hajime Tsujimoto[2] and Yasuyuki Endo[1]. [1]Kagoshima University, Kagoshima, Japan. [2]The University of Tokyo, Tokyo, Japan.

Feline immunodeficiency virus (FIV) infection is one of the most important infectious feline diseases. Although the vaccine against FIV has been developed in the United States, a causal treatment strategy against FIV has not been established until now. Recent findings showed that the progression of the clinical stage of FIV infection coincided with the elevation of the blood viral RNA load and therefore the establishment of a causal treatment strategy for FIV infection is essential in feline practice. In the present study, we focused on the potent reverse transcriptase (RT) inhibitor R-9-(2-phosphonomethoxypropyl) adenine (PMPA). We evaluated its inhibitory potential on FIV replication *in vitro* and in FIV naturally infected cats. In addition, we examined *in vivo* the toxicity of the oral form, tenofovir (Viread, Gilead, Foster City, CA).

The feline interleukin (IL)-2 dependent lymphoblastoid cell line, Kumi-1 cells, were exposed to FIV clinical isolates, FIV-Sendai1 or FIV-Shizuoka strain at a multiplicity of infection (MOI) rate of 0.01 with or without PMPA in culture supernatant. RT activities in culture supernatants were measured to determine the virus replication efficiency. The viral replication reached its peak 7 days after FIV-Sendai1 or FIV-Shizuoka exposure in PMPA untreated control cells; however, no virus replication was observed in either FIV-Sendai1 or FIV-Shizuoka infected cells which were treated with PMPA at concentrations ranging from 10 to 100 μM. Treatment with 1 μM PMPA completely inhibited the FIV-Sendai1 replication but partially permitted FIV-Shizuoka growth. In addition, PMPA did not show any cytotoxicity. These findings suggest the possible use of PMPA as a causal therapeutic against FIV infection. We therefore proceeded to evaluate the therapeutic effects of PMPA on two FIV naturally infected cats at asymptomatic carrier stage in accordance with the Guidelines for Animal Experimentation of Kagoshima University. Both cats were positive for anti-FIV antibody but their viral RNA load was at an undetectable level on RT-PCR analysis. Therefore, the proviral DNA level was evaluated to assess the inhibitory effects of PMPA. These cats were treated with PMPA at the dosage rate of 25 mg/kg/day subcutaneously for 7 days. At day 10, the proviral DNA levels in both cats were reduced to 1% of their pretreatment level and no adverse effects were observed in these cats. Furthermore, the *in vivo* toxicity of the oral medication form of the PMPA derivative, tenofovir was also evaluated, and no abnormalities were observed during physical and hematological examinations of three cats.

These findings suggest that PMPA is a potent causal therapeutic for FIV infection. However, further studies on the emergence of the PMPA-resistant strain of FIV and the effects on the viral RNA load are necessary.

ABSTRACT #180

CD4$^+$ T-LYMPHOCYTES COUNT AND CD4$^+$:CD8$^+$ RATIO IN A COLONY OF CATS WITH CHRONIC GINGIVITIS AND NATURALLY-INFECTED WITH FELINE IMMUNODEFICIENCY VIRUS. A Reche Junior, K Haipek. Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, Brazil.

Chronic and intractable gingivitis in FIV-infected cats is a relatively common clinical problem in veterinary practice. The role of FIV in the aetiology of persistent stomatitis is still undetermined. Oral manifestations often found in HIV-infected people are frequently the first clinical sign of the infection and can be considered as indicators of the progression of the HIV infection.

The purpose of this study was to evaluate the CD4$^+$ T-lymphocytes count and CD4$^+$:CD8$^+$ ratio in a colony of cats with chronic gingivitis and compare the findings with severity of oral lesions and FIV status.

To achieve these goals a colony of forty-eight domestic shorthair cats were used. All cats had some degree of gingival inflammation with scores ranging from 1 through 4 (WATERS et al., 1993). Twenty cats were FIV-positive and twenty-eight FIV-negative. CD4$^+$ and CD8$^+$ T-lymphocytes counts were performed by means of flow cytometry in all forty-eight cats and results compared.

FIV-infected cats had a lower CD4$^+$ count than FIV-negative cats, with no difference in the CD8$^+$ count in both groups. A declining CD4$^+$ lymphocyte count was associated with an increasing severity of gingival disease among the FIV-infected cats. There was no difference in the CD4$^+$ lymphocyte count among the cats with gingivitis but FIV-negative despite the degree of gingival inflammation. The CD4$^+$:CD8$^+$ ratio was also lower in the FIV-infected cats when compared to FIV-negative cats.

One can conclude that oral soft tissue lesions were common among FIV-infected cats and lower CD4$^+$ count and CD4$^+$:CD8$^+$ ratio was presented in cats with more severe gingival inflammation and such lesions can be considered as indicators of the progression of the FIV infection in cats.

ABSTRACT #181

THE EFFECT OF ANESTHESIA AND SURGERY ON SEROLOGICAL RESPONSES TO VACCINATION IN KITTENS. JK Levy, MJ Reese, EV Patterson, SJ Tucker. University of Florida College of Veterinary Medicine, Gainesville, FL.

Current animal shelter protocols call for immunization of cats against panleukopenia virus (FPV), calicivirus (FCV), and herpes virus (FHV) immediately upon admission and sterilization before adoption. To accomplish these goals, it is common practice to vaccinate cats when they are under anesthesia for sterilization surgery. Studies in puppies and rodents suggest that concurrent anesthesia and surgery may suppress the immunological response to vaccination, thus leaving patients at greater risk for contracting infectious diseases in the shelter environment. The purpose of this study was to determine which factors, if any, affected serological response to vaccination in kittens, including use of modified live (MLV) vs. inactivated (IA) vaccines, maternal antibody interference, and timing of vaccination in relation to surgery.

SPF seronegative kittens received either MLV (n = 8) or IA (n = 8) vaccines against FPV, FCV, and FHV subcutaneously at 8, 11, and 14 weeks of age. Serum was collected at 8, 9, 11, 14, and 17 weeks of age to measure antibody titers for FPV, FCV, and FHV. Kittens (n = 32) that were seropositive for persistent maternal antibodies were vaccinated with MLV vaccines only and tested for antibodies according to the same schedule. These kittens were sterilized at 7 weeks (1 week prior to vaccination), 8 weeks (vaccinated while under anesthesia), 9 weeks (1 week after vaccination), or not at all. Results were compared by the Mann-Whitney rank sum test and Fisher exact test. $P < 0.05$ was considered significant.

In seronegative kittens, protective titers appeared in 75% (FPV), 50% (FCV), 0% (FHV) by 1 week after MLV vaccination compared to 0% (FPV), 25% (FCV), 0% (FHV) after IA vaccination. In seropositive kittens, maternal antibodies suppressed the rate and magnitude of response to FPV and FHV such that mean titers were significantly lower at all time points and only 75% (FPV) and 50% (FHV) of kittens reached protective titers by 17 weeks. Anesthesia and surgery did not affect response to vaccination.

MLV vaccines should be used when rapid protection of kittens in a high-risk environment is required. Maternal immunity significantly suppresses response to vaccination and this suppression persists past the age currently suggested for final vaccination of kittens (12 weeks). Kittens should be removed from shelters as quickly as possible to avoid infections caused by delayed immunological responses. Surgical sterilization does not inhibit response to vaccination.

ABSTRACT #182

PREVALENCE OF *RICKETTSIA FELIS* IN THE BLOOD OF CATS AND THEIR FLEAS IN THE UNITED STATES. JR Hawley,[a] SE Shaw,[b] MR Lappin.[a] From the Department of Clinical Sciences,[a] Colorado State University, Ft.Collins, Colorado and the University of Bristol, UK.[b]

Rickettsia felis is a spotted fever group organism occasionally associated with fever, headache, myalgia, and macular rash in people. It has been detected in *Ctenocephalides felis*, *C. canis*, and *Pulex irritans*; these fleas have a worldwide distribution. While *R. felis* prevalence rates have been determined in *C. felis* collected from cats in several countries around the world, to our knowledge, *R. felis* prevalence rates in *C. felis* and the cats from which they were collected has not been determined in the United States.

Cat blood in EDTA and their corresponding flea samples (92 pairs; 1–14 fleas per cat) were collected by veterinarians in Alabama, Maryland and Texas and assayed for *Bartonella spp.*, hemoplasmas, *Ehrlichia* spp., and *Anaplasma phagocytophilum* DNA in a previously completed study. The DNA samples were stored at −20°C until used in this study. Two *R. felis* PCR assays were performed using adaptations of previously described protocols. One assay utilized primers that amplify the citrate synthase gene (gltA) and the other utilized primers that amplify the outer membrane protein B (ompB) gene. Endpoint sensitivity was 1.10 fg DNA and 0.110 fg DNA for the gltA and ompB assays, respectively. Positive samples were sequenced and evaluated using the BLAST program on the National Institutes of Health (NCBI) website.

An amplicon consistent with that of *R. felis* was detected in 62 of 92 flea groups (67.4%); 59 of 62 (95.2%) samples were positive in both PCR assays. Both genes of interest were amplified from flea digests from all three states. *Rickettsia felis* DNA was not amplified from any cat blood sample. Of the 62 flea digests with an appropriately sized amplicon in one or both PCR assays, 52 had ample DNA available for genetic sequencing. Results of sequencing showed that all samples had homology with *R. felis* (GenBank Accession number CP000053.1 *Rickettsia felis* URRWXCal2, complete genome).

These results show *R. felis* infection is common in *C. felis* residing on cats in the United States. Results from the cat blood suggest the cats were not infected, eliminated the infection, or were infected at a level below the sensitivity limit of the PCR assays.

ABSTRACT #183

PREVALENCE OF *RICKETTSIA FELIS* INFECTIONS IN CATS WITH AND WITHOUT FEVER. DB Bayliss, J Hawley, M Brewer, MR Lappin. From the Department of Clinical Sciences, Colorado State University, Ft. Collins, Colorado.

Fever of unknown origin is common in cats, and infectious diseases are thought to be the most common cause. *Rickettsia felis* has recently been identified as a cause of fever and illness in people, and antibodies against *R. felis* have been identified in the serum of febrile cats. Cats are likely to be exposed to this organism, as it is carried by the cat flea, *Ctenocephalides felis*. In one of our prior studies, *R. felis* DNA was amplified from 62 of 92 (67.4%) flea groups collected from cats in the United States. The purpose of this study was to compare the prevalence of *R. felis* DNA in the blood of cats with and without fever.

Blood in EDTA from 77 cats with a body temperature of >102.5°F (39.2°C), and 77 cats without fever from the same clinics served as the fever group and control group, respectively. Information collected included state, age, and history of flea exposure. DNA was extracted and *R. felis* PCR assays were performed using oligonucleotide primers for the citrate synthase gene (gltA), and outer membrane protein B gene (ompB). The analytical sensitivities of the 2 assays were determined to be 0.110 fg and 1.1 fg, respectively. Most of the cats came from states with a high flea density (128 cats) and were allowed to go outdoors (50 cats) or were known to have fleas (30 cats). However, all blood samples were negative for *R. felis* DNA.

These results suggest that *R. felis* was not a cause of fever in this group of cats. However, the infection level may have been below the sensitivity limit of the assays utilized. Additional studies, including immunofluorescence assays to determine the seroprevalence rates for *R. felis* in cats with and without fever are indicated to further evaluate for an association.

ABSTRACT #184

MARBOFLOXACIN FOR THE TREATMENT OF EXPERIMENTALLY-INDUCED *MYCOPLASMA HAEMOFELIS* INFECTION IN CATS. AM Ishak, KL Dowers, JR Hawley, D Bachman, SV Radecki, MR Lappin. Colorado State University, Fort Collins, CO.

Administration of tetracyclines or enrofloxacin is associated with improved clinical and laboratory abnormalities in cats infected with *Mycoplasma haemofelis*, but no treatment protocol has consistently eliminated the organism. Additionally, because anti-microbial susceptibility varies among *M. haemofelis* isolates, the continued search for effective therapies is warranted.

Twelve cats were inoculated IV with 2.0 ml of heparinized blood from 2 cats used to amplify *M. haemofelis*. All cats were examined daily; blood for CBC and hemoplasma PCR assay was collected 3 times prior to inoculation, weekly for weeks 1–3 after infection, and twice weekly for weeks 3–6 after infection. Treatment with marbofloxacin (1.25 mg/lb, PO, daily for 14 days) was initiated in 6 randomly selected cats the day the PCV was <30 or the body temperature was >102.5°F (39.2°C). Cats that were PCR positive on day 7 of therapy were treated a total of 28 days.

A number of significant differences between groups were noted; the most clinically significant difference was a higher PCV detected in treated cats on multiple days. Of the treated cats, 5 cats were administered marbofloxacin for 28 days. At the end of the treatment period, 4 of 5 untreated cats (one cat never become PCR positive) and 4 of 6 treated cats were PCR positive. Toxicity associated with marbofloxacin was not noted.

Results of the study indicate that marbofloxacin has clinical effects against this strain *of M. haemofelis* that are similar to those of other drugs, but the protocol utilized did not consistently eliminate infection.

ABSTRACT #185

DETECTION OF ANTI-ERYTHROCYTE ANTIBODIES USING DIRECT COOMB'S TESTING IN CATS EXPERIMENTALLY INFECTED WITH *MYCOPLASMA HAEMOFELIS*. KL Dowers, AM Ishak, CB Webb, KW McCord, DE Bachman, MR Lappin. Colorado State University, Fort Collins, CO.

Mycoplasma haemofelis is an epicellular organism of erythrocytes that is commonly associated with hemolytic anemia in cats. Anti-*M. haemofelis* antibodies are produced in the acute phase of the disease and it is believed that anemia is due at least in part to phagocytosis of antibody-coated erythrocytes. While anti-erythrocyte antibodies were assessed in some earlier studies, sequential evaluation of experimentally infected cats inoculated with a genetically characterized strain of *M. haemofelis* has not been performed to our knowledge. The purpose of this study was to attempt to correlate clinical disease, laboratory abnormalities, and presence of *M. haemofelis* DNA in blood to results of direct Coomb's test and flow cytometry in cats experimentally inoculated with *M. haemofelis* and sampled over time.

Twelve mixed sex, adult, DSH cats were infected with *M. haemofelis* as part of an antibiotic efficacy study. Six infected cats were administered a drug with presumed anti-hemoplasma activity, daily for 28 days after anemia (PCV < 30) or fever (T > 102.5° F) was detected. The remaining six cats served as untreated controls. Blood was collected from all 12 cats for performance of CBC, *M. hemoplasma* PCR assay, and direct Coomb's test at 37°C using previously reported techniques prior to inoculation and on days 15, 21, 24, 28, 35 and 42 after inoculation. On select samples before and after inoculation, blood samples were processed for flow cytometry and assayed for anti-erythrocyte antibodies (FITC-labeled goat anti-feline IgG and IgM antibodies and thiazine orange for the detection of reticulocytes) utilizing an adaptation of a technique validated for use with canine blood in the laboratory of a coinvestigator (CBW).

Of the 12 cats, 11 became persistently PCR assay positive, 10 had microscopic evidence of *M. haemofelis* on the surface of erythrocytes, and 8 developed a PCV < 30%. However, none of cats developed anti-erythrocyte antibodies detectable by direct Coomb's test or flow cytometry.

In previous studies, anti-erythrocyte antibodies have been detected with tests performed at both 4°C and 37°C. We believe our results suggest that the levels of anti-erythrocyte antibodies were below the sensitivity limits of the assays used, only cold agglutinins were induced by this strain of *M. haemofelis*, or that other mechanisms of anemia exist and may vary with different strains of *M. haemofelis*.

ABSTRACT #186

MOLECULAR CHARACTERIZATION OF MULTIPLE-DRUG RESISTANT *ESCHERICHIA COLI* AT A VETERINARY TEACHING HOSPITAL. CM Gurnee, K O'Shea, RP Groman, SC Rankin. Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania.

Multiple-drug resistance (MDR) is an emerging epidemic. It was recently found that 27% of *E. coli* isolates at the Matthew J Ryan Veterinary Hospital (MJRVH) were resistant to amoxicillin/clavulanic acid. This very high rate of beta-lactam resistance prompted further investigations into the mechanisms of resistance and epidemiology of these infections.

Thirty-nine multi-drug resistant *E. coli* isolates were studied; all specimens were collected between January and June 2005. Thirty-five isolates were from clinical cases at MJRVH, and four isolates were external submissions to our microbiology laboratory. Antibiogram data were generated using a MicroScan™ Walkaway system. Pulsed-field gel electrophoresis (PFGE) was performed using a standardized protocol to determine the number of strains of *E. coli*, and BioNumerics™ software was used to determine the phylogenetics of the population. Polymerase Chain Reaction (PCR) was performed to detect blaCMY, blaTEM and blaSHV genes.

There were 38 different antibiograms. Two of the isolates had the same antibiogram and identical PFGE profiles. PCR revealed that 3 isolates were positive for a blaSHV gene and these 3 isolates also contained a blaTEM gene. In total, 20 isolates were positive for blaTEM and thirty isolates were positive for a blaCMY gene; twelve blaCMY positive isolates also contained a blaTEM gene. One isolate was negative for all three genes and an alternate mechanism for beta-lactamase resistance is suspected.

Thirty unique PFGE profiles were generated following restriction with *Xba*I. Three clusters of three identical isolates and three clusters of two identical isolates were identified. One of these was from a single patient although the isolates were obtained from different sites of infection. All of the other clusters were comprised of at least two different patients.

The clusters of identical pulsed-field profiles observed in multiple patients suggest that nosocomial infection may have occurred. However, the majority of patients were infected with different strains of *E. coli*, and this suggests that community-acquired infection with multiple drug resistant *E. coli* strains may be more widespread than previously expected.

The multiple different antibiograms in the face of several similar pulsed-field profiles suggests that antibiogram data alone, as a phenotypic characteristic, is insufficient to distinguish relapse from recurrence of infection in a given patient.

In conclusion, there appear to be several mechanisms, including some that need further characterization that confer beta-lactam resistance in these bacteria. There was evidence for both community-acquired and nosocomial infection with MDR *E. coli*. Further work is required to determine the source of multiple beta-lactam resistance genes in these MDR *E. coli* infections.

ABSTRACT #187

ESCHERICHIA COLI ANTIMICROBIAL RESISTANCE IN SMALL ANIMALS: THE SCOPE OF THE PROBLEM. Dawn Boothe, Timothy Smaha, Auburn University, Auburn, AL.

As continues to be demonstrated in lay and scientific media, antimicrobial resistance remains a global concern for both human and veterinary medicine. In veterinary medicine, the focus is on public health related to use of antibiotics in food animals. However, this focus is shifting toward antimicrobial use and subsequent resistance in small companion animals. A previous study of 300 small animal pathogens from labs throughout the USA documented an alarming 39% resistance rate of *Escherichia coli* to five common fluorinated quinolones. A retrospective study (n = 310, time= 2 yr) last summer of six academic laboratories identified potential geographic differences in the rates of resistance to small animal *E coli* (limited to UTI). The current report further examines these differences, studying small animal *E coli* pathogens prospectively among geographic areas and between laboratory types. All sample isolates were collected during a 5 month period, represented various tissues (including urine) and were processed by one laboratory. Sample isolates (n = 350) were provided by 9 veterinary microbiology laboratories within the United States. Commercial laboratories [n = 4, total 211 isolates] were California (CA; n = 60), Indiana (IN; n = 42), Massachusetts (MA; n = 57), Wisconsin (WI; n = 52) and academic [n = 5, total 140 isolates] were Alabama (AL; n = 43), Kansas (KS; n = 25), Mississippi (MS; n = 25), North Carolina (NC; n = 25), and Washington (WA; n = 20). Using CLSI guidelines, each isolate was subjected to MIC determination using the Epsilon Test (E-test™), which tests a wider MIC range compared to standard antibiograms. Drugs for which MIC were determined included amoxicillin (AMX), amoxicillin-clavulanic acid (AMXC), doxycycline (DC), enrofloxacin (ENR), cefpodoxime (CFP), trimethoprim-sulfadimethoxine (TMS), and gentamicin (GM). Overall resistance (R or I based on CLSI guidelines) for each lab to all seven antimicrobials ranged from a low of 11% (CA) to a high of 47% (AL). Overall susceptibility for all labs for each drug was AMX (49.4%), AMXC (58.3%), CFP (74.6%), DC (75.1%), ENR (76.4%), TMS (78.7%), and GM (84.2%). Susceptibility was greater in commercial laboratories (83%) compared to academic laboratories (65%). Regionally (averages), West Coast laboratories (WS, CA) had the highest susceptibility for all drugs combined (81%), followed by the Central US (KS, IN, WI: 78%), East Coast (MA: 76%), and the South East (AL, MS, NC: 61%). WI had the lowest MIC_{90} for all drugs save TMS and ENR (CA lowest for these) and AL had the highest MIC_{90} for all drugs, with each drug $MIC_{90} > MIC_{BP}$. This study suggests geographical factors influence antimicrobial resistance and resistant *E coli* are more commonly isolated at academic institutions compared to commercial laboratories.

ABSTRACT #188

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) COLONIZATION IN VETERINARY PROFESSIONALS. B Hanselman, J Rousseau, S Kruth, JS Weese. Dept. Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

MRSA is an important cause of hospital-acquired infections in humans worldwide and is increasingly being identified as a community-associated pathogen. The objective of this study was to determine the prevalence of nasal MRSA colonization in veterinary personnel attending an international veterinary conference. Nasal swabs were collected from 417 volunteers at the 2006 ACVIM Forum. Swabs were enriched in 2 ml of broth consisting of 10 g/L Tryptone T, 75 g/L sodium chloride, 10 g/L mannitol and 2.5 g/L yeast extract for 24 hours at 35°C. An aliquot of broth was then inoculated onto mannitol-salt agar with 10 µg/ml cefoxitin and incubated at 35°C for 48 hours. Isolates were identified as *S. aureus* based on colony morphology, Gram stain appearance, catalase and coagulase reactions and latex agglutination test (LAT). Methicillin-resistance was confirmed via PBP2a LAT and the MIC of oxacillin was determined via Etest. Isolates were typed via *smaI* pulsed-field gel electrophoresis (PFGE). Isolates were tested for the presence of the Panton-Valentine leukocidin (PVL) via *lukF-lukS* real time PCR. Risk factors were assessed via stepwise forward logistic regression.

MRSA was isolated from 27/417 (6.5%) volunteers including 22/376 (5.8%) veterinarians, 5/34 (14.7%) technicians and 0/7 others. No colonized individuals reported a history of recent hospitalization or previous diagnosis of MRSA infection. Large animal practice was the only significant independent risk factor for colonization (OR = 2.9; 95% CI 1.2–6.6) with 12/271 (4.4%) small animal personnel, 15/96 (15.6%) large animal personnel and 0/52 others colonized ($P < 0.001$). Two predominant clones were identified using PFGE. Canadian epidemic MRSA-2 (equivalent to USA100) was isolated from 11 small animal and two large animal personnel from the US (n = 12) and Denmark (n = 1). In contrast, CMRSA-5 (equivalent to USA 500) was isolated exclusively from large animal personnel ($P < 0.001$) from the US (n = 10), UK (n = 2) and Germany (n = 1). One other isolate, possibly related to CMRSA-2, was recovered from a US small animal veterinarian. A cluster of 5 colonized individuals was identified from one facility. Four large animal personnel were colonized with CMRSA-5 and 1 small animal veterinarian was colonized with CMRSA-2 on PFGE from this cluster. No isolates were identified as carrying the genes for PVL production. All MRSA isolates were sensitive to vancomycin and mupirocin.

MRSA colonization may be a significant occupational risk for veterinary personnel and colonized personnel could be sources of infection for patients. Widespread CMRSA-5 colonization in large animal personnel supports previous studies, that suggest this isolate is endemic in the horse population and transmission between horses and humans occurs. In contrast, the predominance of the common CMRSA-2 clone in veterinary personnel in this study, and in household pets in other studies suggests that household pet MRSA is a closer representation of the types of MRSA in the general human population in a given area.

ABSTRACT #189

METHICILLIN-RESISTANT STAPHYLOCOCCI IN HEALTHY DOGS AND HORSES IN SLOVENIA. M. Vengust[1], V. Cestnik[1], M.E.C. Anderson[2], J.S. Weese[2]. [1]University of Ljubljana, Veterinary Faculty, Slovenia, SI-1000; [2]Department of Clinical Studies, University of Guelph, Ontario, Canada.

Staphylococci are one of the major groups of bacterial commensals isolated from skin, skin glands, and mucous membranes of mammals. Coagulase positive staphylococci (CoPS) such as *S. aureus, S. intermedius* and *S. schlieferi* are important opportunistic pathogens. Methicillin-resistant strains of *S. aureus* (MRSA) in particular, and other CoPS and coagulase negative staphylococci (CoNS) are of concern because of their virulence, resistance to multiple antimicrobials, ability to transfer genetic determinants of antimicrobial resistance, and their role as nosocomial and community pathogens. MRSA is an important pathogen in humans in Slovenia; yet, MRSA infections in veterinary species have not been reported. The objective of this study was to evaluate the prevalence of methicillin-resistant staphylococcal (MRS) colonization in clinically normal dogs and horses in Slovenia.

A total of 300 healthy horses of various breeds from 14 farms were enrolled. Horses selected were housed in publicly open establishments used for tourist/show purposes (n = 100), riding schools and recreational facilities (n = 100), and competition horses (n = 100). One nasal swab was collected from horses.

A total of 200 healthy dogs of various breeds were enrolled and 400 swabs collected (agility competition (n = 70), rescue/working dog training camps (n = 70), and household dogs (n = 60)). Two swabs were taken from dogs from: 1) anterior nare, and 2) a combination of perineal area and 0.5 cm into the anus.

Direct culture was performed by inoculating swabs onto mannitol-salt agar with 2 µg/ml oxacillin. Enrichment culture was performed by incubating swabs in broth containing 7.5% NaCl for 24 hours prior to inoculation onto mannitol-salt agar with 2 µg/ml oxacillin.

MRSA was not isolated from any sample. Methicillin-resistant CoNS (MRCoNS) were present in 126/300 (42%) horse samples. MRS were isolated from 26/200 dogs (13%). Methicillin-resistant *S. intermedius* was isolated from nasal swab from one dog and from perianal/anal swab from two dogs. MRCoNS were isolated from the remaining 23 dogs: 15 from nasal swabs only, 6 from rectal/perineal swabs only and, 2 from both swabs.

MRCoNS typically only cause disease in compromised hosts; however, there is a concern that they can serve as reservoirs in the community and could be associated with emergence of novel MRSA strains. Continued surveillance is indicated to determine whether MRSA will emerge in the animal population and become a concern for animal disease and zoonotic infection.

ABSTRACT #190

SEROPREVALENCE OF *BORRELIA BURGDORFERI* ANTIBODIES IN DOGS AT A VETERINARY TEACHING HOSPITAL IN A LYME ENDEMIC AREA. MP Littman, U Giger, TJ Nolan. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

The clinical diagnosis of borreliosis and other vector-borne diseases can be difficult based on serology in Lyme endemic areas. We determined the prevalence of antibodies against *Borrelia burgdorferi* (Bb) as well as *Ehrlichia canis* (Ec), and a positive heartworm antigen (Hw) result in dogs seen as patients at our referral clinic (mean 6.7 ± 3.8 yrs) and healthy canine blood donors (>23 kg, mean 4.6 ± 2.4 yrs) at the University of Pennsylvania; we then compared the data in three common large breeds (Labrador and Golden retrievers and German shepherds).

Between May 2001 and October 2004, 2526 samples were tested using the SNAP-3 (IDEXX) test. Duplications due to paired sampling and annual

rechecks were excluded so that a single representative test day was chosen for each dog (n = 2290). Among 1812 canine patients, 15 positive test results were found for Hw antigen (<1%), 37 positive for Ec antibodies (<2%), and 648 positive for natural exposure Bb antibodies (28%). Of the 478 healthy donors, 5 positive Ec (1%) and 114 positive Bb (24%) results were found, and none were Hw antigen positive.

BORRELIA BURGDORFERI ANTIBODY STATUS IN % IN SELECTED BREEDS						
	Labradors		Golden Retrievers		German shepherds	
Clinical signs	n	% Pos	n	% Pos	n	% Pos
Lameness	32	50*	17	71*	21	33
Neuro-ocular signs	29	28	15	67	8	38
Protein losing nephropathy	34	76*	24	96*	2	50
Hemolytic anemia	17	24	3	67	3	33
Thrombocytopenia	32	44*	21	71*	4	0
Cardiopulmonary	16	0	5	40	4	0
Other illness, eg cancer	37	19	27	37	14	29
Asymptomatic	99	17	47	38	42	24
Total =sick + healthy dogs	251	32	134	53	92	27

*p<0.05, significant by Fisher's Exact test, when compared to healthy dogs of that breed

Lameness, protein-losing nephropathy, and thrombocytopenia were clinically associated with positive Bb serology in Labrador and Golden retrievers, but not in German shepherds in the patient population. No association was found for a positive Hw antigen or Ec antibody test result. The causal relationship between Bb antibody positivity, actual infection, and specific clinical signs remains to be further determined in Labrador and Golden Retrievers.

ABSTRACT #191

SEROPREVALENCE OF *EHRLICHIA CANIS* AND *BORRELIA BURGDORFERI* INFECTION IN DOGS, SOUTH KOREA. SE Lee, KH Song, J Liu, SJ Park, DH Kim; College of Veterinary Medicine, Chungnam National University, Daejeon, South Korea.

Ehrlichia canis infection is a tick-borne disease of dogs. Clinical signs usually described in canine ehrlichiosis are fever, depression, anorexia, thrombocytopenia, leukemia and anemia. Lyme disease is caused by *Borrelia burgdorferi*, which comprises several species that affect human and dogs worldwide. Clinical illness begins 2 to 5 months after tick exposure. Clinical signs include fever, inappetence, lethargy, lymphadenopathy, and episodic shifting limb lameness related to polyarthritis. This study was performed to investigate the seroprevalence of *E. canis* and *B. burgdorferi* infection by ELISA kit in asymptomatic dogs in South Korea. Two-hundred and thirty six dogs (116 females and 120 males, 79 dogs in Gangwon, 53 dogs in Gyunggi, 36 dogs Chungnam and 68 dogs Gyungnam provinces) were examined for detection *E. canis* and *B. burgdorferi* antibody in South Korea. Whole blood sample was collected from 236 dogs and tested for detection of *E. canis* and *B. burgdorferi* antibody using commercial ELISA kit (SNAP test, IDEXX Laboratories, USA). The mean overall seropositive rate of *E. canis*, *B. burgdorferi* and coinfection of them were 9.8%, 4.2% and 1.3% using antibody detecting ELISA kits, respectively. Seropositive rate of *E. canis*, *B. burgdorferi* and coinfection of them were 11.4%, 3.8% and 1.3% in Gangwon province, 5.7%, 5.7% and 1.9% in Gyunggi province, 16.7%, 2.8% and 2.8% in Chungnam province and 16.2%, 4.4% and 0% in Gyungnam province by ELISA kits, respectively. The results indicate that the overall seroprevalence of *E. canis* and *B. burgdorferi* is low. To our knowledge, this is the first detection of *E. canis* and *B. burgdorferi* infection by ELISA kit in dogs in South Korea to date.

ABSTRACT #192

BARTONELLA SEROPREVALENCE IN DISEASED DOGS AND HEALTHY BLOOD DONOR DOGS IN THE NORTHEASTERN UNITED STATES. JS Diroff[1], WD Hardy, Jr[2], EE Zuckerman[2], KJ Drobatz[1], TJ Nolan[1], E Withnall[1], U Giger[1], MP Littman[1]. [1]University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA. [2]National Veterinary Laboratory, Inc, Franklin Lakes, NJ.

The prevalence and clinical importance of *Bartonella spp.* infection remains unclear in sick and healthy dogs. Currently blood donors are not routinely screened for *Bartonella* infections. Between May 2004 and November 2005 the seroprevalence of antibodies against *Bartonella spp.* was determined in 202 healthy large breed dogs used in a volunteer blood donor program (donors, median age 4.5 yrs) and 186 sick dogs (patients) hospitalized at the University of Pennsylvania (median age 6.0 yrs). An immunoblot analysis for antibodies to *Bartonella spp.*, utilizing *B. henselae* as the antigen, along with other routine tests and serology were used in both groups and the clinicopathologic findings of *Bartonella* seropositive and negative patients were compared.

Seroprevalence for antibodies against *Bartonella spp.* was significantly ($p \leq 0.001$) higher in patients (20.4%) than in donors (7.4%). Among the patient group, dogs with peripheral lymphadenopathy were more likely to be *Bartonella* seropositive ($p \leq 0.05$). No statistically significant associations were found in this patient population with other clinical findings such as ocular disease, fever, thrombocytopenia, hyperglobulinemia, anemia, lameness, proteinuria, pyogranulomatous disease, hepatitis, endocarditis/pericarditis, and meningitis. *Bartonella* seropositive patients were more likely to be seropositive to *Anaplasma phagocytophilum* ($p \leq 0.025$), while there were no significant associations in seropositivity observed to *Ehrlichia canis*, *Rickettsia rickettsii*, or *Borrelia burgdorferi*. Seropositivity to *A. phagocytophilum* did not significantly affect the prevalence of peripheral lymphadenopathy. As required, all blood donors tested serologically negative for all other screened infectious disease microorganisms, except 23.8% were *B. burgdorferi* seropositive.

In conclusion, sick dogs in the northeastern United States have more likely been exposed to *Bartonella spp.* than healthy dogs from the same region, but seropositivity does not document active infection or a disease association. The higher frequency of peripheral lymphadenopathy as well as the trend for some other clinical manifestations in seropositive versus seronegative sick dogs may suggest a causal relationship. However, larger surveys with additional screening methods for active infection and response to therapy studies are needed to confirm any disease associations. Sick *Bartonella spp.* seropositive dogs are also more likely to be seropositive to the arthropod-borne agent *A. phagocytophilum*, but not more likely to be seropositive to others, such as *B. burgdorferi*. This may be related to similarities in the arthropod vector route or the required contact time for transmission of *Bartonella spp.* and *A. phagocytophilum*. Finally, healthy dogs may also be seropositive to *Bartonella spp.* in this region and recommendations for the management of such emerging diseases and the use of exposed donors needs to be developed.

ABSTRACT #193

MOLECULAR PREVALENCE OF *BARTONELLA* SPP. IN BRAZILIAN DOGS. PPVP Diniz[1], DS Schwartz[1], RG Maggi[2], MB Cadenas[2], EB Breitschwerdt[2]. [1]School of Veterinary Medicine and Animal Science, São Paulo State University (FMVZ – UNESP), Botucatu, São Paulo, Brazil. [2]College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

Bartonella spp are emerging vector-borne pathogens that cause persistent, often non-clinical bacteremia in mammalian hosts including dogs, cats, mice, rabbits and human beings. Dogs and wild canids constitute an environmental reservoir for human exposure to *B. vinsonii berkhoffii* and potentially *B. henselae*. The tropical and sub-tropical weather in South America favors multiplication of ticks and fleas which are known or proposed vectors for *Bartonella* spp. As there is minimal information on bartonellosis in South America, the purpose of this study was to investigate the molecular prevalence of *Bartonella* spp. infection in sick dogs from Brazil. One hundred ninety eight consecutive dogs with clinicopathological abnormalities consistent with flea or tick-borne infections were sampled at a Veterinary Teaching Hospital population in Botucatu, southeastern Brazil. Inclusion criteria included at least three of the following: presence of ticks, fever, bleeding, neurological signs, inflammatory ocular disorders, anemia, leukopenia, thrombocytopenia and/or hyperproteinemia. Using primers targeting the 16S-23S rRNA gene intergenic transcribed spacer (ITS), a 604 bp product of *Bartonella* DNA was amplified from only 1/198 samples. A stored frozen blood sample from this dog was inoculated in *Bartonella*-alpha *Proteobacteria* liquid growth medium (BAPGM) and cultured at 35°C for 9 days, at which time BAPGM was sub-inoculated onto blood agar plates for colony isolation. Conventional ITS PCR using samples of liquid and solid media again yielded a 604 bp product. All three 604-bp PCR products were purified and cloned after which the plasmid DNA was sequenced. Sequences aligned using Basic Local Alignment Search Tool (BLAST version 2.0) were identical and were 99% homologous with *B. henselae* ITS gene sequences available in the GenBank database. For secondary confirmation, a *B. henselae* bacteriophage-associated gene (Pap31) was amplified from the original genomic DNA, the liquid culture and the plate isolate. The 564-bp Pap31 gene product was 100% homologous to *B. henselae*. The sequences obtained for ITS region and Pap31 gene was deposited in GenBank as *B. henselae* strain Brazil-1, under accession numbers DQ346666 and DQ351240, respectively. Our results indicate that molecular prevalence of *Bartonella* spp. infection among clinically ill dogs examined at a Teaching Hospital in Brazil was 0.51%. Although infrequent in this dog population, *B. henselae* was successfully isolated and characterized genetically, which to our knowledge represents the first sequences for a Brazilian isolate.

ABSTRACT #194

EXPERIMENTAL INOCULATION OF DOGS WITH A HUMAN ISOLATE (NY18) OF *ANAPLASMA PHAGOCYTOPHILUM* AND DEMONSTRATION OF PERSISTENT INFECTION FOLLOWING DOXYCYCLINE THERAPY. A Alleman[1], R Chandrashekar[2], M Beall[2], K Cyr[2], A Barbet[1], A Lundgren[1], H Sorenson[1], H Wamsley[1], S Wong[3]. [1]University of Florida College of Veterinary Medicine, Gainesville, FL. [2]IDEXX Laboratories, Westbrook, ME. [3]Wadsworth Center, Albany, NY.

The purpose of this study was to monitor experimental infection of *A. phagocytophilum* in dogs. Two adult male Beagle dogs were inoculated intravenously with the NY18 strain of *A. phagocytophilum*, cultivated in fetal rhesus (*Macaca mulatta*) RF/6A endothelial cells. Once inoculated, the animals were monitored by physical exam, CBC, and biochemical profile at decreasing time intervals over the 1 year period of the trial. Seroconversion was monitored by ELISA using the in-clinic test SNAP®4Dx™, recombinant major surface protein 5 (rMSP5) and IFA testing. Infectivity was confirmed by nested PCR using *msp2* of *A. phagocytophilum*, and real-time PCR assays amplifying the Gro*El* gene and the *msp2* of *A. phagocytophilum*. Clinical, hematological or biochemical abnormalities were not observed during anytime of the trial period. Both animals seroconverted to a positive status by day 8 PI (SNAP®4Dx™ and IFA) and day 35 PI (rMSP5 ELISA) and remained seropositive throughout the duration of the trial. Both animals were positive on all PCR assays by day 8 PI. The animals tested PCR-positive on various samples collected from day 1 to 35 PI. From days 39 to 224 the animals were rarely positive by PCR analysis. Between days 230 and 243 PI, prednisolone was administered orally at 2 mg/lb/day. Both dogs were PCR positive using nested and real time-PCR assays based on *msp2* by day 13 post-immunosuppression (243 days PI). Treatment with doxycycline was initiated from days 261 to 275 PI at an oral, daily dose of 10 mg/kg. All PCR assays were negative from days 261 to 298 PI, up to 5 weeks after treatment with doxycycline. On day 316 PI, prednisolone was again administered at an oral dose of 2 mg/lb/day for 10 days. Both dogs tested PCR-positive using nested and RT-PCR *msp2* assays between days 11 to 14 post-prednisolone therapy, up to 340 days PI. We conclude that persistent infection can be established in dogs using a human isolate of cultivated *A. phagocytophilum*. In addition, as seen with *Ehrlichia canis* and *Anaplasma marginale* infections, doxycycline therapy may not eliminate the organism from infected animals.

ABSTRACT #195

POSSIBLE TREATMENT STRATEGY AND CLINICAL ESTIMATION FACTORS FOR ONSET, RELAPSE AND PROGNOSIS OF *BEBESIA GIBSONI* INFECTION. Koretoki Suzuki, Haruna Wakabayashi and Yasuyuki Endo. Kagoshima University, Kagoshima, Japan.

Babesia gibsoni (*B. gibsoni*) is still an important pathogen in small animal practice in Japan. To date, diminazene aceturate has been widely used for the treatment of *B. gibsoni* infection, however, treatment with diminazene often fails to eliminate *B. gibsoni* from affected dogs and a high incidence of relapse of babeisosis has been observed. Development of a clinical tool for estimating the onset, relapse and prognosis of canine babesiosis therefore seems necessary. In the present study, we evaluated the effectiveness of a combination therapy using clindamycin, metronidazole and doxycyclin, which have been reported to be effective against part of canine, human and mouse babesiosis. In an attempt to ascertain clinically useful tests for the onset, relapse and prognosis of canine babesiosis, we examined platelet count, measured the serum C-reactive protein (CRP), degree of parasitemia and performed polymerase chain reaction (PCR) for the detection of *B. gibsoni* derived genome DNA.

Four clinically healthy dogs were experimentally infected with *B. gibsoni*. Splenectomy was performed in all four dogs: dogs No. 1–3 underwent splenectomy before experimental inoculation with *B. gibsoni* and dog No. 4 after experimental inoculation. All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation of Kagoshima University. After inoculation, dogs 1–3 showed thrombocytopenia prior to the development of severe anemia. These dogs were administered diminazene when their PCV dropped below 15%. Their clinical features showed temporal improvement, however, two of them suffered a relapse of severe anemia with thrombocytopenia. These dogs then received a combination therapy of clindamycin, metronidazole and doxycyclin. Clinical symptoms disappeared and hematological findings also recovered and stabilized within an average of 65 days after initiation of this therapy. Dog No. 4 developed severe anemia with thrombocytopenia 43 days after splenectomy. This dog received combination therapy alone and showed a recovered PCV 18 days post treatment. None of the four dogs showed any evidence of relapse after initiation of the combination therapy. Clinical estimation factors were then evaluated. Thrombocytopenia was observed 7 days and 6 days prior to the development of severe anemia at the time of primary onset and relapse, respectively. The serum CRP level increase coincided with the progression of anemia but decreased with the PCV recovery. Twelve days after the initiation of combination therapy dog No. 4 showed negative PCR results and the presence of parasites in the blood smears of all 4 dogs became negative on average 12 days after initiation of combination therapy. Our results indicate that the combination therapy is an effective alternative strategy in the treatment of *B. gibsoni* affected dogs. Estimation of the primary onset seems to be difficult, however, measuring CRP and PCR analysis would be useful indicators in the prediction of relapse and prognosis of *B. gibsoni* infection.

ABSTRACT #196

DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR THE DETECTION OF PATHOGENIC LEPTOSPIRES IN CANINE URINE. K Kasper, KF Lunn, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

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* and *L. kirschneri*, and the consequences of infection in dogs range from subclinical to peracute disease, with typical presentations including acute renal failure or acute hepatic disease. The tests most commonly used to diagnose canine leptospirosis include the microscopic agglutination test on serum samples, and polymerase chain reaction (PCR) on urine samples. The aim of this study was to develop a real-time PCR assay for detection of pathogenic leptospires in the urine of dogs. Real-time PCR technology is a flexible, cost-effective, sensitive, specific and quantitative method for the diagnosis of infectious disease. The development of a sensitive and quantitative test for canine leptospirosis will enhance our ability to diagnose this zoonotic disease and will provide an opportunity to compare the effects of different treatment protocols on shedding of organisms in the urine of infected dogs.

The PCR primers and probe were generated using Beacon Designer Probe and Primer Design Software (Premier Biosoft International), and synthesized by Integrated DNA Technologies (IDT, Coralville, IA). Based on previously published data,[1] primers were selected to amplify an 87 base pair product from a region of the *Leptospira spp*. 16S ribosomal DNA sequence. The fluorogenic probe included the reporter dye 6-carboxy-fluorescein (FAM) at the 5′ end, and the quencher dye 6-carboxy-tetramethyl-rhodamine (TAMRA) at the 3′ end.

DNA was extracted from reference cultures of the pathogenic leptospiral serovars canicola, icterohaemorrhagiae, grippotyphosa, pomona, bratislava, and hardjo. Real-time PCR reactions were run on a Bio Rad iCycler iQ (Hercules, CA). The assay was positive for all 6 serovars, and product was detected over ranges of up to 6 ten-fold dilutions of the initial cultures. The assay did not produce a detectable product from DNA isolated from *Staphylococcus spp*, *Streptococcus spp*., *Pseudomonas spp*. or *E. coli*. Urine samples from a normal dog were spiked with aliquots of reference cultures of the serovars canicola, grippotyphosa and pomona. The real-time PCR reaction was run on DNA isolated from the urine samples. Product was detected from urine spiked with each of the serovars used, over a range of 4 ten-fold dilutions of the organisms in urine.

Previous results have suggested that the primers and probes used in this study do not detect non-pathogenic leptospiral serovars.[1] The results of present study indicate that this real-time PCR assay detects at least 6 leptospiral serovars that are pathogenic in dogs, and the assay is able to detect organisms in canine urine samples.

[1]Smythe, LD, Smith, IL et al. A quantitative PCR (Taqman) assay for pathogenic Leptospira spp. BMC Infectious Diseases 2 (1):13–19.

ABSTRACT #197

EVALUATION OF CHROMATOGRAPHIC IMMUNOASSAYS FOR THE DIAGNOSIS OF *GIARDIA* SPP. AND *CRYPTOSPORIDIUM* SPP. OF CATS AND DOGS. DE Bachman, M Brewer, JR Hawley, and MR Lappin. Colorado State University, Fort Collins, CO.

Cryptosporidium spp. and *Giardia* spp. have been recognized as significant infectious agents that infect a variety of animals including humans. Prevalence rates in dogs and cats with diarrhea vary by the study, but are generally >5% for both organisms. Because of their small size, identification can be difficult on routine fecal flotation. For these reasons, rapid and accurate point-of-care diagnostic tests are needed for small animal practitioners. The purpose of this study was to compare the results of an immunofluorescent assay (Merifluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, OH), for detection of *Giardia* cysts and *Cryptosporidium* oocysts in human feces, 2 lateral flow chromatographic immunoassays for the detection of *Giardia* and *Cryptosporidium* antigen

(AG) in human feces (Immunocard STAT! *Cryptosporidium*/*Giardia*, Meridian; XpectÖ *Giardia*//*Cryptosporidium*, Remel Inc., Lenexa, KS), and a lateral flow chromatographic assay for detection of *Giardia* AG in dog and cat feces (SNAP® *Giardia*, IDEXX Laboratories, Westbrook, ME).

A convenience sample set of previously frozen feces (n = 17), fresh feces (n = 15), and feces stored at 4°C for 21 days (n = 4) that had been collected from dogs or cats were assayed. Assays were performed according to the manufacturer's instructions. Percentage concordance between the assays was calculated.

Based on IFA testing, *Giardia* spp. cysts were identified in 17 of 36 samples (47.2%) and *Cryptosporidium* spp. oocysts were detected in 14 of 36 samples (38.9%). For *Giardia*, the percentage concordances between assays were: 1. IFA versus Meridian AG test (94.4%); 2. IFA versus IDEXX AG test (94.4%); 3. IFA versus Remel AG test (91.7%); 4. Meridian AG test versus IDEXX AG test (100%), 5. IDEXX AG test versus Remel AG test (97.2%); and 6. Remel AG test versus Meridian AG test (97.2%). There were 2 samples (previously frozen) that were negative for *Giardia* cysts by IFA but AG test positive in all 3 assays, suggesting the IFA was falsely negative. For *Cryptosporidium*, the percentage concordances between the assays were: 1. IFA versus Meridian AG test (55.6%); 2. IFA versus Remel AG test (61.1%); and 3. Meridian AG test versus Remel AG test (94.4%). However, only 2 samples each were positive in the Meridian and Remel *Cryptosporidium* AG tests.

Results suggest that all 3 *Giardia* AG tests are suitable for point-of-care use with feces from dogs and cats. However, the 2 chromatographic assays for detection of *Cryptosporidium* spp. did not detect AG in feces of most dogs or cats with oocysts documented by IFA. Differences between assay results may relate to antigenic differences between the common *Cryptosporidium* spp. found in dogs and cats (*C. canis*, *C. felis*) and those found in people (*C. parvum*, *C. hominus*).

ABSTRACT #198

PREVALENCE OF SELECT INFECTIOUS AGENTS IN DIARRHEA SAMPLES FROM DOGS AND CATS IN NORTH CENTRAL COLORADO ANIMAL SHELTERS. ME Spindel,[1,2] L Riggenbach,[3] MR Lappin.[1] From Colorado State University, Fort Collins, CO,[1] Larimer Humane Society, Ft. Collins, CO,[2] and Colorado Humane Society, Englewood, CO.[3]

Diarrhea in animals housed in humane societies is common. It is often assumed that infectious agents are the cause, but there have been few infectious agent prevalence studies published. Furthermore, to our knowledge, limited information concerning geographical differences in prevalence rates is available. The purpose of this study is to report the results of a battery of infectious disease tests performed on fecal samples from shelter animals with diarrhea admitted to two animal shelters in North-central Colorado.

Between April and December of 2005, fecal samples were collected from a convenience sampling of surrendered and stray animals greater than 4 weeks of age with diarrhea but no vomiting. Those animals suspected to have parvovirus infections were excluded. Fresh fecal samples were collected and stored at 4°C until assayed. Tests included microscopic examination after zinc sulfate centrifugation flotation (FF), electron microscopy (EM), parvovirus antigen testing (SNAP® Parvo Test, IDEXX Laboratories, Westbrook, ME), rotavirus antigen testing (Sure-Vue* Rota Test Kit, Fischer Scientific, Pittsburg, PA) aerobic bacterial culture, immunofluorescent antibody testing (IFA) for *Cryptosporidium* oocysts and *Giardia* cysts (Merifluor® *Cryptosporidium*/*Giardia*, Meridian Bioscience, Inc., Cincinnati, OH) and *Giardia* antigen (SNAP® *Giardia*, IDEXX).

Eighteen feline samples and 19 canine samples were collected from a total of 45 animals (several litters were group sampled). The majority of animals were <6 months of age. Time of sampling relative to days from admission was available for animals from one shelter when the abstract was written. Sampling at this shelter was conducted within 24 hours of diarrhea, and occurred from 1 to 38 days (mean = 12 days) after admission. Of the feline samples, 2 of 18 (11.1%) were positive for *Isospora felis* or *I. rivolta* by FF and 1 of the 9 samples tested (11.1%) was positive for rotavirus by antigen testing; all other test results were negative. Of the canine samples, 13 of 19 (68.4%) were positive for at least one agent. *Giardia* spp. (13 samples; IFA), *Isospora ohioensis* (3 samples; FF), *Cryptosporidium* oocysts (3 samples; IFA), and *Salmonella* spp. (1 sample; culture) were detected. Results of EM, parvovirus antigen test, and rotavirus antigen test (9 samples tested) were all negative. Percentage concordance between *Giardia* test results was calculated: 1. IFA versus antigen (97.3%); 2. IFA versus FF (70.6%); and 3. antigen versus FF (73.5%).

In the study population, parasites, but not viruses, *Salmonella* or *Campylobacter* spp. were common. Results of the IFA and antigen tests for *Giardia* spp. had a high percentage concordance but the results suggest that FF was commonly falsely negative for *Giardia* cysts.

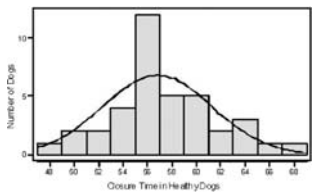
ABSTRACT #199

ESTABLISHMENT OF A REFERENCE INTERVAL AND EVALUATION OF HEMODILUTION EFFECTS ON CLOSURE TIME IN DOGS USING THE PFA-100 PLATELET FUNCTION ANALYZER. A Nicastro, S Burton, B Horney, A MacKenzie. Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PE, Canada.

Traditional tests evaluating platelet function in dogs have inherent drawbacks. The aim of this study was to establish a laboratory specific reference interval for closure time in dogs using collagen-ADP cartridges on the PFA-100 platelet function analyzer. The study also evaluated effects of *in vitro* hemodilution in individual canine samples to determine the level of anemia that interferes with analyzer results. Blood samples from 21 female and 19 male dogs of various breeds and ages (0.5–13 years) with no appreciable abnormalities on physical examination, CBC, serum biochemical profile and von Willebrand factor analysis were drawn into 3.2% Na citrate tubes. Closure times were determined in duplicate. Platelet rich plasma from 20 dogs was added to the original citrated samples to achieve platelet counts $> 150 \times 10^9$/L and PCV values of 35%, 25% and 15%, then closure times were determined in duplicate.

Following outlier exclusion of 2 points, the normally distributed closure time data had a reference interval of 48–66 seconds. Compared to the original closure time, a repeated measures ANOVA followed by a Dunnett's test revealed no significant difference in the 35% PCV (p = 0.54) group and significant differences in the 25% PCV (p = 0.00) and 15% PCV (p = 0.00) groups.

The reference interval for closure time agreed with the few previously reported, suggesting consistency of analyzer performance at various sites. Reliability of results decreased between the PCV values of 35% and 25% and further refinement of cut-off value would be optimal.



ABSTRACT #200

THE DIAGNOSTIC UTILITY OF BONE MARROW CYTOLOGY IN CANINE THROMBOCYTOPENIA. MD Miller, KF Lunn. Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Bone marrow cytology has been recommended in canine patients with non-regenerative anemia, persistent thrombocytopenia, persistent neutropenia, and atypical cells on peripheral blood smears. To our knowledge, the diagnostic value of bone marrow cytology in canine thrombocytopenia has not been investigated. The most common cause of severe thrombocytopenia is immune-mediated platelet destruction. Bone marrow cytology in these patients is predicted to show megakaryocytic hyperplasia as an appropriate response to peripheral platelet destruction. Bone marrow disorders that could lead to thrombocytopenia include myelophthisis and dysthrombopoiesis. We hypothesized that bone marrow cytology does not commonly identify a cause of severe thrombocytopenia.

The medical records database at the Colorado State University Veterinary Teaching hospital was searched from 1999 through 2004 for canine patients with thrombocytopenia that had bone marrow cytology performed. Cases were excluded which had neutropenia or had received previous therapy with immune-suppressive drugs. 48 cases met the selection criteria. The cases were divided into dogs with severe thrombocytopenia (<20,000 platelets/μL) and mild to moderate thrombocytopenia (≥20,000 but <200,000 platelets/μL). The diagnostic utility of the bone marrow cytology was compared between groups. 31 dogs had severe thrombocytopenia. Bone marrow cytology did not demonstrate the cause of thrombocytopenia (such as myelophthisis or dysmyelopoiesis) in any of these dogs. 19 dogs had mild to moderate

thrombocytopenia. Bone marrow cytology in 4 of these dogs showed myelophthisis. Significantly fewer dogs with severe thrombocytopenia had evidence of myelophthisis on bone marrow cytology ($p = .02$).

Based on the results of this study, we conclude that bone marrow cytology rarely provides a specific diagnosis in dogs with severe thrombocytopenia. Bone marrow cytology may not be an efficient use of resources in the initial workup of these patients.

ABSTRACT #201

AN OPTIMAL CRYOPRESERVATION METHOD FOR CANINE CD34$^+$ BONE MARROW STEM CELLS. K Ide, S Matsuura, Y Fujino, K Ohno, H Tsujimoto. University of Tokyo, Tokyo, Japan.

CD34$^+$ cells purified from bone marrow (BM) were shown to differentiate into multilineage mature blood cells in dogs as shown in humans. CD34$^+$ cells are conceivably useful for the stem cell transplantation in conjunction with radiation therapy or dose-intensified chemotherapy for lymphoid malignancies. The cells will be also utilized for *ex vivo* gene therapy. To know an optimal condition for the cryopreservation of canine CD34$^+$ BM cells, efficiency of 4 different methods were compared. CD34$^+$ cells were purified from BM mononuclear cells by a magnetic beads-sorting method using a monoclonal antibody directed to canine CD34. We used 2 different cryoprotective solutions: solution A containing 10% dimethylsulfoxide and solution B containing 5% dimethylsulfoxide, 6% hydroxyethyl starch, and 4% albumin. For each of the cryopreservation methods using solutions A and B, one half of the cells were frozen with a rate-controlled freezer and another half of them were put into a freezing container placed in an ordinary freezer. The results indicated that the cryopreservation using a cryoprotective solution B and a rate-controlled freezer produced the highest cell viability (trypan blue dye exclusion) (75.0 $\pm$ 19.4%), cell recovery rate (56.3 $\pm$ 57.7%), *in vitro* colony forming unit recovery rate (101 $\pm$ 67%), and purity of CD34$^+$ cells (82.5 $\pm$ 6.3%) after cryopreservation for 4 weeks. The cryopreservation study period will be extended to 6 months. The present study will provide an efficient cryopreservation method for the clinical use of stem cell transplantation in dogs.

ABSTRACT #202

THE EFFECT OF ACEPROMAZINE AND PROPOFOL ON HEMOSTASIS IN HEALTHY DOGS. D.M. Martin, D.G. Allen, A. Abrams-Ogg, P. Gentry. Ontario Veterinary College, Guelph, Ontario, Canada.

Acepromazine and propofol are two drugs commonly used in veterinary medicine for sedation or anesthesia. Limited studies using acepromazine in dogs have suggested that this drug may prolong platelet aggregation and therefore have a negative effect on primary hemostasis. Propofol has been extensively studied in human medicine, in which it has been variably associated with decreased or unaltered platelet aggregation. There has been little investigation of these potential adverse effects in dogs.

The purpose of this study was to evaluate the effect of these two drugs, alone and in combination, on primary and secondary hemostasis in dogs. Eight healthy purpose-bred Beagles received saline control, acepromazine (0.05 mg/kg IV), propofol (4 mg/kg IV) or the combination of acepromazine and propofol in a randomized, blinded study design. All dogs received each treatment once, with a two-week washout period to minimize possible carryover effect. Blood was collected prior to and after the administration of the drug. Platelet aggregation was evaluated by optical aggregometry and whole blood aggregometry using a platelet function analyzer (PFA-100®). Other parameters evaluated included prothrombin time, partial thromboplastin time, fibrinogen level, platelet count, total solids, hematocrit and total white blood cell count.

No effect on platelet aggregation was noted after administration of these drugs, individually or in combination. Acepromazine, alone and in combination with propofol, was associated with a significant decrease on the hematocrit, red blood cell count, platelet count and total solids ($p < 0.05$). Acepromazine, alone and in combination with propofol, was also associated with a decrease in the prothrombin time. All post-treatment parameters remained within the normal reference ranges.

In summary, neither acepromazine nor propofol administration caused a defect of primary or secondary hemostasis in normal dogs. Though statistically significant, the magnitude of the changes in platelet count and prothrombin level is unlikely to be clinically relevant in healthy dogs.

ABSTRACT #203

PROSPECTIVE PILOT STUDY ON PERFORMANCE AND PROGNOSTIC VALUE OF A NEW HUMAN, ISTH BASED SCORING SYSTEM FOR IDENTIFYING NON-OVERT DISSEMINATED INTRAVASCULAR COAGULATION IN DOGS. B Wiinberg[1], AL Jensen[1], R Rojkjaer[2], P Johansson[3], AT Kristensen[1]. [1]Small Animal Clinical Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark, [2]NNRUS, NJ, USA, [3]Rigshospitalet, Copenhagen, Denmark.

A correct diagnosis of disseminated intravascular coagulation (DIC) in dogs is hampered by the lack of a simple, yet accurate, diagnostic scoring system. A template for such a scoring system, for non-overt DIC in humans, has been proposed by the International Society of Thrombosis and Hemostasis (ISTH). It was the objective of this pilot study to asses the applicability of a modified ISTH scoring system for diagnosing non-overt DIC in dogs suffering from a disease known to predispose to the development of DIC and to assess the prognostic capability of the scoring system compared to the common veterinary diagnostic approach to DIC.

24 patients suffering from a disease known to predispose to DIC that were admitted to our intensive care unit (ICU) were assessed. Heparinized patients were excluded. Citrated blood samples were collected daily during hospitalization. Tests performed were platelet count, activated partial thromboplastine time (APTT), prothrombin time (PT), fibrinogen, D-dimer, and antithrombin (AT). Reference ranges were determined from a daily pool of 5 healthy dogs. The modified ISTH scoring system was: *1. Risk assessment*: patient suffering from underlying disease? yes = 2p no = 0p and *2. Specific criteria:* platelet count <100 = 1p; PT $>$ 20% prolonged compared to pool = 1p; D-dimer $>$ 0.5 mg/L = 1p; AT activity $<$ 80% compared to pool = 1p. A score of 5 or more was considered as diagnostic of DIC. The results of the ISTH score were compared to the traditional veterinary approach, with diagnosis of DIC when 3 or more criteria were fulfilled (a platelet count $<$ 200 and/or any of the hemostasis test deviated $>$20% from the pool of healthy dogs). The highest score derived from each patient was included in the study. Furthermore total ICU days and survival at 28 days post discharge was recorded for each patient.

12 of 24 dogs (50%) had DIC based on traditional veterinary scoring. Only 6 dogs (25%) had DIC based on the modified ISTH scoring system, all of which also had DIC based on the veterinary scoring system. Overall 28 day mortality rate was 46% (11 of 24). There were 8 and 5 deaths within the veterinary score- and modified ISTH score groups, giving mortality rates of 67% and 83% respectively. Total number of ICU days was similar for both groups with 2.75 days for the veterinary score group and 3.0 days for the modified ISTH score group.

In this study, DIC and mortality rates of dogs scored with the modified ISTH system were similar to results from a recent prospective evaluation of the ISTH scoring system in humans. In contrast, the traditional veterinary diagnostic approach, though previously proven sensitive for DIC, appears to be less specific, especially with regard to mortality. Further prospective studies are needed to assess the clinical applicability of the modified ISTH scoring system for the diagnosis and prognosis of DIC in dogs.

ABSTRACT #204

BIPHASIC TRANSMITTANCE WAVEFORM IN APTT COAGULATION ASSAY IDENTIFIED IN DOGS WITH DIC BY MEANS OF A HIRUDIN-MODIFIED AUTOMATED ASSAY. M Kjelgaard-Hansen, B Wiinberg, AL Jensen, AT Kristensen. Small Animal Clinical Sciences, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

A decrease in light transmittance independent of clot formation (biphasic waveform [BPW]) in coagulation assays, such as the activated partial thromboplastin time (APTT), has been correlated with the onset of disseminated intravascular coagulation (DIC) in humans (Downey et al., 1997) and identified as a promising tool to assist in the diagnostic management of DIC. A Calcium-dependant precipitation of C-reactive protein (CRP) and very low density lipoprotein (VLDL) is responsible for the BPW (Toh et al., 2002). The VLDL-component in patients with BPW has over twice the normal prothrombinase activity of VLDL due to a structural change (Dennis et al., 2003), thus also having a possible pathogenic role in disseminating the process of DIC. The aim of the study was to investigate whether BPW could be identified in dogs with DIC.

An automated APTT assay (ACL9000) was hirudin-modified (recombinant, final concentration 2 μM) (Toh et al., 2002) to ensure measurements independent of clot formation. Citrated plasma from (a) 8 dogs with DIC (underlying disease predisposing for DIC and 3 or more of the following criteria were fulfilled (a platelet count $<$200 10^9/L and/or any of the hemostasis tests deviated $>$20% from a pool of healthy dogs [APTT, prothrombin time, fibrinogen, D-dimer, and antithrombin]), (b) 4 diseased

non-DIC dogs and (c) 4 clinically healthy dogs and a pool of citrated plasma from 5 healthy dogs, were run in the assay (groups b and c, included in study as controls). Absorbance was measured continuously over 200 seconds.

The median drift in baseline absorbance observed over 200 seconds in groups (a), (b) and (c) were 2.3%, 1.1% and 1.2%, respectively. The difference was significant between group (a) and a pool of groups (b) and (c) at the 200 second point-estimate ($P = 0.02$, Mann-Whitney). Two dogs with DIC had a marked change (an over 7% change in absorbance after 200 seconds).

In conclusion, a significant change in absorbance independent of clot formation was observed in a hirudin-modified APTT assay of dogs with DIC compared to healthy and non-DIC dogs. Two dogs with DIC had marked changes. These observations are similar to those made in human DIC patients caused by a CRP-VLDL precipitate. Further investigations are warranted to clarify whether the aetiology of BPW in canine DIC patients is identical to the precipitate identified in human DIC patients. Canine CRP is recognized to have a response pattern to disease processes very similar to human CRP and is often used as a model in comparative studies.

The identification of a similar CRP-VLDL complex in canine DIC patients could have diagnostic importance. Furthermore, the VLDL could have a possible pathogenic role, as indicated in research in human DIC thus being of possible importance to the diagnostic management of canine DIC and as further evidence of the dog being a useful model for research in human DIC and related diseases.

ABSTRACT #205

CANINE RED BLOOD CELL SURVIVAL FOLLOWING PHOTOTREATMENT WITH THE NOVEL PHOTOSENSITIZER THIOPYRYLIUM. A Balch[1], A Mackin[1], S Wagner[2], A Skripchenko[2], CR Boyle[1], L Pinchuk[1]. [1]Mississippi State University, Starkville, MS. [2]American Red Cross Blood and Cell Therapy Development Laboratory, Rockville, MD.

Transfusion medicine has become an advanced field in both human and veterinary medicine. Many advances are related to the detection and prevention of transfusion-associated infection. Blood supply safety can change dramatically following the emergence of new pathogens such as HIV, West Nile Virus or *Babesia* species. The development of safe and effective methods of pathogen reduction that maintain the integrity of blood components will be a major advance in transfusion safety. The American Red Cross, in collaboration with Mississippi State University, has recently developed a pathogen reduction system for red cell concentrates using thiopyrylium. Thiopyrylium is a novel photosensitive agent that undergoes a photodynamic reaction if exposed to light of a certain wavelength. Thiopyrylium has been shown to effectively eliminate common bacterial and viral pathogens in human and canine blood products. The purpose of our study was to assess pre- and post-transfusion viability of thiopyrilium-treated red cells in a dog model.

In vitro assays of red cell viability were performed on stored units of blood from 19 greyhounds, while 17 dogs were used for post-transfusion cell survival studies. A unit of blood (450ml) was removed from each dog, and used to manufacture a unit of leukodepleted packed red cells. Each packed cell unit was either phototreated with thiopyrylium (10 units) or sham phototreated (control; 9 units). For *in vivo* survival testing, a portion of the packed cells was labeled with biotin and transfused back to the original donor. Following transfusion, serial blood samples were collected until negligible amounts of biotinylated red cells were observed by flow cytometry. Post-transfusion survival of phototreated ($n = 9$) and control ($n = 8$) red cells was compared via a two-tailed independent samples t test. No significant difference in cell survival between phototreated and control samples was detected ($p = 0.61$). Mean survival of phototreated red cells was 36.2 days (SD 15.9, median 28.0), while mean survival of control cells was 38.9 days (SD 13.3, median 38.5). Mean difference between groups was −3.7 days, with a 95% confidence interval of −18.8 to 11.5 days. No post-transfusion side effects were detected in either phototreatment or control groups. *In vitro* measures of red cell viability (glucose, potassium, hemoglobin, lactate, pH, hemolysis and cell morphology) were evaluated on paired stored samples and analyzed using ANOVA for a randomized complete block design. Significant differences ($p < 0.05$) were found between control and phototreated units of packed red cells after 35 days of storage for the following: glucose (control 395 mg/dL; treated 677 mg/dL), potassium (3.9 mmol/L; 2.2 mmol/L), lactate (24.2 mmol/L; 11.1 mmol/L), pH (6.7; 7.1), and hemolysis (0.50%, 3.05%).

In conclusion, thiopyrylium-treated cells appear to have normal survival following transfusion, and are well-tolerated by recipient dogs. Future studies on transfusion of stored cells are warranted based on differences in laboratory viability assays between control and treated samples.

ABSTRACT #206

MICROVETTE®: A NEW BLOOD SAMPLING TECHNIQUE FOR HEMATOLOGY IN DOGS AND CATS. KG Boudet[1,2], M Faucher[1,2], A Geffré[1], C Germain[1,2], HP Lefebvre[1,2], B Reynolds[1]. [1]Department of Clinical Sciences and [2]UMR 181 INRA-ENVT Experimental Physiopathology and Toxicology, National Veterinary School of Toulouse, France.

Vacutainer sampling (V) is largely used in small animals. Its major limits however are animal stress, vein collapsus by vacuum, and hematoma risk. Microvette® sampling (M) (200 µL tubes, Sarstedt AG, Nümbrecht, Germany) appears to be an interesting alternative, especially when a small blood volume is needed. The objective was to compare both techniques for hematological examination in dogs and cats.

7 dogs and 6 cats were sampled four times on the same day according to a cross-over design. Blood was taken in EDTA tubes using V with a 22G needle from the jugular vein and M with a 25G needle from the cephalic vein. A complete hematological examination (blood smear and automatic blood count on Scil Vet ABC) was performed. The technique effect was assessed by ANOVA.

M was very well tolerated. Eosinophils on blood smear were slightly lower in dog for M (2.4 ± 1.99 vs. 3.9 ± 2.40%, $P < 0.05$)). For automatic blood count, a difference ($P < 0.05$) between M and V was observed for monocytes (mean difference: −12%), hemoglobin (+3.8%), and PCV (+3.5%) in dogs, and erythrocytes (+8.9%), hemoglobin (+8.6%), and PCV (+8.9%) in cats. PCV determined by the microhematocrit method were similar.

In conclusion, M offers a new alternative for hematological examination in cats and small dogs.

ABSTRACT #207

EFFECTS OF RECOMBINANT HUMAN ACTIVATED FACTOR VII AND CANINE FRESH FROZEN PLASMA IN BEAGLES WITH HEREDITARY COAGULATION FACTOR VII DEFICIENCY. Elanor Withnall and Urs Giger, Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

Many hereditary coagulopathies have been reported in various canine breeds, and hemorrhagic episodes are generally treated with canine fresh frozen plasma (FFP) or, where appropriate, cryoprecipitate. Due to safety concerns and limited blood resources, recombinant coagulation factors have recently been introduced for the treatment of various coagulopathies in human patients. In this study we compared the effects of recombinant human activated factor VII (rFVIIa) and FFP in Beagles with hereditary FVII deficiency. Affected Beagles have <5% plasma FVII activity compared to normal controls due to a missense mutation. This defect can cause an increased bleeding tendency, but is often discovered incidentally by an isolated prothrombin time (PT) prolongation. This is an autosomal recessive trait and a recent limited survey suggests a relatively high prevalence of the mutant allele in the show as well as research Beagle population.

A total of 4 male and 2 female adult FVII-deficient research Beagles (weighing 7–11 kg) were treated. On separate occasions (2–7 days apart with 4–6 dogs per group) each dog received an FFP infusion at 10 ml/kg over <15 min and a bolus of rFVIIa, at 5 µg/kg (Low) or 30 µg/kg (High). Under mild sedation a cuticle bleeding time (CBT) test was performed prior to and 15 min post-treatment. Citrated blood samples were collected at time 0, 15, and 90 min as well as 24 hours post-treatment for immediate PT measurement in whole blood (SCA2000), and subsequent PT, partial thromboplastin time (PTT), FVII and FXI (Stago) determination on frozen plasma samples.

The pre-CBT values ranged from 4.3 to 9 min (m ± SEM, 6.1 ± 0.4) and were significantly shorter after the administration of both doses of rFVIIa (High 3.1 ± 0.3, Low 3.3 ± 0.4), but not following FFP (5.2 ± 0.7). The prolonged whole blood pre-PT values (27.4 ± 0.7 sec, reference range 12–17 sec) were shortened into the near normal range at 15 min and 90 min, but were again prolonged by 24 hours. Similar results were obtained with the plasma PT test measurements. Plasma FVII activity ranged from 2–5% (4.2 ± 0.4) in deficient dogs compared to normal controls and rose at 15 min in all cases (High 136 ± 14%, Low 26 ± 4%, FFP 30 ± 2%). The PTT and FXI values stayed within the normal range throughout the investigation. No adverse reactions were observed with human rFVIIa, but one dog experienced a mild reaction to canine FFP treatment.

We conclude that a single treatment with rFVIIa was safe and highly effective in shortening CBT and normalizing PT in FVII-deficient Beagles, while FFP appeared to cause a similar or lesser effect. Minute amounts (5 µg/kg) of rFVIIa appeared to be sufficient to exert these shortenings of CBT and PT, presumably due to its ability to initiate the extrinsic cascade with subsequent sustained activation of the intrinsic cascade. It needs to be determined whether human rFVIIa will also control clinical bleeding in FVII-deficient Beagles, before it can be recommended as an alternative to FFP. (Supported in part by NIH RR 02512 and Novo Nordisk)

ABSTRACT #208

COAGULATION FACTOR XI DEFICIENY IN KERRY BLUE TERRIER DOGS IS CAUSED BY AN EXONIC SINE INSERTION. Eva Tcherneva, Angela M. Huff, and Urs Giger. Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Many hereditary coagulopathies have been reported in canine breeds and these hemostatic defects are important to consider as differential diagnoses in any bleeding dog. The molecular defects causing these disorders have been determined only for Beagles with factor VII deficiency and a few hemophilic dogs. Factor XI (FXI) deficiency has been reported in the Kerry blue terrier (KBT) breed and an isolated family of Great Pyrenees and English springer spaniel dogs (Knowler et al. JAVMA, 295:1557, 1994). Affected dogs have a mild bleeding tendency characterized by late post-traumatic or post-surgical hemorrhage, but many are recognized incidentally by finding a persistently prolonged partial thromboplastin time. Plasma FXI activities appear to vary greatly in FXI deficient bleeding animals, and heterozygote dogs cannot be definitively differentiated from homozygote affected or normal dogs based on that assay. The FXI deficiency seems to be inherited as an autosomal trait with incomplete penetrance. Here we describe the molecular defect responsible for FXI deficiency in KBT dogs.

Four related FXI-deficient KBTs (incl. mother and one of her male and female offspring) were studied. They had either no or mild bleeding tendencies (prolonged bleeding following nail clipping), but an affected female relative (not available for these studies here) bled excessively following her spay surgery. Their partial thromboplastin times, but not their prothrombin times, were markedly prolonged and their plasma FXI activities were $<10\%$ of normal healthy controls. Fresh EDTA blood samples were obtained from these affected and related KBTs as well as from some dogs of unrelated breeds. Genomic DNA and cDNA were prepared and sequenced utilizing primers for the canine FXI sequence derived from the published canine genome sequence. The first non-coding and 10 of the 14 coding exons of the sequenced canine FXI gene were identical between the normal KBTs' DNA and the published canine genome sequence. However, the seventh coding exon differs between normal and affected animals. It is normally 110 bp long, but in affected KBTs it contains a short interspersed nucleotide element (SINE) insertion. This exonic SINE is 90 bp long, consisting mostly of adenines coding for lysine which is presumed to affect the 3rd apple domain of the FXI gene. Interestingly, the canine genome contains a large number of SINE insertions, which seem to continue to retrotranspose in the canine genome such that FXI deficiency in KBTs, narcolepsy in Doberman pinschers and centronuclear myopathy in Labrador retrievers are examples of the deleterious results of SINE insertions. Further studies are in progress to characterize the effects of this SINE mutation on FXI function. Finally, a simple genomic DNA test can be developed to screen the KBT population to determine the mutant allele frequency within the breed and its association to any clinical bleeding tendencies. Supported in part by NIH RR 02512.

ABSTRACT #209

COMPARATIVE STABILITY OF CANINE HEMOSTATIC FACTORS IN FREEZE-THAW-CYCLED FRESH FROZEN PLASMA. Yaxley PE[1], Beal MW[1], Jutkowitz LA[1], Brooks MB[2], Parr A[1], Hauptman J[1]. [1]Michigan State University College of Veterinary Medicine, East Lansing, MI. [2]Cornell University College of Veterinary Medicine, Ithaca, NY.

Canine fresh frozen plasma (FFP) is commonly used to treat a wide variety of hemostatic defects in dogs. There are numerous circumstances in which FFP is thawed, but not transfused. The purpose of this study was to evaluate the stability of canine hemostatic factors in freeze-thaw-cycled (FTC) FFP.

Whole blood from nine healthy blood donor dogs was collected and processed to packed red blood cells and FFP according to standard methods. Each unit of FFP was then subdivided into equal aliquots and frozen ($-41^{\circ}C$) in pediatric blood component transfer bags. One aliquot from each donor was then removed and thawed in a rocking/circulating water bath ($37^{\circ}C$) for fifteen minutes and held at $4^{\circ}C$ for one hour. This FTC FFP was then refrozen ($-41^{\circ}C$) until the time of analysis. FTC FFP from each donor was then assayed in duplicate with aliquots that had remained frozen (NFTC). The hemostatic proteins assessed included coagulation factors, anticoagulant factors [antithrombin (AT) and Protein C (PC)], and adhesive proteins [fibrinogen and von Willebrand factor (vWF)]. The coagulant activities of Factors II, VII, VIII, IX, X, XI, and XII were measured in modified one-stage aPTT (Factors VIII, IX, XI, XII) or PT assays. AT and PC activities were measured in chromogenic substrate assays. Clottable fibrinogen was measured via Clauss method, and vWF concentration was measured in an ELISA. A paired t-test was utilized to identify differences in factor activity or concentration between FTC FFP and NFTC FFP.

No clinically or statistically significant differences (all $p > 0.05$) in hemostatic factor levels were identified between FTC FFP and NFTC FFP. Refreezing FFP within one hour of initial thawing appeared to have no deleterious effects on the factor content of that unit. Based on these results, transfusion of FTC FFP is expected to provide the recipient with comparable replacement of hemostatic proteins as FFP that has remained frozen.

ABSTRACT #210

VOMITING IN DOGS RECEIVING CHEMOTHERAPY WITH CYCLOPHOSPHAMIDE, EPIRUBICIN, VINCRISTINE, AND PREDNISOLONE FOR LYMPHOMA. TA Cave. Cave Referrals, Axbridge, Somerset, United Kingdom.

Gastrointestinal toxicity from systemic chemotherapy is undesirable. Identification of risk factors for gastrointestinal toxicity may allow targeted use of prophylactic medications.

A retrospective review of the clinic database was performed for dogs undergoing Madison-Wisconsin scheduled chemotherapy with cyclophosphamide, epirubicin, vincristine, and prednisolone for lymphoma. Dogs were excluded if they had received prior cytotoxic drug therapy other than corticosteroids. Vomiting following each treatment cycle had been prospectively graded for severity using a previously peer-reviewed 4 point scale. Thirty-four dogs undergoing 253 treatment cycles were identified. Dogs had undergone a median of 8 protocol weeks before therapy was changed either because of disease progression or because they were lost to follow up. Data concerning signalment, initial presenting problems, initial physical signs, initial clinical pathology, inter-therapy haematology, cytotoxic drug dosage, and supportive therapy were collated. Multivariable ordinal logistic regression was then used to identify associations between these data and vomiting severity following each treatment cycle.

Sixteen dogs (47.1%) did not develop vomiting at any stage during the course chemotherapy. Six (17.6%) dogs each vomited to at least grade 1, 2, and 3 following chemotherapy. A median of 1 treatment cycle per dog (range 0–3) was associated with vomiting. Vomiting severity fell significantly as dogs progressed through the chemotherapy protocol. Vomiting severity was not associated with a specific cytotoxic drug but generalised cytotoxic drug dose intensification was associated with increased vomiting severity. Hypoproteinaemia (<54 g/l) and an eosinophil count greater than 0.6×10^9/l prior to chemotherapy were strongly associated with significantly greater severity of vomiting during subsequent treatment. Prophylactic prescription of metoclopramide (median dose 0.41 mg/kg q8h rs for 7 days [0.27–0.91]) or sucralfate (median dose 100 mg/kg q8 hrs for 7 days [60–150 mg/kg]) was associated with a significantly reduced vomiting severity during the subsequent inter-cycle period.

Vomiting occurred following Madison-Wisconsin chemotherapy in 52.9% of dogs. Pre-treatment hypoproteinemia or a high normal eosinophil count were predictors of vomiting. No specific cytotoxic drug was associated with vomiting severity but generalised dose intensity was. Both prophylactic metoclopramide and sulcralfate significantly reduced the severity of vomiting. A prospective randomised controlled clinical trial is required to further evaluate the benefits of prophylactic supportive care.

ABSTRACT #211

EQUIVALENCE ASSESSMENT OF IMPORTED MELPHALAN TABLETS. Butch KuKanich, Shirley Arck, Laura Garrett. Kansas State University, College of Veterinary Medicine, Manhattan, KS.

Melphalan is a bifunctional alkylating agent used in veterinary medicine for the treatment of plasma cell tumors, multiple myeloma, and lymphocytic leukemia. Melphalan is rapidly absorbed following oral administration to dogs with maximum serum concentrations occurring at 30 minutes. The elimination half-life of melphalan in dogs is 66 minutes with high concentrations of active drug eliminated in the bile and 8% of the dose eliminated unchanged in the urine. Adverse effects associated with melphalan are typically mild, with myelosuppression being the main concern. The purpose of this study was to assess the equivalence of melphalan imported by a client using an internet pharmacy from Canada, to tablets manufactured in the United States.

Melphalan tablets (Alkeran, Celgene Corp., Summit, NJ, USA) from a domestic source were used as the reference product, whereas the test product was the imported drug (Alkeran, Celgene, Heumann Pharma Gmbh, Feucht, Germany). Melphalan reference standard (Sigma-Aldrich, St. Louis, MO, USA) was used as a comparison for both sources. Three tablets (2 mg) from each source were dissolved individually in 4 mL of

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

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

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

ABSTRACT #212

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

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

ABSTRACT #213

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

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

ABSTRACT #214

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

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

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

Herein report on the *HMGA2* expression patterns determined by real-time quantitative RT-PCR in prostatic tissues from 16 dogs with different histological findings. The results show that expression of *HMGA2* is low in tissues with no abnormality detected, rises in benign neoplasms and increases at least 19-fold in carcinomas. In our study all malignant neoplasias showed expression levels beyond 50,000 transcripts per 250 ng total RNA, whereas none of the non-malignant tissues showed expression levels beyond this value. These results indicate that *HMGA2* expression analysis using real-time quantitative RT-PCR may provide a potential tool for better differentiation between varying degrees of malignancy in prostate carcinomas.

ABSTRACT #215

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

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

Anti-DEA 1.1 0.3%, Anti-DEA 3 1.2%, Anti-DEA 5 0.8%,

Anti-DEA 7 9.8%, nonspecific agglutinating 2.0%

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

considered clinically significant. 2) These low preliminary numbers confirm the importance of careful donor selection. Samples submitted to the laboratory for screening are predominantly from dogs of know history selected as optimum candidates for donation; i.e., dogs not exposed to pregnancy, previous transfusion, disease or parasites, all of which could induce antibody formation. 3) These findings may reduce the cost of donor testing by corroborating that antibody screen is necessary to ensure minimal destruction of recipient erythrocytes only if large numbers of plasma donors are to be utilized. Antibody screening of potential canine donors is ongoing and future results accumulate a broader basis for evaluating the probability of donor antibody adversely affecting the outcome of transfusion.

ABSTRACT #216

EVALUATION OF CC CHEMOKINE RECEPTOR 4mRNA EXPRESSION LEVEL IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM DOGS WITH ATOPIC DERMATITIS. DH Bhang, CH Baek, EW Choi, KW Seo, JY Jung, CY Hwang, HY Youn. Departments of Internal Medicine, College of Veterinary Medicine, Seoul National University, San 56-1, Sillim-dong, Seoul, 151-742, Korea.

The purpose of this study was to evaluate expression level of CCR4 mRNA in peripheral blood mononuclear cell(PBMC) obtained from dogs with atopic dermatitis(AD) and normal dogs using TaqMan real-time RT PCR, and to investigate whether CCR4 expression level correlated disease severity in canine AD.

Peripheral blood mononuclear cells (PBMCs) were isolated from 5 dogs with AD that were recruited from the Veterinary Medical Teaching Hospital of Seoul National University. Expression levels of CCR4 mRNA in PBMCs from dogs with AD were evaluated by relative standard curve method real-time RT PCR and compared with that of normal controls ($n = 5$). In addition, to assess the correlation with disease activity, the severity of AD were estimated in the dogs with AD using scoring system.

The average of normalized amount of CCR4 mRNA in normal dogs was 0.35 ± 0.099, while the average in dogs with AD was 1.12 ± 0.61; the relative level of CCR4 mRNA expression of dogs with AD is higher (about 3.2 fold) than that of normal dogs. Expression level of CCR4 mRNA was significantly correlated with disease severity ($r = 0.9$, $P < 0.05$).

Consequently, CCR 4 would be a good maker for evaluation of disease severity in canine AD and the role of CCR4 in canine AD may be similar to that in human AD.

ABSTRACT #217

HYPOINFLAMMATORY STATE IN CRITICALLY-ILL DOGS. CB Webb, K McCord, SW Dow. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Studies in rodent models have suggested that following severe bacterial infection and sepsis, the innate immune system becomes functionally inactivated, resulting in a *hypoinflammatory state*. Therefore, we conducted studies to: (1) develop functional assays to rapidly assess innate immunity and neutrophil (PMN) function in dogs; and (2) assess neutrophil function in healthy dogs and dogs with sepsis. The ability of PMNs to eliminate pathogens depends on their production of reactive oxygen species (ROS) and effective phagocytosis. Previous assays to measure these functions in PMN have been cumbersome and time-consuming. Therefore, we developed flow cytometric assays to quickly assess these properties of PMN rapidly, using small volumes of blood. Opsonized Alexa 488-labeled *E. coli* was used to measure PMN phagocytosis. The Fc OxyBURST® assay, which uses 2′,7′-dichlorodihydrofluorescein (DCHF) covalently linked to bovine serum albumen and complexed to rabbit polyclonal anti-BSA IgG, was used to quantitate PMN oxidative burst activity. Dihydrorhodamine 123 was used to determine ROS production in activated PMNs. Incubation of PMN with viable bacteria and subsequent determination of bacterial counts was used to quantitate intracellular bacterial killing ability. Monochlorobimane (mBCl) was used to determine the relative amount of intracellular reduced glutathione, as an indirect measure of oxidative stress.

We found that use of the flow cytometric assay of PMN oxidative burst activity allowed dog PMN to be classified into 3 distinct populations based on their in vitro responses. High responder PMN had high levels of conversion of DCHF to a fluorescent intermediate, while low responder PMN exhibited little DCHF conversion and moderate responders exhibited intermediate levels of fluorescence. Similar results were also found with the flow cytometric assay of PMN phagocytosis. In critically-ill dogs that have been evaluated to date, we have observed low levels of PMN neutrophil phagocytic activity, which improved during treatment. Reduced levels of intracellular GSH have also been documented in septic patients. We conclude therefore that flow cytometric assays of PMN function can provide rapid assessment of PMN function in dogs. Moreover, these assays also allow much greater discrimination of functional differences than previous assays. Our early results also suggest that PMN in critically-ill dogs with sepsis may exhibit substantial functional impairment resembling the hypoinflammatory state. Therefore, flow cytometric assays may allow rapid and precise determination of PMN function in clinical samples from critically-ill dogs.

ABSTRACT #218

PRO-INFLAMMATORY TNF-α, IL-1β, AND COX-2 EXPRESSION IS INHIBITED BY CHONDROITIN SULFATE IN CHONDROYCTES, MONOCYTES-MACROPHAGES, AND FIBROBLASTS. PV Phan[1], RY Au[1], M Grzanna[1], M Shaya[1], K Lahiji[1], T Al-Talib[1], D Griffin[1], CG Frondoza[1, 2]. [1]Nutramax Laboratories, Inc., Edgewood, MD. [2]Johns Hopkins University, Baltimore, MD.

The inflammatory cytokines TNF-α, IL-1β, and the enzyme COX-2 have been demonstrated to mediate chronic inflammation in a wide variety of disorders such as arthritis in animals. Tissue cells express increased levels of these mediators upon activation by stimuli including bacterial endotoxin and cytokines. Chondroitin sulfate (CS) has been reported to regulate pro-inflammatory mediators produced by chondrocytes in articular cartilage. Little is known about its effect on other tissue cells that produce pro-inflammatory markers. The joint is lined with synovial tissue that contains fibroblasts and macrophage-like cells which plays a role in maintaining cartilage integrity. The present study tested the hypothesis that CS inhibits the expression of cytokines and COX-2 in chondrocytes, monocytes-macrophages, and fibroblasts.

Human and bovine chondrocytes, THP-1 monocytes-macrophages-surrogate which exhibit features of their normal counterpart, and human dermal fibroblasts were used. These cells were plated at 5×10^5/well. CS (TRH122®, Nutramax Laboratories Inc., Edgewood, MD, USA) at physiologic concentration of 20 μg/ml was used. The cells were incubated with: (i) control media alone, or (ii) CS (20 μg/ml) for 24 or 72 hrs. Cells were re-incubated with control media alone, or with activator lipopolysaccharide (20 ng/ml, LPS) at 37°C, 5% CO_2 for 1 hr. Cells were lysed and total RNA was subjected to reverse transcription-polymerase chain reaction (RT-PCR). Bovine or human primers specific for TNF-α, IL-1β, COX-2 and GAPDH as the housekeeping gene were used. The gels containing ethidium bromide were electrophoresed and DNA bands were visualized under UV light.

CS inhibited activation of gene expression of TNF-α, IL-1β, and COX-2 in chondrocytes. Activation of TNF-α, IL-1β, and COX-2 expression was also reduced in THP-1 monocyte-macrophages. Similar suppression of LPS-induced activation was observed for COX-2 transcript levels in fibroblasts.

The observation that CS blocks pro-inflammatory mediators in three cell types supports the notion of its broader anti-inflammatory action. CS thus exerts a protective-prophylactic effect on more than one tissue cell type involved in the inflammatory pathway. The present study may help identify the possible mode of action of CS in interrupting the inflammatory cascade.

ABSTRACT #219

THE USE OF HETEROLOGOUS ANTIBODIES TO DETECT TOLL-LIKE RECEPTORS IN CANINE WHITE BLOOD CELLS. IA Burgener[1], TW Jungi[2]. [1]Department of Clinical Veterinary Medicine and [2]Division of Veterinary Immunology, Vetsuisse Faculty, University of Bern, Switzerland.

Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) on microbes including bacteria, fungi, viruses and parasites. They are mainly expressed by cells belonging to the innate immune system, such as macrophages and dendritic cells. Ligand recognition ultimately results in activation of nuclear factor-kappaB, thereby leading to the production of pro-inflammatory cytokines and reactive oxygen and nitrogen intermediates. Amongst others, dysregulated expression of TLRs recognizing bacterial products (lipoproteins by TLR2, lipopolysaccharide by TLR4, flagellin by TLR5 and CpG-containing DNA by TLR9) and abnormal activation of cells of the innate immune system by PAMPs have been implicated in the pathogenesis of human inflammatory bowel disease (IBD). The purpose of this study was to investigate antibodies developed against human and murine TLR2–5 and 9 for their use in dogs.

A total of 22 antibodies (13 monoclonal and 9 polyclonal) were tested for their potential to recognize TLRs on canine monocytes (Mo), granulocytes

(Gr) or lymphocytes (Ly) by flow cytometry. After lysis of erythrocytes, white blood cells were either left untreated or were treated with various fixation methods (0.4% formaldehyde, 4% paraformaldehyde, methanol) or permeabilization methods (IC-Flow Kit (Imgenex) or BD Perm (BD Biosciences)) considering the different possible localizations of the TLRs. Incubation with unstained primary antibodies was followed by a phycoerythrin-labeled secondary antibody. Measurements were conducted on a FACS cytometer (Becton Dickinson) and the results compared with isotype-matched controls and other antibodies against the same TLR.

Nine monoclonal antibodies were identified to detect canine TLRs on different subpopulations:

- TLR2 (on Mo + Gr): clones TL2.1 and TL2.3 (both eBioscience)
- TLR4 (on Mo + Gr): clones HTA125 (eBioscience) and 76B357.1 (Imgenex)
- TLR5 (on Mo + Gr): clone 85B152.5 (Imgenex)
- TLR3 (on Mo + Gr + Ly): clones TLR3.7 (Hbt) and 40C1285.6 (Imgenex)
- TLR9 (on Mo + Gr + Ly): clones 26C593.2 (Imgenex) and 5G5 (Hbt)

As expected from previously published studies in humans and mice, TLR2, 4 and 5 were only detected on the cell surface (untreated, 0.4% formaldehyde and methanol: epitopes positive; all others negative) and TLR3 and 9 were only detected inside cells (positive with permeabilization of the cells, otherwise negative). These results were confirmed with immunocytochemistry.

Our study revealed that canine TLRs can be detected on white blood cells with heterologous antibodies. As observed in other species, TLR2, 4 and 5 are probably localized on the cell surface, whereas TLR3 and 9 are only detected after permeabilization of the cells.

ABSTRACT #220

DOES IODINE INTAKE PLAY A ROLE IN THE PATHOGENESIS OF FELINE HYPERTHYROIDISM? J.Wakeling, J.Elliott, H.Syme; Royal Veterinary College, London.

Hyperthyroidism (HTH) in cats has strong similarities to toxic nodular goitre in humans. This is the most frequent cause of thyrotoxicosis in elderly people, and is particularly prevalent in iodine deficient areas. Previous studies in healthy cats have established that urinary iodine excretion (UI) is reflective of dietary iodine intake, and epidemiological studies have linked the occurrence of HTH with consumption of moist diets that may have variable iodine content. The purpose of this study was to compare UI in HTH and non-HTH cats.

Urine samples were collected by cystocentesis from cats presenting to first opinion clinics in London. Cats were diagnosed with HTH on the basis of compatible clinical signs and total thyroxine (tT4) concentration >55 nmol/l. Cats were included in the non-HTH group if they were >8 years old and were considered healthy on the basis of a complete history, physical examination, complete biochemical screen and tT4 concentration <40 nmol/l. UI was also measured prior to, and 3–6 months after, successful treatment for HTH to determine if UI was altered, since in particular it was considered that polyphagia would increase iodine intake. UI was measured using a modified Sandell-Koltoff reaction and values normalised to a urine specific gravity of 1.010 to correct for the effect of variable urine concentration. Inter and intra-assay CVs ranged from 3.7–12.1% and the sensitivity of the assay was 6.9 µg/L. UI was not normally distributed so results are expressed as median [25^{th}, 75^{th} percentiles]. Logarithmic transformation was performed prior to statistical analysis using the independent (comparison of HTH and non-HTH) and paired (comparison pre and post treatment) student t-tests. Additionally, since studies in human patients and rat models have shown that UI is decreased by chronic renal failure, comparison of UI in HTH and non-HTH groups was repeated excluding cats from the analysis that were documented to be azotemic (plasma creatinine concentration >2.0 mg/dl) before, or in the first 3 months following, treatment for HTH.

UI was not significantly different in the HTH (213 [126, 314] µg/L; n = 64) and non-HTH (236 [160, 392] µg/L; n = 52) cats (p = 0.08). When cats with azotaemia (n = 27) were excluded from the HTH group there was still no significant difference between the HTH and non-HTH groups (p = 0.30). Successful treatment for HTH did not significantly alter UI (pre-treatment 238 [159, 337] and post-treatment 257 [145, 412] µg/L; n = 24, p = 0.75).

The results of this study do not support the hypothesis that a difference in iodine intake exists between HTH and non-HTH cats. However, studies of cats with high and low lifetime iodine intake would be required to determine if there is an effect on the overall disease prevalence within the population. Estimations of iodine intake based on UI measured in this study, and assumptions regarding faecal iodine output, do not indicate that cats in London, UK are iodine deficient.

ABSTRACT #221

INSULIN SENSITIVITY MEASURES IN CATS EXHIBIT HIGH INTER-DAY VARIABILITY. M Coradini[1], JS Rand[1], JM Morton[1], AL Litster[1], JM Rawlings[2]. [1]Centre for Companion Animal Health, University of Queensland, Australia. [2]WALTHAM Centre for Pet Nutrition, Leicestershire, UK.

In cats with underlying low insulin sensitivity, obesity is a major risk factor for type 2 diabetes. Strategies to prevent the onset of type 2 diabetes could be implemented if these cats could be identified. Currently, two labour-intensive and complex methods have been used to measure insulin sensitivity in research studies: the hyperinsulinemic euglycemic clamp (Clamp) and the minimal model analysis (MINMOD) of a frequently-sampled intravenous glucose tolerance test. However, simpler measures are required in practice. Validation of simple measures requires a well-established method with minimal inter-day variability. The aims of this study were to determine the inter-day variability of the current methods of measuring insulin sensitivity in cats, and to assess the relationship between these tests and simpler measures of insulin sensitivity.

Three lean [body condition score (BCS) 4–5/9] and 3 obese cats (BCS ≥ 7.5/9), estimated to be aged between 3 and 5 years were used.

The Clamp and MINMOD were performed in all cats, and each test was repeated 2–3 days apart in 4 cats. The Clamp and MINMOD were performed 4 months apart. Body weight and body condition scores of individual animals did not differ between tests. Insulin sensitivity from the Clamp was calculated as the amount of glucose metabolised per unit of plasma insulin concentration after steady-state hyperinsulinemia was reached. Insulin sensitivity from MINMOD was calculated using MINMOD Millennium. Simple measures of insulin sensitivity were baseline insulin concentration (I_0), baseline glucose/insulin ratio (G_0/I_0), HOMA [$(I_0 \times G_0)/405$], QUICKI [$1/(\log_{10} I_0 + \log_{10} G_0)$] and Bennett index[$1/(\log_{10} I_0 \times \log_{10} G_0$]. These were calculated using data from each of the insulin sensitivity tests.

Although the Clamp had lower inter-day variability [mean coefficient of variation (CV) = 0.22] compared to MINMOD (mean CV = 0.33), both methods were highly variable. There was a strong correlation between insulin sensitivity measured by MINMOD and all simple measures [I_0 ($r = -0.85$; $P = 0.03$), G_0/I_0 ($r = 0.97$; $P < 0.01$), HOMA ($r = -0.83$; $P = 0.04$), QUICKI ($r = 0.91$; $P = 0.01$) and Bennett index ($r = 0.94$; $P < 0.01$)]. The simple measures of insulin sensitivity were not significantly correlated ($P \geq 0.72$) with the results of the Clamp.

The results of this study are consistent with the hypothesis that inter-day variability in insulin sensitivity in cats is high, irrespective of whether it is measured using the MINMOD or Clamp technique. Simple measures of insulin sensitivity are a reasonable alternative to MINMOD but were not strongly correlated with estimates based on the euglycemic clamp. Further research is required to understand this lack of correlation with the Clamp technique.

ABSTRACT #222

ACCURACY OF PURINA GLUCOTEST™ FOR MONITORING OF GLUCOSURIA IN CATS. JM Fletcher[1], EN Behrend[1], HP Lee[1], E Welles[2]. [1]Department of Clinical Sciences, [2]Department of Pathobiology, Auburn University, College of Veterinary Medicine, Auburn AL.

Monitoring control of diabetes mellitus is challenging, especially in cats. Purina Glucotest™ is a litter additive that supposedly can be used to accurately assess glucosuria. The purposes of this prospective study were to evaluate the accuracy of Purina Glucotest™ for estimation of urine glucose concentrations when the chips were first exposed to urine and to determine whether the color change was stable for 8 hr as claimed by the manufacturer.

Glucose was added to previously frozen feline urine samples to achieve 40 urine samples with approximate glucose concentrations of 50, 150, 300 and 600 mg/dL. Eight additional samples were from diabetic cats and contained glucose at the time of freezing. Purina recommends the urine sample be evaluated within 15 minutes of collection. The absolute glucose concentration in the samples was also determined with the Hitachi 911 Chemistry Analyzer. Three Glucotest™ pieces were added to clay litter-filled Petri dishes and soaked with one of the urine samples. An estimate of the urine glucose concentration was determined by a trained, blinded observer by comparing the color change of the pieces to the color chart in the Glucotest™ package insert immediately and at 30, 60, 120, 240, 360, 480 and 600 minutes.

Reference ranges that correlate with the colors on the Glucotest™ color chart (Glucotest™ 0 mg/dL [0 to 25 mg/dL], 50 mg/dL [26–100 mg/dL], 150 mg/dL [101–225 mg/dL], 300 mg/dL [226–450 mg/dL] or 600 mg/dL [>450 mg/dL]) were devised because of lack of published concentration ranges for the Glucotest™. According to our ranges, the glucose concentration was accurately measured immediately after soaking in 29 of 48 samples. Of the 19 inaccurate measurements, 17 were overestimated.

Glucose concentrations in all 19 samples were between 50 and 300 mg/dL. At 8 hours, readings in 16 samples decreased into the next lower range. These changes resulted in an increase in overall accuracy. The majority of the color changes occurred within 6 hours (range of 1 to 8 hours). Fifteen of the color changes occurred in the 40 spiked samples and 1 occurred in a sample from a diabetic cat.

Results suggest the Glucotest™ tends to overestimate urine glucose concentrations in the midranges (50–300 mg/dL), even when exposed to urine glucose concentrations that directly correlate with the published color chart. The 8 hr measurements were more accurate than the initial readings, but the change over time is not consistent with the labeled 8-hr color stability claim.

ABSTRACT #223

ASSOCIATION BETWEEN ATHEROSCLEROSIS AND GLOMERULONEPHRITIS IN DOGS. RS Hess,[1] PH Kass,[2] TJ Van Winkle.[1] [1]University of Pennsylvania, Philadelphia, PA. [2]University of California, Davis, CA.

The purpose of this study was to investigate whether glomerulonephritis is observed more commonly in dogs with spontaneous atherosclerosis compared to dogs that do not have atherosclerosis. Renal glomerular disease increases the risk for atherosclerosis in human beings. The association between atherosclerosis and glomerulonephritis has not been reported in dogs.

37 dogs with histopathological evidence of atherosclerosis were included in the case group. 279 control dogs with results of a complete necropsy and no histopathological evidence of atherosclerosis were frequency matched on age and year of necropsy. The 279 control dogs included 142 dogs with a final necropsy diagnosis of neoplasia, 71 dogs that were randomly chosen from the hospital population, and 66 dogs that had a diagnosis of diabetes mellitus, hypothyroidism, or hyperadrenocorticism. A retrospective mortality prevalence case-control study was performed. Proportionate changes in the prevalence of glomerulonephritis were calculated using prevalence odds ratios (POR), 95% confidence intervals (95% CI), and p-values.

Eleven of 37 dogs (30%) with atherosclerosis had glomerulonephritis, 6 of 142 dogs (4%) in the neoplasia control group had glomerulonephritis, 9 of 71 (13%) dogs in the random diagnosis control group had glomerulonephritis, and 6 of 65 (9%) dogs in the endocrine control group had glomerulonephritis. Dogs with atherosclerosis had significantly more glomerulonephritis than dogs in the neoplasia control group (POR = 20.5, 95% CI 4.6–127.8, $p < 0.0001$), dogs in the random diagnosis control group (POR = 4.8, 95% CI 1.5–15.2, $p = 0.0078$), and dogs in the endocrine control group (POR = 6.8, 95% CI 2.0–22.8, $p = 0.0019$).

It is concluded that glomerulonephritis is more prevalent in dogs with atherosclerosis compared to dogs without atherosclerosis.

ABSTRACT #224

INSULIN RESISTANCE IS NOT ASSOCIATED WITH GLUCOSE INTOLERANCE IN DOG MADE OBESE BY OVERFEEDING. François Briand[1,2], Khadija Ougerram[1], Michel Krempf[1], Patrick Nguyen[2]. Centre de Recherche en Nutrition Humaine de Nantes, [1]: INSERM U539, CHU Nantes, France, [2]: Unité de Nutrition et Endocrinologie, Ecole nationale vétérinaire de Nantes, France.

In human obesity and type 2 diabetes (T2D), insulin resistance and glucose intolerance are generally associated. Given that canine obesity is current while T2D is not, this close association would not be so evident in dog. To give further insights into this canine specificity, insulin sensitivity and glucose tolerance were assessed before and after an overfeeding period in beagle dogs.

Seven healthy male beagle dogs were overfed (1.6 fold the NRC recommendation) for 25 weeks. Insulin resistance was assessed during euglycemic hyperinsulinemic clamp. Glucose tolerance was measured with intravenous glucose tolerance test. Plasma lipids were also measured before and after diet.

Body weight was higher after diet (13.9 ± 1.2 vs 19.6 ± 1.7 kg, $p < 0.05$). Total cholesterol was also higher (1.74 ± 0.12 vs 2.23 ± 0.14 g/L, $p < 0.05$), as well as triglycerides (0.34 ± 0.03 vs 1.07 ± 0.07 g/L, $p < 0.05$). Glucose infusion rate to maintain basal blood glucose during clamp was lower (16.5 Mp 1.5 vs 11.2 ± 1.4 mg/kg/min, $p < 0.05$) as well as insulin sensitivity (0.159 ± 0.04 vs 0.068 ± 0.007, $p < 0.05$). Intravenous glucose tolerance test showed no change in glucose tolerance (Kg value). However, insulin resistant dogs showed higher triglycerides area under the curve (AUC) after glucose load (185 ± 33 vs 324 ± 38, $p < 0.05$), as well as free fatty acids AUC (429 ± 80 vs 622 ± 60, $p < 0.05$).

We concluded that overfeeding and weight gain induce insulin resistance but not glucose intolerance in dog. This may explain why prevalence of T2D is lower than prevalence of obesity in dogs.

ABSTRACT #225

THE EFFECTS OF ANESTHESIA AND SURGERY ON THYROID FUNCTION TESTS IN DOGS. MA Wood[1], DL Panciera[1], SH Berry[1], WE Monroe[1], KR Refsal[2]. [1]Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA. [2]Michigan State University College of Veterinary Medicine, East Lansing, MI.

The purpose of this investigation was to evaluate the effects of anesthesia alone and anesthesia combined with abdominal exploratory surgery on thyroid function tests in dogs. Fifteen euthyroid purpose bred mongrel dogs were randomly assigned to one of three groups: control, general anesthesia, and general anesthesia plus abdominal exploratory surgery. Dogs in the anesthesia group were pre-medicated with acepromazine 0.05 mg/kg IM and morphine 0.5 mg/kg IM, were induced with propofol 4 mg/kg IV to effect, then were maintained on isofluorane inhalant (0.8–1.25%) for 100 minutes. Dogs in the surgery group were pre-medicated and anesthetized for 100 minutes as the dogs in the anesthesia group, and had an exploratory laparotomy performed which lasted 60 minutes from initial skin incision to closure. Dogs in the anesthesia and surgery groups received morphine 0.5 mg/kg SC at the end of anesthesia. Blood samples were collected from each dog immediately prior to pre-medication, 20 minutes after pre-medication, 55 minutes after anesthesia induction, at the end of anesthesia, 4, 8,12, 24, and 36 hours post-anesthesia induction, once daily for 6 additional days, and 14 days post-procedures. The dogs in the control group did not receive any treatment, but had blood samples drawn on the same time schedule as the other dogs. Total T4 (T4), total T3 (T3), free T4 by equilibrium dialysis (fT4), canine TSH (cTSH) and reverse T3 (rT3) were measured in all samples. Mean difference from baseline to each sample time was compared among the 3 groups using mixed effects repeated measures ANOVA with a Bonferroni correction for multiplicity comparisons.

Results of all thyroid function tests were not significantly different between control and anesthesia groups. Serum T3 was significantly lower in the surgery group compared to control at 8, 12 and 72 hours, and compared to the anesthesia group at 8, 24, 120, 144 and 168 hours. Serum T4, fT4, and rT3 for the surgery group were increased significantly compared to control at times 24, 36, 48, and 96 hours, 168 hours, and 8 and 24 hours, respectively. Serum T4, fT4, and rT3 for the surgery group were increased significantly compared to the anesthesia group at times 12 and 24 hours, 48 hours, and 8, 12, 24, and 36 hours, respectively.

We conclude that surgery has a significant effect on thyroid function tests, while the anesthetic protocol used in this study does not.

ABSTRACT #226

INHERITED HYPERPARATHYROIDISM IN THE KEESHOND: A CANDIDATE GENE APPROACH. BJ Skelly and RJM Franklin, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, UK.

Keeshonds have a breed predisposition to primary hyperparathyroidism but the mode of inheritance of this disease and the molecular genetic basis are unknown. A questionnaire was sent to 350 keeshond breeders and owners in order to identify the disease prevalence in the UK and participating dogs were blood sampled. Hypercalcaemic keeshonds were assessed for renal insufficiency and diagnosis of hyperparathyroidism was confirmed by measurement of parathyroid hormone (PTH). The calcium sensing receptor gene (CaSR) and the multiple endocrine neoplasia 1 gene (MEN1), were selected as candidate genes on the basis of their roles in the pathogenesis of primary hyperparathyroidism in humans. Fresh, frozen parathyroid glands and blood samples from affected and normal keeshonds were used to clone both genes. The coding sequences (exonic DNA) and splice junctions were analysed for mutations using direct sequencing analysis.

180 keeshonds have been included in this study. Thirty-seven have been shown to be hypercalcaemic and most (30/37) have had hyperparathyroidism confirmed by measurement of PTH levels. The genes encoding CaSR and MEN1 have been cloned in normal and affected keeshonds and the two sequences compared. Although several polymorphisms have been identified in these genes, none segregates with the disease phenotype.

We have ruled out mutations in CaSR and MEN1 as potential explanations for primary hyperparathyroidism in the keeshond. The study of a third candidate, HRPT2, encoding parafibromin, is underway. Future work will begin to use mapping techniques to identify positional candidate genes for hyperparathyroidism.

ABSTRACT #227

ADRENAL ASYMMETRY IN 14 DOGS WITH HYPERADRENOCORTICISM. Pauline de Fornel-Thibaud[1], Delphine Rault[2], Yannick Ruel[2], Juliette Besso[2], Amélie Cohen[2], Juan Hernandez[2] and Dan Rosenberg[1]. Internal Medicine Unit[1], Medical Imaging Unit[2], National Veterinary School of Alfort, Maisons-Alfort, France.

Numerous reports confirm the value of abdominal ultrasonography in the evaluation of Cushing's syndrome in dogs in order to distinguish pituitary-dependent hyperadrenocorticism (PDH) and adrenocortical tumor hyperadrenocorticism (ATH). This distinction is based on the observation of symetrical (often hypertrophied) adrenal glands in case of PDH and adrenal mass with controlateral atrophy in case of ATH, even if, to the knowledge of the autors, no cutoff for adrenal atrophy was determined so far. However equivocal adrenal asymmetries are sometimes identified. This retrospective study described 14 cases of adrenal asymetry objectivized by means of ultrasonography in dogs with hyperadrenocorticism without obvious vascular invasion or organ metastasis. The aim was to determine in that situation the value of ultrasonographic criteria in differentiating PDH from ATH.

The inclusion criteria were: a history and a physical examination consistent with hyperadrenocorticism associated with at least one endocrine test corroborating the diagnosis (ACTH stimulation test and/or low-dose dexamethasone suppression test) ; an adrenal asymmetry defined as a difference greater than 20% between the maximal width measured ultrasonographically of each adrenal gland; the univocal determination of the cause of hyperadrenocorticism established on the basis of unsuppressed basal ACTH plasma levels and suppression of cortisol on high-dose dexamethasone suppression test for PDH and suppressed basal ACTH plasma levels and histologic examination of the greatest adrenal gland for ATH. The size, shape, echogenicity and relation with adjacent structures were studied for each adrenal gland.

Fourteen dogs were included, 8 with PDH and 6 with ATH. A major overlap of the maximal width of the greatest adrenal gland was observed between the two groups (range, median: ATH: 11 to 88 mm, 30 mm; PDH: 9 to 35 mm, 19 mm). Maximal width of the smallest adrenal gland in dogs with ATH (range, median: 3 to 5 mm, 3.9 mm) was significantly lower than width of the smallest adrenal gland in dogs with PDH (range, median: 5 to 15 mm, 7.6 mm). Shape, echogenicity and relation with adjacent structures were not considered as relevant distinction criteria as, for example, round and/or irregular shape, heterogeneous echogenicity and compression of adjacent vessels were identified in both groups.

These results emphasize the interest of the precise evaluation of the adrenal gland controlateral to an adrenal hypertrophy. Our findings indicate that in a dog with hyperadrenocorticism and an adrenal mass, visualization of an atrophied opposite gland (width lower than 5 mm) is necessary to establish a diagnosis of a functioning adrenocortical tumor. In the other cases, further evaluation should be performed.

ABSTRACT #228

COMPARISON OF SYNTHETIC DEPOT ACTH WITH SYNTHETIC AQUEOUS ACTH FOR ADRENAL STIMULATION TESTING IN NORMAL DOGS. ACG Abrams-Ogg, S Atkinson, D Bienzle. University of Guelph, Guelph, Ontario, Canada.

Our purpose was to describe the blood cortisol response to synthetic depot ACTH (Synacthen Depot) compared to that obtained with synthetic aqueous ACTH for injection (Cortrosyn). Synacthen Depot contains the same ACTH molecule (cosyntropin/tetracosactrin/tetracosactide) as Cortrosyn, but complexed to zinc phosphate, which retards absorption.

Thirteen dogs (23–40 kg) were randomly assigned to Cortrosyn (0.25 mg IV) or Synacthen Depot (1 mg IM) in a crossover design with a 1-week washout period. Seven dogs received Cortrosyn first, and 6 dogs received Synacthen Depot first. Blood samples were obtained prior to injection, hourly over the subsequent 8 hours, and 24 hours after injection. Serum samples were frozen at −70 C and serum cortisol concentrations were assayed in batch by chemiluminescent immunoassay. Data were analyzed by ANOVA for repeated measures with $p \leq 0.05$.

No adverse reactions occurred. Mean ± SD serum cortisol concentrations (nmol/L) were:

Time (hr)	0	1	2	3	4	5	6	7	8	24
Cortrosyn	62 ± 57	313 ± 137	317 ± 120	167 ± 81	81 ± 41	53 ± 41	39 ± 22	37 ± 26	46 ± 44	46 ± 17
Synacthen Depot	48 ± 13	325 ± 97	462 ± 154	498 ± 166	513 ± 151	524 ± 137	524 ± 152	480 ± 138	452 ± 156	53 ± 40

Mean cortisol concentrations differed between ACTH formulations at each time point except at 0, 1 and 24 hours.

With Cortrosyn, peak cortisol concentrations ranged from 218–562 (355 ± 113) and occurred at 1 hour (8 dogs) or 2 hours (5 dogs). For dogs with 1-hour peak values, differences between 1 and 2 hour values ranged from 1–105. For dogs with 2-hour peak values, differences between 1 and 2 hour values ranged from 21–268. Mean 1 and 2-hour values were not different.

With Synacthen Depot, peak cortisol concentrations ranged from 311–860 (581 ± 159) and occurred at 3 hours (1 dog), 4 hours (1 dog), 5 hours (2 dogs), 6 hours (7 dogs), and 7 hours (2 dogs). Mean 3–8 hour values were not different. For all dogs 2-hour values were greater than 1-hour values, with the differences ranging from 57–319, and mean 1 and 2 hour values were different. For all dogs with a 1-hour peak value with Cortrosyn, the closest value with Synacthen Depot also occurred at 1 hour. For 3 dogs with a 2-hour peak value with Cortrosyn, the closest value with Synacthen Depot occurred at 2 hours, while it occurred at 1-hour with the other 2 dogs.

In conclusion 1) Synacthen Depot resulted in higher and prolonged blood cortisol concentrations; 2) Synacthen Depot yielded a result comparable to Cortrosyn at 1 hour after injection; 3) Cortisol assays 2 hours after each product provided comparable results for some dogs.

ABSTRACT #229

HYPOADRENOCORTICISM IN 98 DOGS. JA Adler, KJ Drobatz, RS. Hess. University of Pennsylvania, Philadelphia, PA.

The purpose of this study was to report variables such as ionized Ca (iCa), ionized magnesium (iMg), and venous pH, not previously reported in a large group of dogs with hypoadrenocorticism (HA) along with the clinical signs, clinicopathologic abnormalities, treatment, and outcome associated with canine HA. To this end, a retrospective study of all dogs newly diagnosed with HA at the MJR Veterinary Hospital between 1997–2005 was performed. Signalment of dogs with HA was compared to the signalment of 894 control dogs randomly selected from the group of all other dogs examined at the same time period.

Decreased iCa or iMg was observed in 22% or 4% of dogs, respectively and increased iCa or iMg was observed in 16% or 28% of dogs, respectively. Venous pH was less than 7.35 in 54% of dogs. Median age of 98 dogs with HA (3.8 years) was significantly lower than that of control dogs (7 years, $p = 0.0038$). Females (59%) were over represented in the group of dogs with HA compared to control dogs (46%, $p = 0.011$). Standard Poodle dogs and Great Danes were over-represented compared to the control group ($p < 0.001$ and $p < 0.001$, respectively). Eighty-one dogs (85%) were examined with a history of acute clinical signs. The most common clinical signs were lethargy (97%), inappetance (92%), vomiting (73%), and diarrhea (37%). Seizures associated with hypoglycemia were reported in 5 dogs (5%). Abnormal physical examination findings included dehydration (71%), underweight body condition (41%), tachycardia (47%), bradycardia (12%), hypothermia (35%), fever (5%), and increased respiratory rate (35%). Indirect systolic, diastolic, or mean arterial blood pressure was low in 41, 22, or 24 dogs, respectively. Complete blood counts were normal in most dogs. Anemia, observed in 34% of dogs resolved at the time of follow-up in 62% of dogs tested. Elevations of BUN, creatinine, potassium, or phosphorous concentration were observed in 56%, 40%, 72%, 49% of dogs respectively, and resolved at the time of follow-up in 87%, 100%, 80%, 93% of dogs tested, respectively. Decreased sodium, chloride, cholesterol, or albumin concentration was observed in 72%, 61%, 63% and 39% of dogs, respectively and resolved at the time of follow-up in 82%, 71%, 75% and 81% of dogs tested, respectively. Elevated serum potassium concentration and decreased serum sodium concentration were observed in 83% of dogs. Concurrent conditions included heart disease (11 dogs), pneumonia (8 dogs), and hypothyroidism (6 dogs). All hospitalized and treated dogs survived to discharge (93/93, 100%). Median length of hospitalization was 4 days (range, 0–26 days).

In conclusion, most dogs with HA have an acute onset of clinical signs. Hypocholesterolemia is common and reversible in most dogs with HA. Most dogs have a venous pH less than 7.35. Increased ionized Ca or ionized Mg concentration is observed in 16% or 28% of dogs. Prognosis with treatment is excellent.

ABSTRACT #230

EVALUATION OF THE EFFECT OF LOW DOSES OF ACTH ON CORTISOL CONCENTRATIONS IN CLINICALLY NORMAL DOGS. L Martin[1], EN Behrend[2], K Mealey[1], M Carpenter[3], K Hickey[1]. [1]Washington State University, College of Veterinary Medicine, Pullman, WA; [2]Auburn University, College of Veterinary Medicine, Auburn, AL; [3]Auburn University, College of Sciences and Mathematics, Auburn, AL.

The adrenocortical response of normal dogs to synthetic ACTH (cosyntropin) has been reported using several doses of intravenous ACTH (l µg/kg, 5 7g/kg, 10 µg/kg, and 250 µg/dog). All of these doses have been shown to maximally stimulate cortisol secretion. However, no studies have been performed to determine if a dose lower than 1 µg/kg of cosyntropin will also cause maximal cortisol secretion in dogs. Use of such a dose may be crucial in diagnosing a new syndrome, relative adrenal insufficiency, which has recently been recognized in critically ill human and veterinary patients. The purpose of this study was to determine the lowest dose of ACTH that will produce maximal cortisol secretion in clinically normal dogs.

Five dose-response trials were performed in 10 clinically healthy dogs. Each dog was given 5 doses (1 µg/kg, 0.5 µg/kg, 0.1 µg/kg, 0.05 µg/kg, 0.01 µg/kg) of cosyntropin (Cortrosyn) intravenously with a 2-week wash out period between each dose. Blood samples for determination of serum cortisol concentrations were obtained before and at 10, 20, 30, 40, 50, 60, 120, 240 minutes after cosyntropin administration. Samples were centrifuged after clotting, and the serum was separated and stored at −80°C until analysis. Data were analyzed using the Nonlinear Programming (NLP), Mixed Linear Model (MIXED), and General Linear Model (GLM) procedures in SAS®. Nonlinear dose response models were used to determine the lowest dose of ACTH below 1 µg/kg that produced bioequivalent maximal cortisol secretion.

Mean serum cortisol concentration increased significantly after administration of all 5 cosyntropin dosages when compared to baseline. Based on one-way Analysis of Variance (and subsequent multiple comparisons) mean post-ACTH serum cortisol concentration was significantly lower when ACTH doses of 0.01 and 0.05 µg/kg were administered in comparison to administration of 0.1, 0.5 and 1 µg/kg ACTH ($p < 0.001$). However, mean post-ACTH serum cortisol concentration did not differ significantly in response to the 0.1, 0.5 and 1 µg/kg doses. In a direct comparison, there was statistical evidence that a dose close to 0.1 µg/kg will produce bioequivalent results to the 0.5 and 1 µg/kg doses. Higher dosages of cosyntropin resulted in more sustained elevations in serum cortisol and a later time of peak response.

In conclusion, cosyntropin administered at a dose less than 0.5 µg/kg intravenously produces maximal cortisol secretion in clinically normal dogs. Serum cortisol concentration was reliably increased in all dogs after the administration of each dose of cosyntropin. These results can be used in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in normal and critically ill dogs

ABSTRACT #231

TISSUE DOPPLER EVALUATION OF LEFT VENTRICULAR DIASTOLIC FUNCTION IN HEALTHY, NONSEDATED CATS OF VARIOUS AGES. S Disatian, JM Bright, and J Boon. Department of Clinical Sciences, Colorado State University, Fort Collins, CO.

Tissue Doppler imaging (TDI) is an ultrasound technique used to evaluate left ventricular (LV) diastolic function in cats. However, LV diastolic TDI indices may be affected by age. Data describing the effect of age on LV diastolic myocardial velocities in healthy cats are currently unavailable. Therefore, this study was created to evaluate the relationships between age and LV diastolic myocardial velocities determined by TDI and to provide normal reference values.

Fifty nonsedated cats 3 months and older without clinical or laboratory abnormalities were studied. For analysis the cats were grouped by age: 3 to <6 months, 6 months to <3 years, 3 to <6 years, 6 to <12 years, and 12 years or older. LV myocardial velocities were measured at a point midway to the apex and at the mitral annulus on the LV wall and the ventricular septum using a left apical four chamber imaging plane. LV myocardial velocities were also measured at the endocardium between the papillary muscles using a right parasternal transverse imaging plane. The effect of age on diastolic LV TDI parameters was assessed using simple linear regression, ANOVA and ANCOVA. TDI parameters evaluated LV diastolic function included peak early diastolic velocity (E'), peak late diastolic velocity (A'), isovolumic relaxation time (IRT), deceleration rate of early diastolic motion (DR), the ratio of peak early diastolic to late diastolic velocities (E'/A' ratio), and the ratio of peak velocity of transmitral early diastolic flow velocity to tissue Doppler peak early diastolic velocity (E/E' ratio).

There was a weak but significant effect of aging on E' and DR measured at some sites on the LV wall. A', E'/A' ratio and E/E' ratio were not effected by age. Based on ANCOVA ($P < 0.05$), the regional IRT values were significantly less in kittens than in the adult cats. The LV diastolic myocardial velocities measured at the mitral annulus on LV wall were not affected by age suggesting that this site is an appropriate site for obtaining TDI measurements of diastolic function in cats.

In conclusion, the effect of age on LV diastolic TDI myocardial velocities in cats is minimal and not clinically important particularly when longitudinal velocity data is obtained at the mitral annulus on the LV wall. In addition, this study provides reference values of LV diastolic myocardial velocities for healthy nonsedated cats.

ABSTRACT #232

COMPARISON OF TWO METHODS TO MEASURE TISSUE DOPPLER VELOCITIES. G Wess, M Killich, TB Wagner, K Hartmann. Clinic for Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

Tissue Doppler imaging (TDI) includes several different techniques to evaluation the systolic and diastolic myocardial function, such as Strain, Strain Rate and tissue velocity imaging (TVI). The direction and velocity of the myocardial wall has been measured in veterinary medicine using either spectral pulsed wave TVI (PWTVI), or color Doppler TVI (CDTVI) methods. In a clinical setting it is necessary to compare the values of a patient to reference values; however, it is unclear, if the reference values using one method (i.e. CDTVI) are valid for the other method.

The objective of this study was to compare velocity information derived from normal contracting myocardium by both pulsed wave and color Doppler myocardial imaging, to investigate if the results can be used interchangeably and to compare different myocardial walls.

The study included 71 healthy dogs. All dogs had a normal ECG, a normal 2D-echocardiographic examination and a normal blood pressure. The echocardiographic examination was performed using a GE Vivid 7 machine. A left apical 4-chamber view was obtained and data were acquired for off-line analysis using the GE-EchoPac software from the interventricular septum and left ventricular free wall (FW) at the mitral annulus, using both methods.

Both methods showed a systolic S-wave, an early diastolic E-wave and a late diastolic A-wave. The normal values derived by both TVI methods are shown in the table. Maximum velocities of the S-, E- and A-wave were significantly ($p < 0.001$) lower using the CDTVI method, as compared to the PWTVI method. Mean velocities were about 2.1 cm/s or 25% lower using the CDTVI method. When both myocardial walls were compared using the same TVI method, E- and A-waves were significantly higher in the left ventricular free wall, as compared to the septum.

This study proved an inherent difference between PWTVI and CDTVI techniques for velocity estimation. Care should be taken when analyzing and comparing peak velocity data collected by the pulsed wave and color Doppler techniques and when comparing different myocardial walls.

PW TVI	N	Mean (cm/s)	SD		Color TVI (cm/s)	Mean	SD
S Septum	71	10.97*	3.65		S Septum	8.94*	3.13
E Septum	71	7.41*,‡	1.47		E Septum	5.51*,‡	1.29
A Septum	71	7.16*,‡	2.09		A Septum	4.79*,‡	1.63
S FW	71	10.89*	2.77		S FW	8.60*	2.30
E FW	71	10.41*,‡	2.94		E FW	8.20*,‡	2.71
A FW	71	9.06*,‡	2.47		A FW	6.52*,‡	2.24

FW= free wall, * statistically significant (P<0.05) difference between PW and color TVI method; ‡ statistically significant difference between myocardial walls using the same TVI method

ABSTRACT #233

ASSESSMENT OF THE TEI INDEX OF MYOCARDIAL PERFORMANCE IN CONSCIOUS HEALTHY DOGS. MG Sousa, GB Pereira-Neto, D Paulino-Júnior, JPE Pascon, F Gava, AA Camacho. College of Agricultural and Veterinary Sciences, São Paulo State University, Campus of Jaboticabal, Brazil.

The Tei index of myocardial performance combines systolic and diastolic time intervals to assess global cardiac function in a single number. However, although studied extensively in healthy and diseased humans, it has not been well characterized in veterinary patients. Tei index of myocardial performance (TEI) is defined as (Isovolumic contraction time + Isovolumic relaxation time)/Left-ventricular ejection time. Because information regarding the index in dogs is still lacking, we studied how TEI performs in healthy conscious dogs.

Twenty-nine mature Beagles (19 males; 10 females), with mean weight of 12.3 Kg, were enrolled in the study. With dogs positioned in left lateral recumbency on an echocardiography table, apical five-chamber view images were acquired. To acquire inflow and outflow velocity spectra during the same cardiac cycle, the Doppler sample volume was placed midway between mitral inflow and left ventricular outflow in the apical five-chamber view. This image was used to measure the mitral closing-to-opening time, which was named interval *A*. Left-ventricular ejection time (LVET) was determined as the duration of left ventricular outflow profile. Therefore, TEI was calculated as (*A* – LVET)/LVET. Heart rate was calculated from simultaneous lead II electrocardiogram. To check for interobserver agreement, every dog underwent echocardiographic evaluation performed by two individuals, which were blinded to the results of the other. Also, one of the observers performed the same exams one week later to check for interday reproducibility.

Results are listed in Table 1. Unpaired t test showed no difference ($P = 0.4741$) between the index calculated in males and females. Pearson's correlation coefficient demonstrated significant interobserver ($r^2 = 0.4490$; $P < 0.0001$) and interday ($r^2 = 0.5736$; $P < 0.0001$) correlations. However, no correlation existed between TEI and heart rate ($r^2 = 0.0228$; $P = 0.4335$), as well as between the index and body weight ($r^2 = 0.0028$; $P = 0.7822$).

Results allowed concluding that TEI is a heart rate-independent indicator of systolic and diastolic function in dogs. It also does not depend on body weight and may be reproduced satisfactorily. Since this non-invasive index may be easily calculated from standard echocardiographic images, it may prove useful in the evaluation of heart function in animals. Further studies are needed to determine how the index performs in dogs with cardiac diseases.

Table 1 – Tei index of myocardial performance in healthy conscious Beagles.

	Males (n=19)	Females (n=10)	All (n=29)
Tei Index of Myocardial Performance (mean ± SD)	0.28 ± 0.14	0.25 ± 0.11	0.27 ± 0.13
95% Confidence Interval	0.21 – 0.35	0.16 – 0.32	0.22 – 0.32

ABSTRACT #234

THE EVALUATION OF A TEI INDEX IN CATS. Y Hori[1)], M Uechi[2)], A Indou[1)], K Tejima[2)], K Asano[2)]. [1)]Kitasato University, Aomori, Japan, [2)]Nihon University, Kanagawa, Japan.

The aim of this study is to evaluate the TEI index associated with the change of heart rate, alteration of pre- or after-load in clinically healthy cats. Mixed-breed cats were anesthetized. Heart rate was controlled by right atrial pacing. The arterial pressure and the central venous pressure were measured by catheters. The afterload was increased by descending aortic balloon occlusion. Preload was increased by intravenous infusion with lactate ringer (40 ml/kg/h). FS, E/A ratio, TEI index were measured at each condition. In regulated heart rate by the atrial pacing, the ejection time was significantly shortened from baseline, the regression coefficient was -0.82 ($p < 0.01$). The isovolumic time was shortened, the regression coefficient was -0.48 ($p < 0.05$). The TEI index was not changed by heart rate. The arterial blood pressure was significantly elevated ($p < 0.05$) by the aortic occlusion. The ejection time was not changed, but the isovolumic time was significantly prolonged ($p < 0.05$). The TEI index was significantly elevated by increased afterload ($p < 0.05$). With the increased preload by the intravenous infusion, FS was significantly elevated. Whereas the ejection time was significantly prolonged ($p < 0.05$), the isovolumic time was not changed. The TEI index was not changed. Present study indicated that the TEI index is independent from heart rate and afterload was highly significant determinants of the TEI index.

ABSTRACT #235

ECHOCARDIOGRAPHIC REFERENCE VALUES FOR SEDATED HEALTHY CYNOMOLGUS MONKEYS. Gaughan JM, Burkett DE, Sleeper MM. Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania, Philadelphia, PA.

Non-invasive echocardiographic imaging is used in both veterinary and human medicine to evaluate cardiac structure and function. An animal model of human cardiovascular disease, the Cynomolgus monkey (*Macaca fascicularis*) is a commonly used nonhuman primate species in biomedical research. Currently echocardiographic reference ranges for this species are not readily available. Echocardiographic evaluation, using standard 2D and M-Mode imaging planes, was performed on 84 male and 82 female Cynomolgus monkeys weighing 1.9 to 6.3 kg sedated with ketamine HCl (10 mg/kg IM). Reference ranges were determined (mean +/− SD) for heart rate (HR), LV size (Interventricular Septum in Diastole (IVSd), LV Internal Diameter in Diastole (LVIDd), LV Free Wall in Diastole (LVFWd) and LV Internal Diameter in Systole (LVIDs)), LV function (Fractional Shortening (FS)), Doppler derived indices of LV function (Aortic Peak Flow Velocity (AVMax), LV Ejection Time (LVET) and LV Pre-Ejection Time (LVPET)), left atrial diameter (LA) and aortic diameter (AO). The results are: HR 161 +/−29.39 bpm; IVSd 0.38 +/−0.06 cm; LVIDd 1.42 +/−0.19 cm; LVFWd 0.38 +/−0.07 cm; LVIDs 0.86 +/−0.15 cm; FS 39.07 +/−6.81%; LA 0.93 +/−0.17 cm; AO 0.85 +/−0.10 cm; LA:AO 1.07 +/−0.16 cm; AVMax 0.75 +/−0.12 m/s; LVET 0.2 +/−0.03 sec; LVPET 0.03 +/−0.01 sec; LVPET:LVET 0.15 +/−0.04. These values should prove useful for future biomedical research using this species, especially where non-invasive serial evaluation of cardiac structure and function are indicated.

ABSTRACT #236

EFFECT OF BODY POSITION (STANDING VERSUS RIGHT LATERAL RECUMBENCY) ON ELECTROCARDIOGRAPHIC VARIABLES IN DOGS. J.A. Stern, K.W. Hinchcliff, P.D. Constable*. College of Veterinary Medicine, The Ohio State University, Columbus, OH; *School of Veterinary Medicine, Purdue University, West Lafayette, IN.

We tested the hypothesis that electrocardiographic variables obtained with dogs standing would differ from those obtained with the dog in right lateral recumbency.

Electrocardiograms (ECGs) were recorded from 65 fit Alaskan sled dogs, 39 males, 2 male castrates, 22 females, and 2 spayed females, aged 4.4 ± 2.2 y (mean ± SD; range 1–9), and weighing 24.2 ± 3.2 kg. ECGs were recorded with the dogs in right lateral recumbency or standing. A coin toss determined the order in which the examinations were performed. After collection of the first ECG the dog's position was changed and the other ECG recorded. A 10 lead, semi-orthogonal lead system was used to record leads I, II, aVF, and V10 simultaneously at a 25 mm/s paper speed. ECG variables were tabulated and Signed Rank Test performed to test for differences between variables in standing and right lateral recumbent ECGs. Three independent evaluators rated ECG quality, scoring each tracing 0, 1, 2, or 3. The scoring system was: 0 = a high quality recording with no baseline wander or small baseline deflections; 1 = intermittent, mild tremors or deflections of baseline +/− baseline wander; 2 = moderate tremors or baseline deflection consistent throughout recording; 3 = severe tremor artifact inhibiting the interpretation of p and t waves. Concordance among investigators was determined by Cohen's weighted kappa statistic. Differences between body position were determined using McNemar's test. Null hypothesis was rejected at $P \leq 0.01$.

The quality of ECG tracings appeared superior in recordings made with the dogs in right lateral recumbency ($P < 0.001$). Standing ECGs contained 28% (18/65) grade 3 recordings while right lateral recumbent ECGs contained only 1 grade 3 recording. Heart rate did not differ between body positions. Statistically significant differences were determined within the standing vs right lateral recumbent ECG variables. R wave amplitude was 0.60 ± 0.87, −0.50 ± 1.45, 0.11± 0.26 mV greater in leads I, aVF and V10 standing ECG recordings respectively when compared to right lateral recumbent ($P < 0.001$). P wave amplitude was 0.10 ± 0.24, 0.07 ± 0.17 mV greater in leads I and V10 standing ECG recordings respectively when compared to right lateral recumbent ($P = 0.01$; $P < 0.001$ respectively). S wave amplitude was −0.15 ± 0.30, −0.17± 0.50, −0.09 ± 0.33, −0.25 ± 0.61 mV more negative across leads I, II, aVF, and V10 standing ECG recordings respectively when compared to right lateral recumbent ($P < 0.001$, $P = 0.039$, $P = 0.019$, $P = 0.002$ respectively). QRS duration was 8.7 ± 24.03 ms longer in lead II standing ECG recordings when compared to right lateral recumbent ($P = 0.01$). Among this homogenous group of dogs significant variation in ECG wave amplitudes and durations occurred in right lateral recumbency versus standing recording positions. Standards of measure derived for right lateral recumbency might not be valid for definitive evaluation of an ECG obtained in a standing position.

ABSTRACT #237

THE EXPERIMENTAL HYPERKALEMIA INDUCED T-WAVE CHANGE OF THE STANDARD LIMB LEADS AND THE NEW LEADS SYSTEM IN DOGS. Y Hori[1)], M Uechi[2)], T Ebisawa[1)], S Yamano[1)], T Mizukoshi[1)]. [1)] Kitasato University, Aomori, Japan, [2)]Nihon University, Kanagawa, Japan.

The aim of this study was to evaluate a new leads system (cardiac leads system) of the electrocardiography about experimental hyperkalemia-induced ECG changes. The normal mixed-breed dogs (n = 9) were

positioned right lateral recumbency, and infused with KCL (4 mEq/kg/h). The limb leads and cardiac axis leads were recorded simultaneously. Potassium concentration (Kc) was significantly increased with administration of KCl ($P < 0.05$). The P-wave durations were prolonged associated with potassium loading. The correlation coefficient between the P-wave durations and Kc was 0.59 ($P < 0.001$) in the limb leads and was 0.53 ($P < 0.01$) in the cardiac axis leads. The P-wave amplitudes were decreased associated with potassium loading. Between the amplitude of the P-wave and Kc had a significant correlation with the limb leads −0.52 ($P < 0.01$) but not the cardiac axis leads −0.29 (NS). In cardiac axis leads, T-wave amplitude was significantly elevated by potassium administration compared with the limb leads. Between the cardiac axis leads and Kc were observed a significant correlation ($r = 0.48$ $P < 0.001$), but not in the limb leads ($r = 0.01$ NS). In the cardiac axis leads, the positive T-wave were showing 100% in $K^+ = 7.9$ mEq/L. In the limb leads, positive T-wave was gradually disappeared by Kc increasing. These results suggested that the cardiac axis leads is useful to evaluate hyperkalemia and ventricular repolarization in dogs.

ABSTRACT #238

EFFICACY OF TRANSESOPHAGEAL AND TRANSGASTRIC CARDIAC PACING IN THE DOG. RA Sanders[1], HW Green III[1], DF Hogan[1], JA Ramos-Vara[1], AS Batra[2]. [1]Purdue University School of Veterinary Medicine, West Lafayette, IN [2]University of California-Irvine, Orange, CA.

Transesophageal atrial pacing (TEAP) is a routinely applied technique for the acute treatment of bradyarrhythmias in human medicine while transgastric ventricular pacing has been described. This study investigated the efficacy of transesophageal and transgastric atrial and ventricular pacing and possible esophageal damage after 24 hrs of pacing in dogs.

Eleven juvenile beagle dogs underwent general anesthesia followed by trans-nasal placement of a multipolar electrophysiology catheter into the esophagus or stomach. Transesophageal and transgastric atrial and ventricular pacing was attempted using bipolar and unipolar configurations with a temporary pulse generator in 9 dogs. All attempts at transgastric and transesophageal ventricular pacing were unsuccessful while TEAP was successful in all cases (10.7 ± 3.7 mA threshold at a 2 ms pulse width) using a bipolar configuration. The reliability of TEAP was inadequate in chronically sedated dogs presumably due to movement with loss of catheter-esophageal contact. To assess esophageal damage, TEAP was then established in a bipolar configuration using a temporary pacing generator with an output of 20 mA and pulse duration of 2 ms at a rate of 140 ppm. Catheters were affixed to the nasal septum and sedatives were given to minimize catheter tip movement during the 24 hr pacing period. Dogs were divided into 2 groups; Group A ($n = 5$) dogs were euthanized immediately following the 24 hr pacing period and Group B ($n = 4$) dogs were euthanized 7 days after the 24 hr pacing period. A control group (Group C, $n = 2$) had pacing catheters placed with no electrical stimulation applied for a 24 hr period to account for the effect of mechanical irritation of the catheter and were euthanized 24 hrs after placement of the catheter. Gross and histological examination of the esophageal tissue was performed at lead-esophageal contact as well as 2–3 cm oral and aboral to lead location. Gross evidence of mild esophagitis was seen in 1/5 dogs from group A while this was absent in all dogs from groups B and C. Histopathologic evidence of mild esophagitis was seen at the site of lead-esophageal contact in 5/5 dogs in group A, 0/4 dogs in group B, and 1/2 dogs in group C. There was no evidence of esophageal stricture or fibrosis in any dog from any group.

We conclude that TEAP using standard and easily acquired cardiac pacing equipment is simple to perform, very effective in the anesthetized dog and a viable alternative to temporary transvenous pacing with supraventricular bradyarrhythmias. Microscopic lesions of mild esophagitis consistently seen after 24 hrs of TEAP, resolve within 7 days and mechanical irritation from the catheter cannot be ruled out as a cause of these changes.

ABSTRACT #239

MYOCARDIAL PERFUSION RESERVE MEASUREMENT BY POSITRON EMISSION TOMOGRAPHY IN CATS WITH HYPERTROPHIC CARDIOMYOPATHY. SD Jenni[1], T Schepis[2], R Jenni[3], R Milovanovic[2], PT Siegrist[2], CE Reusch[4], PA Kaufmann[2], TM Glaus[1]. [1]Division of Cardiology, [4]Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, and [2]Nuclear Cardiology, [3]Echocardiography, Cardiovascular Center, University of Zurich, Switzerland.

In humans with hypertrophic cardiomyopathy (HCM), decreased hyperemic myocardial blood flow (MBF) and myocardial perfusion reserve (MPR) have been demonstrated. MPR is defined as the ratio of stress MBF, usually induced by adenosine (ado) at 0.14 μg/kg/min over 7 minutes, divided by resting MBF. Positron emission tomography (PET) using ^{13}N–ammonia is the gold standard to measure MBF. This can also be used in healthy anesthetized cats where maximal MPR is markedly lower and requires higher adenosine doses than in humans. The purposes of this study were to further refine the protocol to determine MBF and MPR by PET in cats, and to test whether abnormal MBF and impaired MPR is found in cats with echocardiographically confirmed HCM.

Twelve cats were studied by PET, 7 healthy cats and 5 with HCM. Standardized anesthesia was induced and heart rate, ECG, oxygen saturation and temperature in all cats, as well as arterial blood pressure in 6/12 cats (3/5 HCM cats) were continuously monitored. MBF was measured at rest in all cats, then cats were challenged one to three times with randomly assigned ado constant rate infusions for 7 minutes at different doses 50 min apart. In healthy cats ado doses were 0.14 ($n = 3$), 0.28 ($n = 3$), 0.56 ($n = 4$), 0.84 ($n = 3$) and 1.12 ($n = 3$) μg/kg/min. In HCM cats, ado doses were 0.14 ($n = 5$), 0.28 ($n = 4$) and 0.56 ($n = 4$) μg/kg/min. Non-parametric tests were used for statistical analysis of MBF and MPR. For MPR only values obtained at ado 0.56 μg/kg/min were calculated.

Global MBF at rest was 1.79 ± 0.75 ml/min/g myocardium in HCM versus 1.32 ± 0.33 ml/min/g in healthy cats ($p = 0.29$). In HCM cats, MBF was 2.14 ± 0.54 at 0.14 μg/kg/min ado and 2.16 ± 0.84 ml/min/g at 0.28 μg/kg/min. At 0.56 μg/kg/min, MBF in HCM cats was 2.58 ± 1.24 ml/min/g versus 2.25 ± 0.17 ml/min/g in healthy cats, and MPR was 1.41 ± 0.19 in HCM cats versus 1.60 ± 0.14 in healthy cats ($p = 0.25$). No differences in septal versus lateral perfusion were found. Oxygen saturation and rectal temperature were constant in all cats throughout the procedure. Heart rate was constant or only mildly fluctuating during ado infusions. ECG abnormalities were episodic ventricular premature complexes (VPC) at all ado doses used in one HCM cat, single VPCs at 0.56 μg/kg/min in one healthy cat, and one short run of 2nd degree AV-block in another healthy cat. Mean arterial blood pressure decreased during 13 of 16 ado infusions by up to 30% (more pronounced in healthy cats), normalizing during or shortly after the end of the ado infusion.

In conclusion, MPR in cats with HCM tended to be lower than in healthy cats, although this fell short of statistical significance. Based on our results, it appears safe and feasible to measure MPR in anesthetized cats with HCM using ado at 0.56 μg/kg/min. A larger number of cats as well as cats with varying severities of HCM need to be studied by PET.

ABSTRACT #240

PLASMA D-DIMER CONCENTRATIONS IN CATS WITH LEFT ATRIAL ENLARGEMENT. Christopher Hoolihan, San Diego, CA.

The purpose of this study was to prospectively evaluate plasma D-dimer concentration in cats with left atrial enlargement to see how plasma D-dimer concentration related to LA size severity and risk for thrombogenesis. Plasma D-dimers concentration is thought to be specific for thrombogenesis. It is well known that cats with an enlarged left atrium (LA) are at increased risk for thrombus formation in the LA. It has been proposed that cats with spontaneous contrast (SC) in the LA are at increased risk of developing a thrombus. We hypothesized that plasma D-dimer concentration would be elevated in cats with a moderate to severely enlarged LA, with SC, or with clinical evidence of a thrombus or thromboembolus (TE).

Thirty cats were entered into the study. All cats had cardiac disease and examiners noted SC, presence of a thrombus or TE and congestive heart failure (CHF) in results. Cat's with an LA:Ao ratio greater than 1.5 were eligible for inclusion in the study. The cats were grouped into three groups of LA enlargement severity. Plasma D-dimer concentration severity was compared to the LA severity. Using a Chi Square analysis of the data, there was a poor correlation and predictive value comparing plasma D-dimer concentration to left atrial enlargement severity. Chi-square = 2.14644565960355, $p = 0.05$. For significance at the 0.05 level, chi-square should be greater than or equal to 12.59.

Plasma D-dimer concentration v.s. LA:Ao ratio

	Normal	Mild D-dimer	Mod D-dimer	Sev D-dimer	Total
Mild LA:Ao	3	1	0	0	4
Moderate LA:Ao	6	1	0	0	7
Severe LA:Ao	13	3	2	1	19
Total	22	5	2	1	30

We concluded that plasma D-dimer concentration is a poorly sensitive test for severity of LA enlargement in cats with known cardiac disease. Also, in the 5 cats with SC, only 2 of 5 cats had an elevated plasma D-dimer concentration. Additionally and most discrediting to our hypothesis, every cat with known thrombus or thromboembolus present had a completely

normal D-dimer concentration showing a zero sensitivity for active clotting in our test subjects. One of the more interesting findings of the study was that all cats in CHF had an elevated plasma D-dimer concentration and all were moderately to severely elevated. Further studies need to be done to assess the usefulness of plasma D-dimer in cats for assessing CHF.

ABSTRACT #241

PREVALENCE OF CARDIOMYOPATHY IN APPARENTLY HEALTHY CATS. CF Paige[1], JA Abbott[1], RL Pyle[1], F Elvinger[2]. [1]Department of Small Animal Clinical Sciences, [2]Department of Large Animal Clinical Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Subclinical cardiomyopathy (CM) sometimes is identified after abnormalities are detected during auscultation of apparently healthy cats. However, little is known regarding the prevalence of CM in this population. Furthermore, the clinical importance of auscultatory abnormalities in apparently healthy cats is unclear. In order to determine the prevalence of murmurs and CM, we prospectively evaluated a sample of apparently healthy cats. Cats with systemic hypertension or hyperthyroidism were excluded. Therefore, 103 cats were subject to physical and echocardiographic examinations which were performed by two different investigators; the echocardiographer was unaware of the physical findings. Left ventricular wall thickness was determined by 2-D echocardiography in short- and long-axis planes. Left ventricular hypertrophy (LVH) was defined by an end-diastolic wall thickness $\geq$ 6 mm. Cats with LVH but without left ventricular dilation were considered to have hypertrophic CM (HCM). Cardiomyopathy was identified in 16 cats (15.5%; 95% Confidence Interval (CI): [9.2, 24.0]); 15 had HCM and one had arrythmogenic right ventricular cardiomyopathy. Murmurs were detected in 16 cats (15.5%; 95% CI: [9.2, 24.0]). Of the cats with murmurs, 5 had CM. Of 15 cats with HCM, 11 had segmental LVH and in three of these cats, hypertrophy was localized to the basilar septum. Three cats had diffuse LVH. One cat with marked systolic anterior motion of the mitral valve and maximal ventricular wall thickness of 5.9 mm was classified as HCM. The prevalence of feline subclinical CM in Southwest Virginia is near 16%; approximately a third of these cats had murmurs.

ABSTRACT #242

COMPARISON OF METHODS FOR MONITORING PLATELET FUNCTION AND ANTIAGGREGATION THERAPY IN CATS. TJ Morrison, JM Bright, C Olver. Colorado State University, Fort Collins, CO.

Point-of-care platelet monitoring devices developed for human use could advance platelet function monitoring in cats receiving platelet antiaggregation agents; however, these devices have not been validated in this species. The purpose of this study was to compare the Platelet Function Analyzer (PFA-100)[a] and the Rapid Platelet Function Assay (RPFA)[b] with standard whole blood platelet aggregometry before and after administration of the GP IIb/IIIa antagonist, abciximab.

Six healthy research cats were each studied 3 times with at least 1 week between studies. Platelet count and mucosal bleeding times were measured on each study day at time 0 (baseline) and after 120 minutes of drug infusion. Platelet function was measured via multiple methods, twice at baseline and then singly at 10, 60, and 120 minutes during the abciximab infusion. Each cat received abciximab intravenously to inhibit platelet function with increasing doses on different study days: 0.125 mg/kg followed by 0.0625 ug/kg/min, 0.25 mg/kg followed by 0.125 ug/kg/min, and 0.5 mg/kg followed by 0.25 ug/kg/min. Venous blood samples were obtained from an indwelling 18 g jugular catheter.

Rate of platelet aggregation was measured by standard whole blood impedance aggregometry using ADP and ADP with collagen as platelet agonists. The PFA-100 device measures the time required for the anticoagulated blood flowing under low sheer stress to occlude a 150 µL aperture on a surface coated with platelet agonists. Platelet function is reported as occlusion time. PFA cartridges containing collagen with epinephrine and cartridges containing collagen with ADP were used at all sampling times. The RPFA device measures platelet aggregation by measuring the change in light transmission through anticoagulated blood after automated mixing with a platelet agonist and fibrinogen-coated microbeads. Arachidonic acid and ADP were used as agonists. Results are reported by the device as platelet aggregation units (PAU).

Statistical comparisons of the platelet function monitoring devices were made using local polynomial linear regression modeling between time points followed by pair-wise comparisons of the slopes using independent student t-tests for each abciximab dose. Significant difference was defined as $p < 0.05$. The PFA-100 device failed to provide occlusion time measurements consistently after administration of the antiplatelet agent; therefore, data from this device was omitted from statistical analysis.

Results of this research reveal that the PFA-100 is not a useful method of quantifying platelet function in cats receiving platelet antagonists. However, measurements of platelet aggregation were obtained by RPFA at all levels of platelet inhibition. Furthermore, abciximab-induced variations in platelet aggregation measured by RPFA using either ADP or arachidonic acid cartridges did not differ significantly from variations in aggregation rates measured by whole blood aggregometry. Therefore, the RPFA may be a clinically useful method of measuring platelet function in cats for optimal dosing of anti-platelet agents.

[a]Dade Berhing, (Deerfield, Illinois) [b]Verify Now™, Accumetrics (San Diego, California)

ABSTRACT #243

TRANSDERMAL ATENOLOL IN CATS: PLASMA CONCENTRATIONS AND PHARMACODYNAMIC EFFECTS. JM MacGregor,[1] JE Rush,[1] EA Rozanski,[1] D Boothe,[2] AA Belmonte,[3] LM. Freeman. [1] [1]Tufts University, North Grafton, MA, [2]Auburn University, Auburn, AL, [3]Massachusetts College of Pharmacy and Health Sciences, Boston, MA.

Beta-adrenergic receptor antagonists are commonly prescribed for cats with hypertrophic cardiomyopathy (HCM). Currently, many compounding pharmacies are formulating transdermal atenolol to be prescribed in equal doses to those given orally. This study was designed to evaluate plasma concentrations of atenolol and pharmacodynamic endpoints that might be affected by beta-blockade following oral and transdermal administration of atenolol in healthy cats.

Seven male-castrated, client-owned cats were assessed as healthy by physical examination, ECG, and echocardiogram. Clinical laboratory results excluded major concurrent illness. Each cat received 6.25 mg of atenolol q 12 hours for one week. Atenolol was administered either orally as ¼ of a 25 mg tablet or in a novel compounded transdermal formulation. The alternative formulation was then administered in a crossover design for one week with a minimum one week washout period between treatment weeks. The order of treatments was randomized.

Blood was collected at baseline and at the end of each one-week study period for 2 and 12 hours post atenolol administration plasma atenolol concentrations (PAC). At each of these timepoints, an ECG and blood pressure measurement (Doppler technique) were performed. The ECG was recorded from 1 minute prior to phlebotomy, during phlebotomy and for at least 30 seconds after phlebotomy to evaluate the effectiveness of beta blockade with phlebotomy intended to serve a stressor for the cats. Plasma atenolol levels were detected using HPLC following validation in feline serum.

	Baseline	2 hr oral	2hr td	12 hr oral	12hr td
PAC(±SD) ng/ml	N/A	579[a] ± 212	177 ± 123	258[a] ± 142	62.4 ± 17
HR (BPM)	180	150[b]	160[b]	170	185
HR Stress (BPM)	190	150[b]	160[b]	175[b]	190
Blood Pressure	148	164	145	165	170

a= significantly different from td, b= significantly different from baseline, td = transdermal

The results of this study support that an equivalent transdermal dose of atenolol does not result in either the same PAC or evidence of beta-blockade as an oral dosage.

ABSTRACT #244

SERUM CHEMISTRY VARIABLES OF HEALTHY CATS RECEIVING SPIRONOLACTONE. JA Abbott, KE Saker. Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Little is known of the pharmacodynamics of spironolactone (SP) in cats. Therefore, we administered SP at a mean dose of 2.7 mg/kg PO BID to 9 healthy cats. Duration of SP administration was 7–9 days. Serum chemistries were evaluated prior to administration of SP and again approximately 12 hours after administration of the final dose of SP. Plasma concentrations of SP and the SP metabolite, canrenone (C), were determined by HPLC using blood samples obtained approximately 12 hours after administration of the final dose of SP.

After administration of SP, there were significant increases in the following serum analytes: K [mean difference, 0.39 mEq/L, $p = 0.013$], calcium [0.48 mg/dL, $p = 0.002$], creatinine [0.22 mg/dL, $p = 0.001$],

phosphorus [0.63 mg/dL, p= 0.032], total protein [0.51 mg/dL, p = 0.0008]. Serum chloride decreased [1.11 mEq/L, p = 0.03]. Except for a single determination of serum phosphorus which exceeded the upper limit of the reference interval by 0.1 mg/dL, all serum chemistry variables were within the laboratory's reference interval prior to administration of SP. After administration of SP, serum K marginally exceeded the upper limit of the reference interval in 6 cats. After administration of SP, mean (range) serum spironolactone concentration was 50 ng/mL (18–116) and mean serum canrenone concentration was 65 ng/mL (24–166). Canrenone concentration was correlated with the change in serum potassium concentration (r = 0.82).

Short-term administration of SP at a mean dose of 2.7 mg/kg PO BID is associated with changes in serum chemistry variables and measurable serum concentrations of SP and C.

ABSTRACT #245

DIURETIC EFFECTS OF FUROSEMIDE CONTINUOUS INTRAVENOUS INFUSION. Masami Uechi[1)], Kayoko Kanakubo[2)], Yasutomo Hori[2)], Shigeki Yamano[2)], Takashi Ebisawa[2)]. [1)]Nihon University, Kanagawa, Japan, [2)]Kitasato University, Towada, Japan.

The diuretic effects of furosemide were compared in healthy dogs (n = 7, 9.4–18 kg), using four different administration routes; bolus intravenous injection (BI), subcutaneous injection (SC), oral administration (PO), and continuous intravenous infusion (CI). These seven study dogs received furosemide 2 mg/kg four times at pre-designated intervals by BI, SC, PO, and CI. Urine samples were collected before furosemide administration and at 1, 2, 4, 6, and 8 hours after the administration for the analysis of urine volume, U.G., and urine Na, K, and Cl levels. Blood samples were also collected for the analysis of CBC, biochemistry, and electrolyte levels. When the dogs received a BI, diuresis reached a maximum of 267 ± 77 ml/hr at one hour, and then declined to 53 ± 28 ml/hr at two hours following the injection. When the dogs received a SC, diuresis reached a maximum of 255 ± 75 ml/hr at one hour, and was 103 ± 40 ml/hr at two hours following the injection, showing a significant diuretic effect. With PO, diuresis reached a maximum of 96 ± 53 ml/hr at two hours, and was significant until four hours (at 59 ± 32 ml/hr) following the administration. While the dogs received a CI, diuresis reached a maximum of 162 ± 57 ml/hr at two hours, and remained significant until eight hours (at 43 ± 22 ml/hr) following the administration, showing a significant increase in PCV and TP levels along with a significant decrease in plasma K levels. These results indicated that CI is a method to produce potent diuresis.

ABSTRACT #246

CARDIOVASCULAR EVALUATION AND cTnI LEVELS IN OBESE DOGS. KP Aptekmann, AS Carvalho Filho, DS Schwartz, UNESP-FMVZ-Botucatu (São Paulo State University), SP, Brazil.

Obesity is considered the most common nutritional disturbance in dogs. The incidence of circulatory diseases is twice as high in overweight dogs compared to normal dogs and can result in cardiac dysfunction and heart failure.

This study aimed to determine the occurrence of cardiac changes in obese dogs and to investigate myocardium injury by dosing cardiac troponin I (cTnI).

Twenty five non-obese and 25 obese dogs were selected from a hospital population, based on body score condition (BSC). Physical exam, electrocardiogram, echocardiogram (systolic function), thoracic radiograph, systemic arterial pressure and TnIc levels were obtained.

There was no statistical difference regarding ECG variables. Systemic arterial pressure values were significantly elevated in 60% of obese dogs ($p < 0{,}0001$). Thoracic radiographs showed increased vertebral heart score ($p < 0{,}01$) with right heart enlargement evidence in 56% of the obese dogs, and changes in pulmonary pattern in 84%, what could be related to decreased pulmonary inflation that occurs in obesity. The lack of significant changes on echocardiogram may be due to the duration of obesity in these dogs, since some changes are only observed on long term obesity. Although non-significant, cTnI was elevated in 20% of obese (0,0–0,4 ng/ml) and 12% of control (0,0–0,13 ng/ml) dogs, and the magnitude of variation was higher among the obese. The observed changes in cTnI levels might be significant if an increased number of dogs were included.

In conclusion, obese dogs, even if asymptomatic, may present some degree of cardiac changes that should be diagnosed early in order to reduce possible cardiac complications secondary to obesity.

ABSTRACT #247

COMPARISON OF CANINE CARDIAC TROPONIN I CONCENTRATIONS AS DETERMINED BY 3 DIFFERENT ANALYZERS. DB Adin[1], MA Oyama[2], MM Sleeper[3], RJ Milner[1]. [1]University of Florida, Gainesville, FL, [2]University of Illinois, [3]University of Pennsylvania, Philadelphia, PA.

Recent interest in the use of cardiac biomarkers in veterinary medicine has led to the validation of several commercial analyzers for cardiac troponin I (cTnI) evaluation in dogs, however these analyzers have not been standardized. The objective of this study was to compare canine cTnI concentrations as determined by 3 different analyzers.

Reconstituted purified canine free cTnI was diluted with canine plasma to 8 concentrations (0.01, 0.1, 0.78, 1.56, 3.13, 6.25, 12.5 and 25 ng/mL), for analysis by 3 analyzers, the Biosite Triage Meter®, the Dade-Behring Stratus®, and the Beckman-Coulter Access AccuTnI®. Plasma samples from 23 dogs with cardiac disease were also analyzed for cTnI concentrations on all analyzers. Samples were shipped on dry ice and frozen at −70° C until analysis.

Analyzer results for cTnI dilutions were highly correlated with each other. Bland-Altman analysis showed moderately good agreement between the Biosite and Stratus for purified samples, however, the Access returned higher values, with greater differences at higher concentrations. Recovery was highest for the Access (334–1467%) and lowest for the Biosite (38–60%) (Stratus 52–233%). Analyzer variability was lowest for the Access (1.2–10.4%) and highest for the Stratus (4.8–33.6%) (Biosite 2.8–16.5%). cTnI concentrations in clinical patients ranged from <0.05–5.72 ng/ml (Biosite), 0.02–11.1 ng/ml (Access), and 0.02–9.73 ng/ml (Stratus). Correlations between analyzers were lower for clinical patients, although still significantly correlated. Bias and limits of agreement between the Access and other analyzers was smaller for clinical patients than purified samples. See table below for statistical values.

Purified dilutions	P value	r value	95% CI for r	Bias	Limits of agreement	Fold diff
Stratus vs Biosite	P < 0.0001	0.9734	0.8554 - 0.9953	-0.24	-2.3 - 1.9	0.8 - 2.2
Access vs Stratus	P < 0.0001	0.9966	0.9764 - 0.9995	9.80	-15.9 - 35.6	3.7 - 6.3
Access vs Biosite	P < 0.0001	0.9840	0.8919 - 0.9977	10.40	-16.2 - 36.9	2.9 - 8.9
Clinical patients						
Stratus vs Biosite	P < 0.0018	0.6157	0.2727 - 0.8199	-0.47	-3.0 - 2.1	0.01 - 8.3
Access vs Stratus	P < 0.0001	0.8923	0.7595 - 0.9537	0.11	-0.5 - 0.8	0.3 - 19.0
Access vs Biosite	P < 0.0019	0.6114	0.2663 - 0.8170	0.58	-2.2 - 3.4	0.03 - 15.2

Results from this study suggest that although canine cTnI values obtained from the Biosite, Stratus, and Access analyzers are correlated, they cannot be directly compared to each other. Differences in results between purified free cTnI samples and clinical samples may be due to circulating complexed cTnI detected by the analyzers. In the absence of a gold standard none of the analyzers can be considered more correct than the others.

ABSTRACT #248

PLASMA CARDIAC TROPONIN I IN NORMAL DOGS, TRAUMATIZED DOGS AND SEVERELY ANEMIC DOGS. A. Diquélou, G. Ragetly, A. Geffré, N. Bourgès-Abella, C. Trumel, JP Braun

Cardiac Troponin I (cTNI) is a highly useful marker of myocardial damage in humans and animals. It has been used in dogs to diagnose myocarditis in various diseases in dogs (cardiac diseases, gastric volvulus or babesiosis).

Objective: to compare plasma cTNI in normal dogs, traumatized dogs and dogs suffering severe anemia in order to detect occult myocardial damage.

Material and methods: Heparin plasma were obtained from 25 normal dogs, aged 2 to 14 years (mean ± SD 5.4 ± 2.6 years), 34 dogs suffering trauma, aged 4 months to 13 years (6.2 ± 3.9 years), and 15 dogs aged 1 to 15 years (7.9 ± 3.9 years) suffering severe anemia from hemorrhagic, immune-mediated or myeloproliferative diseases (Hb = 5.6 ± 1.41 g/dL [3–7.98]),. cTNI was assayed on an AIA 360 automate using Tosoh Biosciences cTNI immunoassay.

Results: cTNI was below the lower limit of the assay (<0.1 µg/L) in all normal dogs, which is consistent with the previous studies. In traumatized dogs, plasma cTNI was within the usual values (<0.1 µg/L) in 14 dogs and elevated in 20 dogs (0.1 to 10.08 µg/L, mean ± SD 1.15 ± 2.25). In anemic dogs, 5 had undetectable plasma cTNI; plasma cTNI was elevated in 9 dogs (1.23 ± 1.11 µg/L, range [0.02–3.15]) without significant correlation between Hb and cTNI.

Conclusion: Tosoh AIA 360 cTNI is a useful test to detect myocardial damage in traumatized dogs. Plasma cTNI may also be interesting in determining cardiac hypoxia during severe anemia and may be an help to decision in transfuse.

ABSTRACT #249

TROPONIN I ELEVATIONS IN DOGS WITH THIRD DEGREE ATRIOVENTRICULAR BLOCK. WM Church[1], MA Oyama[1], DD Sisson[2], BJ Bulmer[3], D Owens[4]. [1]Department of Clinical Sciences, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, [2]Department of Veterinary Clinical Sciences, Oregon State University, Corvallis, OR, [3]Department of Clinical Sciences, Kansas State University, Manhattan, KS, [4]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL.

Complete atrioventricular (AV) block is a cardiac rhythm diagnosis that results in significant morbidity and typically requires artificial pacing to relieve the adverse effects of severe bradycardia. The underlying cause of complete AV block is unknown but may involve fibrosis, inflammation, or ischemia of the AV nodal tissue. Cardiac troponin-I (cTnI) is a sensitive and specific marker of myocardial ischemia and cellular injury and is released in proportion to severity of injury. By measuring cTnI in the serum of patients with complete AV block, we hoped to gain insight into the pathogenesis and severity of myocardial injury that accompanies this disease.

We obtained serum cTnI levels in 10 dogs with symptomatic complete AV block. This group of 10 dogs, with ages ranging from 70–140 months, had presented with ventricular escape rhythms between 30 and 60 bpm. Congestive heart failure was present in 3/10 patients. Compared to the normal reference range in our laboratory (<0.11 ng/ml), cTnI concentrations in dogs with complete AV block were markedly elevated (mean cTnI = 1.75 ng/ml, range = 0.21–8.13 ng/ml). These values were generally higher than cTnI concentrations in previously reported populations of dogs with dilated cardiomyopathy, mitral valve disease, or congenital SAS, and as such, suggest relatively extensive amounts of contemporaneous myocardial injury in dogs with this conduction abnormality. This degree of cTnI elevation could be associated with an underlying ischemic etiology of complete AV block or a result of poor myocardial perfusion secondary to bradycardia.

ABSTRACT #250

HEMODYNAMIC EVALUATION AND NT-ANP LEVELS IN HEALTHY DOGS SUBMITTED TO POSTURAL CHANGES. AS Carvalho Filho[1]; DS Schwartz[1]; SL Beier[1], PPVP Diniz[1]; KP Aptekmann[1]; P Solter[2]. [1] UNESP-FMVZ-Botucatu (São Paulo State University), SP, Brazil; [2]University of Illinois, College of Veterinary Medicine, Urbana-Champaign, IL.

Postural change is applied in human medicine for evaluation of patients with syncope, diabetes and heart failure (HF). It activates baroreceptors and causes fluctuations of heart rate (HR) and vasoconstriction, to maintain arterial pressure (AP) and tissue perfusion. N-terminal atrial natriuretic peptide (Nt-ANP) is a neuropeptide released in the presence of cardiac overload/atrial distention. It has been used as an auxiliary laboratorial tool for differential diagnosis and prognostic evaluation of HF in dogs and humans, and it has been shown that ANP levels change depending on the posture. The purposes of this study were to establish AP, central venous pressure (CVP) changes and Nt-ANP levels related to HR fluctuations in healthy dogs submitted to postural changes. Six healthy adult male dogs (20–35 kg) were used. Each dog was submitted to an active head-up (HU) position (70°) for 20 s, and head down (HD) position (50°) for 15 s, starting and returning to a normal standing (quadrupedal) position, repeated 3 times and in a random order. Electrocardiogram (ECG), CVP and AP were continuously measured. The beginning and end of postural change were marked. HR response, AP and CVP were analyzed in relation to time. Blood was collected from the catheter placed within the right atrium for each posture in 2 out of 3 sets for Nt-ANP levels that were measured by Enzyme-Linked Immunosorbant Assay (ELISA). Data was analyzed by ANOVA/Bonferoni test. We found similar HR response pattern as previous work, with significant increase in mean HR during active HU tilt ($p = 0.025$), which decreased to baseline values after returning to horizontal position. The mean HR at HU was 35.9 ± 16.7% higher than baseline values and there was not a significant change in HR for active HD tilt. For the HU, the first peak occurred at 3.89 ± 0.69 s and the first valley at 4.56 ± 0.49 s. The second peak occurred at 7.62 ± 1.1 s and the second valley occurred at 8.63 ± 1.25 s. The fluctuations on AP and CVP were not statistically significant. Although not significant, there was a slight decrease in AP in the first seconds of postural change, which preceded HR increase, and that was enough to elicit a baroreflex. The lack of statistical difference for this decrease in AP may be due to the reduced number of dogs included in the study and large variability in AP values. There was no significant change in Nt-ANP levels for different postures (p = 0,97-HU and p = 0,68-HD) . The absence of mean significant changes in AP during the postural change in these dogs should be expected, since a normal baroreflex should avoid large AP variation at the expense of HR fluctuation and vasoconstriction. This study reproduced the findings in HR response of previous work and showed that Nt-ANP levels do not change in normal dogs submitted to postural change.

ABSTRACT #251

NEUROHORMONE CHANGES IN IRISH WOLFHOUNDS WITH ATRIAL FIBRILLATION. HW Green III, DF Hogan, RA Sanders. Purdue University School of Veterinary Medicine, West Lafayette, IN.

Atrial fibrillation (AF) is a commonly recognized supraventricular tachyarrhythmia in Irish Wolfhounds with and without underlying cardiac disease. Even in the absence of cardiac disease (primary AF), cardiac performance is altered through the loss of atrial kick and irregular ventricular filling. Changes in cardiovascular neurohormones in response to primary AF and its consequences have not been reported in the dog. The goal of this study was to characterize the neurohormone changes with primary AF in Irish Wolfhounds.

Thirty-two Irish Wolfhounds were included in this study; 15/32 (47%) had primary AF and 17/32 (53%) were in sinus rhythm (SR). All cardiovascular medications were discontinued for at least two weeks prior to entry into the study. The neurohormones that were measured included plasma renin activity (PRA), brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), epinephrine (EPI), norepinephrine (NE) and aldosterone (ALD). Normal cardiac anatomy and function was confirmed with echocardiography based on accepted standard parameters for this breed.

Data is presented as mean ± SEM. Of the neurohormones; only ANP was significantly greater ($P < 0.001$) in the primary AF group (1.22 ± 0.12 pg/ml vs 0.46 ± 0.12 pg/ml). There was a statistically insignificant ($P = 0.06$) trend for BNP to be greater in the primary AF group (4.22 ± 1.22 pg/ml vs 1.59 ± 1.10 pg/ml). There was a significant increase ($P = 0.05$) in PRA in females (0.83 ± 0.10 ng/ml/hr vs 0.44 ± 0.12 ng/ml/hr) when both groups were combined, however there was no difference between groups when both sexes were included. The only significantly different echocardiographic parameter between groups was an increased end-systolic left atrial diameter (LADs) in the primary AF group (4.56 ± 0.21 cm vs 3.47 ± 0.21 cm, $P = 0.001$). When dogs in both groups were combined, there was a significant positive correlation between LADs and ANP ($r = +0.60$, $p = 0.001$).

Irish wolfhounds with primary AF have significantly larger left atrial diameters and significantly higher plasma ANP levels than Irish Wolfhounds in SR. These findings are not surprising given that atrial stretch is considered the primary stimulus for the release of the ANP prohormone and suggest that these dogs have increased left atrial pressures. The insignificant trend of increased BNP levels in the primary AF group may be related to combined production from the atria and ventricles or suggest occult ventricular dysfunction. The relatively small sample size may have limited the ability to identify a significant difference. We conclude that primary AF in Irish Wolfhounds is associated with markers of increased left atrial pressure and possibly ventricular dysfunction. Further studies are necessary to determine the chronic effect of primary AF on ventricular function.

ABSTRACT #252

EFFECT OF BODY POSITION ON CARDIAC NEUROHORMONE LEVELS IN NORMAL IRISH WOLFHOUNDS. HW Green III, DF Hogan, RA Sanders. Purdue University School of Veterinary Medicine, West Lafayette, IN.

Several neurohormonal systems are activated in response to cardiac dysfunction and to drugs or events that significantly decrease plasma volume. In humans, the levels of these parameters vary significantly in response to erect or supine positioning at the time of sample collection. In veterinary medicine, it is generally assumed that the small and non-orthostatic stature of our patients does not allow for similar variations in neurohormone levels. However, there have been no studies that have assessed this assumption. Our goal was to assess the effect of changing body position at the time of sample collection on neurohormonal parameters in normal Irish Wolfhounds.

Sixteen normal Irish Wolfhounds (8 males and 8 females) were recruited for this study. Upon admission all dogs were allowed a one-hour adaptation period in a quiet room prior to sample collection. Dogs were then maintained in lateral (L) recumbency for 10 minutes prior to jugular or lateral saphenous venipuncture. Dogs were then allowed to stand and maintained in an upright (U) position for 10–15 minutes prior to repeat sample collection. Plasma renin activity (PRA), plasma brain natriuretic peptide (BNP), plasma atrial natriuretic peptide (ANP), plasma epinephrine (EPI), plasma norepinephrine (NE) and serum aldosterone (ALD) levels

were measured. The height from the floor to the point of the shoulder was measured when the dogs were in the U position. Physical exam, electrocardiography and echocardiography confirmed normal cardiac function.

The mean age was 56.2 ± 31.2 mos (range 9–107 mos) and the mean height to point of the shoulder was 58.0 ± 3.4 cm (51.0–66.7 cm). There were no significant differences in any of the neurohormone parameters although there was an insignificant trend for higher EPI in the U position (66.72 ± 22.2 pg/ml vs 41.1 ± 7.19 pg/ml, P = 0.07).

The data supports the historical assumption that there is no difference in neurohormone levels resulting from different body positions at the time of sampling in normal Irish Wolfhounds. Larger sample sizes and larger breed variation would be needed to expand this conclusion to all breeds. The insignificant trend for higher EPI in the U position may be related to increased anxiety of being maintained in a standing position for 10–15 minutes. A larger sampling may help to further assess this tendency.

ABSTRACT #253

NITRIC OXIDE EXPRESSION IN MITRAL VALVES. SG Moesgaard[1], LH Olsen[1], B Aasted[2], LG Pedersen[1], HD Pedersen[3], AP Harrison[1]. [1]Department of Basic Animal and Veterinary Sciences and [2]Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Frederiksberg. [3]Safety Pharmacology, Novo Nordisk A/S, Maaloev, Denmark.

Despite considerable interest in nitric oxide (NO) and the fact that myxomatous mitral valve disease (MMVD) occurs in many species, there is little known about the role of local NO production in the heart valve. The release of NO from vascular endothelium increases in connection with shear stress, and it appears that there might also be a local "protective" release of NO in mitral valves. In order to help elucidate this field, we have measured the physiological presence and direct release of NO from the porcine mitral valve using a NO microelectrode. Furthermore the expression of endothelial nitric oxide synthase (eNOS) in the mitral valve was studied by the western blot technique and polymerase chain reaction (PCR) with findings being correlated with mild changes in leaflet structure.

Mitral valves were collected from slaughter pigs and sows in a local abattoir. Tissue samples were kept in formalin for histological evaluation and both tissue samples and samples of mitral valve endothelium were stored under liquid nitrogen for western blotting and PCR. Fresh mitral valves reached the laboratory within 2 hrs suspended in a previously oxygenated normal Krebs Ringer (NKR) buffer kept on ice. A calibrated NO microelectrode, capable of detecting nM levels of NO, was used to measure local NO release (within 4 hrs in oxygenated NKR buffer, 37°C). NO release was recorded following addition of bradykinin and the NO synthase inhibitor, N-nitro-L-arginine methyl esther (L-NAME). Relative quantification of eNOS mRNA levels was achieved by real-time PCR using SYBR Green I detection and a LightCycler System. 18S rRNA was chosen as the reference gene.

Addition of bradykinin increased the NO release from the mitral valve (33 ± 5 nM NO, mean ± SEM, $P < 0.001$, N = 10), whereas addition of L-NAME immediately decreased NO release *cf*. basal level (83 ± 7 nM NO, $P < 0.005$, N = 4). Both protein and mRNA expression of eNOS in tissue samples of mitral valves and especially in isolated endothelial cells from mitral valves suggests that NO is mainly released from the mitral valve endothelium. The expression of eNOS was greater in mitral valves from sows compared to slaughter pigs both at the protein (955 ± 29 vs 652 ± 170 densitometric units in mitral valves from slaughter pigs, N = 4, P = 0.05) and mRNA level (3.39 ± 1.03 vs 2.03 ± 0.25 in slaughter pigs, N = 10, P = 0.02). Mitral valves from sows appeared macroscopically thickened and had significantly increased degree of mucopolysaccharide deposition upon histological examination (P = 0.002) compared with slaughter pigs.

It is concluded that; 1) NO is released from normal porcine mitral valves and that eNOS is expressed at both the protein and mRNA level, and 2) the expression of eNOS is higher in mitral valves from sows with increased degree of mucopolysaccharide deposition compared with slaughter pigs, which might suggest that NO plays a role in the pathogenesis of MMVD.

ABSTRACT #254

TOLL-LIKE RECEPTOR-2 (TLR2) AND TLR4 EXPRESSION IN THE CANINE HEART. Annika Linde[1], N. Sydney Moise[2], Frank Blecha[3] & Tonatiuh Melgarejo[1]. Departments of Human Nutrition[1], and Anatomy & Physiology[3], Kansas State University, Manhattan, KS and Department of Clinical Sciences[2], Cornell University, Ithaca, NY.

Toll-like receptors (TLRs) are archetypal pattern recognition receptors of immediate importance for an efficacious innate immune response. TLRs exhibit marked differential tissue activity and their levels within a discrete cell type can be highly dynamic. Interestingly, TLRs also trigger activation of adaptive immunity, making this receptor family an important link between the two branches of the immune system. Of thirteen known mammalian paralogues, three TLRs (TLR2, TLR4, and TLR9) have been identified in the dog. Cardiac TLR expression has been reported in other species, but this study is the first to present evidence that these innate immune receptors are expressed in the canine heart.

Heart tissue samples from left and right atria and ventricles were collected from healthy dogs immediately after euthanasia and stored at −80°C until analysis. Total RNA was extracted with TRI Regent (Sigma-Aldrich). Specific primers were designed for amplification of canine TLR2 and TLR4 based on the previously reported sequences for these genes (GenBank Accession No. NM 001005264 and NM 001002950 respectively): TLR2 forward and reverse primer sequences were 5′- ATGATTCC-TACTGGGTGGAGAAC-3′ and 5′-CGCAGCTTACAGAATCGCTG-3′, respectively. TLR4 forward and reverse primer sequences were 5′-CCTGGAAGGACTGTGCAATT-3′ and 5′-TGCTTCAGTCTGGTTGT-CCC-3′, respectively. Using the designed primers and total RNA (from each heart tissue section) as template, reverse transcription was performed with M-MLV reverse transcriptase (Invitrogen). PCR amplification was then performed and PCR products analyzed by agarose gel electrophoresis. To confirm the specificity of the amplification products, the corresponding single bands were excised from the gel and the cDNA isolated and cloned using the TA Cloning® Kit (Invitrogen).

TLR4 expression was detected in the left ventricle and right atrium, while TLR2 was detectable at low levels in the right atrium only. The identity of the RT-PCR products was confirmed by nucleotide sequencing analysis.

Our findings show that at least two TLR paralogues – namely TLR2 and TLR4 – are expressed in the canine heart. These receptors may play a central role in the intrinsic immune defense of the heart against various incoming noxae. Additional studies are warranted to determine the potential implication of these essential immune receptors in the development of naturally occurring heart disease in the dog.

ABSTRACT #255

ATRIAL SEPTAL DEFECTS IN DOGS AND CATS: A RETROSPECTIVE STUDY OF 156 CASES (2001-2005). V. Chetboul[1,2], V. Gouni[1], V. Charles[1], A. Nicolle[1], C. Carlos Sampedrano[1], R. Tissier[1,2,3], J.-L. Pouchelon[1,2]. [1]Cardiology Unit of Alfort, [2]INSERM U660, [3]Pharmacy-Toxicology Unit of Alfort, National Veterinary School of Alfort, Maisons-Alfort, France.

Atrial septal defect (ASD) is characterized by communication between the two atria owing to a hole in the interatrial septum. ASD is one of the most common congenital heart diseases (CHD) in humans, but is considered relatively rare in veterinary medicine. We hypothesized that the incidence of ASD has been underestimated until now, and that canine and feline ASD can be detected better and earlier today than just a few years ago, thanks to the increasing availability of accurate diagnostic imaging systems and standardized, validated procedures.

The objective of this study was therefore 1) to retrospectively assess the incidence of ASD diagnosed in dogs and cats over the last five years at the Cardiology Unit of Alfort using echocardiography combined with color Doppler mode; and 2) to determine epidemiological, clinical and echo-Doppler features of the disease.

The case records of dogs and cats diagnosed with CHD at the Cardiology Unit of Alfort between 2001 and 2005 were reviewed retrospectively. All animals presenting an ASD confirmed by a trained observer were included in the study. Animals for which color-flow Doppler mode was not used to confirm ASD were excluded, as were those with equivocal color Doppler results. A reference population (entire hospital admission over a 5-year period: 30744 dogs and 21015 cats) was used to study species, breed and sex predispositions to ASD with an odds ratio (OR) analysis.

ASD was diagnosed in 156 animals and represented 37.7% of all canine and feline CHD (n = 414). In 111 of the 156 animals (71.2%), ASD was diagnosed incidentally while performing echocardiographic examination for a symptomatic concurrent congenital or acquired cardiovascular disease. ASD was the most common CHD after mitral dysplasia in both species. The proportion of dogs was significantly higher than that of cats compared to the total hospital admission (OR = 1.8, $p < 0.05$). Boxer and Domestic shorthair were the most common canine and feline breeds affected (OR = 15.3 and 26.3, respectively; $p < 0.05$). No significant sex predisposition was observed. Most defects (98.7%) were secundum-type ASD, without clinical signs in 73.7% of cases. The most common signs included grade 1–3 systolic murmur heard over the left heart base (20.2%), exercise intolerance (7.0%), syncope (5.3%), dyspnea (2.6%) and cough (2.6%). Animals that presented a systolic heart murmur over the left base had a significantly larger ASD

than others ($p < 0.05$), however no correlation was found between ASD size and murmur grade ($r = 0.378$).

In conclusion, the incidence of canine and feline ASD is much higher than previously supposed. ASD should be suspected in instances of soft left basal systolic heart murmur, exercise intolerance, respiratory signs or syncope, even if cardiac auscultation reveals no abnormality.

ABSTRACT #256

EFFECTS OF INHALED FLUTICASONE PROPIONATE ON ENDOCRINOLOGIC, IMMUNOLOGIC AND CLINICAL VARIABLES IN HEALTHY DOGS. AE DeClue, LA Cohn, CR Reinero; University of Missouri, College of Veterinary Medicine, Columbia, MO.

Inhaled glucocorticoids are advocated for treatment of inflammatory airway disease to maximize local effect while minimizing systemic consequences. This study was designed to evaluate systemic endocrine, immune and clinical effects of an inhaled glucocorticoid (IGC) compared to oral glucocorticoid (OGC) and placebo. We hypothesized that IGC would not suppress the hypothalamic-pituitary-adrenal axis, modify immune function or induce clinical signs compared with systemic glucocorticoid administration.

Seven healthy adult dogs were randomized to one of three treatment groups in a crossover design: fluticasone propionate (one 220 µg actuation of a metered dose inhaler delivered via spacer and face mask, q 12 h), placebo (spacer/mask alone, q 12h), or oral prednisone (1 mg/kg, q24h). Each of the three treatments was administered for three weeks followed by a 4 week washout period. Endocrine and immunologic tests were evaluated at baseline, day 28 of the washout period and day 21 of the treatment period. Clinical effects were assessed on days 14–21 of each treatment period. Endocrine testing included measurement of serum cortisol concentrations before and after ACTH stimulation. Immunologic alterations were assessed using white blood cell count and flow cytometric evaluation of lymphocyte phenotype (CD3, CD4, CD8, CD21). Estimated water intake, urine specific gravity and a scoring system for appetite and attitude were used to monitor clinical effects. Statistical analyses were performed using univariate repeated measures ANOVA and post hoc Tukey's test, with a p-value <0.05 considered significant. Estimated water intake and clinical scoring were expressed with descriptive statistics.

Serum cortisol concentrations were significantly different pre-ACTH stimulation for OGC versus placebo (OGC, 0.34 ± 0.3 ug/dl; placebo, 1.61 ± 0.96 ug/dl; $p = 0.016$) and post-ACTH stimulation between all treatment groups (placebo, 12.16 ± 0.98 ug/dl; IGC, 8.37 ± 2.3 ug/dl; OGC, 2.8 ± 2.25 ug/dl). Specifically, post-ACTH serum cortisol concentrations were significantly lower for OGC versus IGC ($p < 0.001$) or placebo ($p < 0.001$), and IGC versus placebo ($p = 0.004$). No significant differences were observed across treatments for any immunologic variable. The OGC group had an increased estimated water intake (87 ± 24 ml/kg/d) compared to IGC (53 ± 12 ml/kg/d) and placebo (48 ± 11 ml/kg/d). Mean urine specific gravity was significantly lower in the OGC group compared to placebo (placebo, 1.053 ± 0.10; OGC, 1.035 ± 0.04, $p = 0.037$). Increased appetite (4/7) and altered attitude (2/7) were noted in the OGC group; no clinical signs were noted in placebo or IGC groups. This study indicates that in healthy dogs inhaled fluticasone suppresses the hypothalamic-pituitary-adrenal axis to a lesser extent than OGC; and that IGC may avert systemic clinical signs associated with OGC administration.

ABSTRACT #257

REFERENCE VALUES FOR CSF TOTAL PROTEIN, ALBUMIN QUOTIENT AND IGG INDEX IN CATS. T.Steinberg, A. Fischer, I.C. Boettcher; Clinic for Small Animal Internal Medicine, Ludwig-Maximilians-University, Munich, Germany.

The evaluation of total protein, albumin quotient and IgG index in the cerebrospinal fluid (CSF) is part of the routine diagnostic workup in human neurology. The albumin quotient is used to asses the integrity of the blood brain barrier (BBB); the IgG index is an indicator for intrathecal IgG synthesis. The purpose of this study was to establish reference values for these parameters in the CSF to evaluate the BBB in cats.

Serum and CSF were taken from 37 neurologically healthy cats before they were euthanized for other clinical reasons. Cats were defined as being neurologically healthy when the neurological examination was normal, no central nervous system (CNS) pathologies were noted during either gross or histological examination of the brain and spinal cord and the CSF had a normal leukocyte count. In addition to conventional CSF analysis (Pándy reaction, leukocyte and erythrocyte numbers and cytospin cell differentiation) the concentrations of total protein, albumin and IgG were measured in CSF and serum with a Behring Nephelometer 100. Trichloracetic acid, cat albumin antiserum and cat IgG antiserum were used as precipitation agents. The analytical method is based on the measurement of the intensity of scattered light emanating from an illuminated volume. The ratio of scattered light intensity to the illuminating intensity was compared with a standard of known properties (human CSF protein standard, standard albumin cat and standard IgG cat).

The albumin quotient is calculated as the ratio albumin CSF: albumin serum. To calculate the IgG Index, the ratio CSF IgG : serum IgG is divided by the albumin quotient. In healthy cats the reference ranges for CSF total protein were 0.06–0.36 g/l (0.15 ± 0.02), for the albumin quotient 0.0006–0.0057 (0.0024 ± 0.0012) and for the IgG index 0.3–0.6 (0.43 ± 0.16). Statistical analysis revealed no significant correlation between the concentration of CSF total protein, albumin quotient or IgG index and breed, age or sex.

In the recent literature there is only little information about the evaluation of the BBB in animals, especially in cats. Regarding the difficulties to diagnose CNS diseases ante mortem, the differentiated protein analysis in the CSF of the cat could be a useful add-on tool to identify CNS pathology.

ABSTRACT #258

NEW INSIGHTS INTO EFFICACY AND SIDE EFFECTS OF POTASSIUM BROMIDE IN EPILEPTIC CATS. Volk HA, Chandler KE, Cappello R and Cherubini GB. The Royal Veterinary College, North Mymms, Hatfield, Herts,. UK.

The standard antiepileptic drugs phenobarbital and diazepam are effective in cats, though they are associated with potential life threatening side effects. Previous reports of bromide use in cats have shown induction of lower airway disease, but its efficacy remains debatable.

The aim of this study was to investigate the efficacy of bromide in feline epilepsy.

Nine clinically normal seizuring cats with unremarkable bloodwork, no abnormalities on magnetic resonance imaging and cerebrospinal fluid were diagnosed with epilepsy and recruited.

Two cats changed to bromide due to unacceptable side effects on phenobarbital. Mean ± SEM values for selected variables included age, 4.1 ± 1.14 years; bromide dose 32.85 ± 3.61 mg/kg/d; bromide serum level, 1.15 ± 0.21 mg/ml and follow up period, 13.63 ± 3.73 months. The mean monthly seizure frequency was 3.93 ± 1.37 pre-treatment, but was reduced significantly post-treatment to 0.52 ± 0.44 ($p < 0.05$; paired t-test). Five cats had no seizures during the trial. One cat maintained on phenobarbital and bromide did not respond.

Six cats developed a cough after 8.2 ± 2.1 months. Interestingly, one cat developed dermatitis similar to human bromoderma. However, this was not steroid responsive and continued after bromide withdrawal. The cat did not respond to phenobarbital and was euthanized. Bromide was discontinued in three other cats and the coughing resolved. Two owners elected to continue with bromide despite the coughing.

In our study bromide is highly effective in feline epilepsy and despite the presence of side effects should be considered as a second line therapy.

ABSTRACT #259

MAGNETIC RESONANCE IMAGING OF FELINE GM_1 – GANGLIOSIDOSIS. LM Tieber, TW Axlund, ST Simpson, HJ Baker, NR Cox, J HudsonJA Hudson, ST Simpson, NR Cox, HJ Baker. Auburn University College of Veterinary Medicine, Auburn, AL.

GM_1 – gangliosidosis is an autosomal ly recessive lysosomal storage disease characterized by a defect in the lysosomal enzyme β-galactosidase. This defect causes accumulation causes accumulation of ganglioside within many cell types including neurons resulting in neurodegeneration and death. This mutation GM_1 – gangliosidosis has been described in Siamese, Korats, and domestic short-hair cats. Affected cats appear normal at birth, but by 2–3 months of age tremors of the head and limbs develop followed by severe neurodegeneration and death by 6–8 months of age. Definitive diagnosis is based on enzymatic activity assay in blood leukocytes and genetic testing. Magnetic resonance imaging (MRI) may be a useful screening test to determine whether to perform more specific investigations like these enzyme assays, and, it may be as well as a potentially valuable noninvasive tool to evaluate disease progression and response to therapy. The goal of this study was to characterize brain MRI changes in cats with GM_1 – gangliosidosis. Magnetic resonance imaging was performed at 16–18 weeks and 28–32 weeks of age in both affected ($n = 6$) and normal ($n = 6$) cats. In

affected cats, gyri appeared widened and sulci depth was decreased compared to normal cats. On T2-weighted and proton-density images normal cats showed hypointense cerebrocortical and cerebellar white matter compared to the more hyperintense grey matter. In contrast, affected cats white matter remained isointense or hyperintense compared to grey matter. These findings support previous reports showing diminished myelination characterized by white matter remaining hyperintense white matter on T2-weighted images. These data suggest that MRI may be a useful tool for the diagnosis and assessment of disease progression in feline GM_1 – gangliosidosis.

ABSTRACT #260

A MISSENSE POINT MUTATION IN N-ACETYLGLUCOSAMINE-1-PHOSPOTRANS-FERASE CAUSES MUCOLIPIDOSIS II IN DOMESTIC SHORTHAIR CATS. Urs Giger, Eva Tcherneva, Jessica Caverly, Adam Seng, Angela M. Huff, Karyn Cullen, Marisa Van Hoeven, Hamutal Mazrier, Mark E. Haskins. Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA.

Mucolipidosis II (ML II), also called I-Cell disease in humans, is a unique lysosomal storage disease caused by deficient activity of the enzyme N-acetylglucosamine-1-phosphotransferase (GNTPA), which leads to a failure to internalize acid hydrolases in lysosomes. We have been studying a colony of domestic shorthair cats with naturally-occurring ML II (Mazrier et al. J. Heredity 94: 353–373, 2003). Clinical manifestations in affected kittens are observed from birth and include failure to thrive, behavioral dullness, facial dysmorphia, retinal changes, decreased muscle tone, and ataxia. Radiographic lesions include metaphyseal flaring, radial bowing, joint laxity, and vertebral fusion. All clinical signs are progressive and euthanasia or death invariably occurs within the first few days to first year of life, often due to upper respiratory disease or cardiac failure. The activities of three lysosomal enzymes from affected kittens, compared to normal adult control cats, are high in serum, but low in cultured fibroblasts that contained inclusion bodies (I-Cells), reflecting the unique enzyme defect in ML II. We describe here the molecular defect responsible for ML II in these cats.

As sources of cDNA and genomic DNA we used blood, cultured fibroblasts, and tissues from affected, carrier, and normal cats. Our primer systems for 5′- and 3′-RACE and for sequencing were based on comparative analyses of published human MGC4170 (GNPTA) mRNA and shot gun sequences from the feline genome sequencing project. The coding sequence of feline GNPTA was 3657 bp long (1219 amino acids). There was close homology (>90%) between our feline and the published human GNPTA cDNA sequence. The cDNA sequences from affected and normal cats were practically identical except for a single missense point mutation C→T at bp 2655, which changes a glycine residue into a stop codon. This leads to premature termination of the coding sequence presumably resulting in a truncated non-functional protein in affected cats.

A genomic DNA screening test was developed by performing PCR amplification of DNA surrounding the mutation and subsequent restriction enzyme digestion: PST1 only cut the 210 bp DNA product from normal cats into a 92 and 118 bp fragment, while carriers with a normal and mutant allele have all three DNA fragments. Screening the cats in the ML II colony showed that the missense mutation segregated completely with the disease. Feline ML II is currently the only species beside humans in which to study the pathogenesis of and therapeutic interventions for this unique lysosomal storage disease. Supported in part by the National MPS Society and NIH RR02512.

ABSTRACT #261

A FRAME SHIFT MUTATION IN *TPP1* IN A JUVENILE DACHSHUND WITH NEURONAL CEROID LIPOFUSCINOSIS. T Awano[1], ML Katz[2], GS Johnson[1], S Khan[1], GC Johnson[1], U Giger[3], and DP O'Brien[1]. [1]University of Missouri, College of Veterinary Medicine, Columbia MO. [2]University of Missouri, School of Medicine, Columbia MO. [3]University of Pennsylvania, School of Veterinary Medicine, Philadelphia PA.

The neuronal ceroid lipofuscinoses (NCLs) are a group of hereditary neurodegenerative disorders characterized by autofluorescent storage granules. Brain, spinal cord, and DNA samples were collected at necropsy from one of two, 12 month old, Dachshund littermates that both died after a 3 month history of intermittent vomiting and progressive ataxia, hypermetria, dementia, and myoclonus.

Sections were examined by light, fluorescent, and electron microscopy. The cerebellum was atrophied with extensive loss and atypical positioning of Purkinje cells. Cytoplasmic granules in neurons stained intensely with PAS and LFB and autofluoresced. EM showed curvilinear bodies characteristic of late-infantile NCL (CLN2), the second most common NCL of humans. We sequenced all 13 exons of *TPP1*, the canine ortholog of *CLN2*. The propositus' sequences from exon 4 contained a single base pair deletion, c.del325delC. This deletion alters the reading frame of the predicted messenger RNA after codon 107 and creates a premature stop codon at codon 114 truncating the enzyme prior to the active site.

Using a pyrosequencing assay, DNA samples from 37 unrelated Dachshunds and 109 other dogs representing 77 other breeds were genotyped. The c.del325delC allele was absent from all dogs other than the propositus, who was homozygous for it.

Previous reports of NCL in Dachshunds differed in age of onset and pathological characteristics. The prevalence of the mutant CLN2 allele in the breed needs to be determined, but the propositus' pedigree showed no common ancestors in four generations. Supported by NIH RR02512, Canine Health Foundation, and Research to Prevent Blindness, Inc.

ABSTRACT #262

MAGNETIC RESONANCE IMAGING (MRI) OF PRESUMED HEPATIC ENCEPHALOPATHY PERFORMED IN CONJUCTION WITH MR PORTOGRAPHY. S.P. Holmes, P.R. Gavin, R.L. Tucker, R.S. Bagley, C.L. West, A.V. Chen, and D.G. Hicks. Washington State University – Veterinary Medicine Teaching Hospital, Pullman, WA.

Definitive diagnosis of portosystemic vascular anomalies (PSVAs) is challenging. In this study, brain changes in dogs with suspected PSVAs were determined using MR evaluation of the intracranial structures.

Twelve dogs with clinical signs consistent with a PSVA based on signalment, clinical laboratory abnormalities, and pre- and post-prandial serum bile acid assays were included. MR imaging was performed on a 1.0T Philips Gyroscan NT imaging magnet. Standard brain MR sequences (T2-, Fluid Attenuation Inversion Recovery/FLAIR-, pre- and post- Gadolinium T1-weighted) were acquired. Concurrently, MR portography was performed with gadolinium-enhanced 2-D time-of-flight (TOF) sequences in the axial plane referenced to T2-weighted axial and sagittal abdominal images. Images were interpreted by a board certified radiologist and neurologist independent of knowledge of clinical evaluations.

MR portography distinguished macroscopic PSVAs (8 patients) from normal portal vasculature (4 patients) with 100% sensitivity. Since the all origins and insertions of the PSVAs were identified, MR portography was 100% specific. The smallest PSVA diagnosed was a partial portal-azygos communication measuring 3 mm in diameter. The information gained through MR portography aided in reducing intra-operative time in these metabolically compromised patients.

In 8 of these dogs, bilateral signal hyperintensity in the cerebral white matter of the coronal radiation and/or immediately adjacent gray matter was present on T2- and FLAIR- weighted sequences. No regional contrast enhancement was present in these areas. The extent and location of hyperintensity varied. Of the 8 dogs with altered signal intensity, five were clinically normal. One of these dogs was subsequently re-imaged following appropriate medical management for PSVA. Neurological examination prior to re-imaging was normal; however, persistence of hyperintensity was seen with reduction in the extent of cerebrum affected. All dogs with this pattern of hyperintensity had a single, extrahepatic PSVA.

Information obtained in these dogs indicates that discrete MR signal abnormalities are present in the brain of some dogs with PSVAs. The presence of these abnormal signal changes within the brain may be subclinical or may be seen in conjunction with neurological signs.

ABSTRACT #263

ALTERATION IN MAGNETIC RESONANCE IMAGING SIGNAL OF THE NUCLEUS PULPOSUS IN DOGS WITH ACUTE ONSET MYELOPATHY. AV Chen, RS Bagley, DG Hicks, PR Gavin, RL Tucker, SP Holmes. Washington State University, Pullman, WA.

Dogs may have acute onset myelopathy from a variety of causes. During evaluation of some dogs with acute onset myelopathy with similar clinical features to those associated with fibrocartilagenous emboli, abnormalities were identified in the magnetic resonance (MR) signal characteristics of the nucleus pulposus and in some instances, the adjacent spinal canal. Medical records and magnetic MR images of 20 such dogs were retrospectively reviewed.

All dogs presented for the evaluation of an acute onset, non-progressive (after 24 hours) paresis or paralysis. The onset of the neurologic signs was witnessed in 15 dogs following running (n = 7), jumping (3), walking (1), and vehicular accidents (4). Breeds represented included Labrador Retriever (8), Golden Retriever (2), Shetland Sheepdog (2), Border Collie (1), German Shepherd (1), Red Heeler (1), Whippet (1), Chihuahua (1), Pomeranian (1), and mixed breed dogs weighing between 30–38 kg (2). The age of the dogs ranged between 1 to 9 years. Neurologic examinations revealed symmetric (8) and asymmetric (12) conscious proprioceptive deficits. Deep pain was present in 19 dogs. Spinal pain was documented in 11 dogs.

Magnetic resonance characteristics included loss of hydration signal of the nucleus pulposus (20), collapse of the intervertebral disk space (20), intramedullary hyperintensity in the spinal cord on T2-weighted study (20), and disruption of the epidural fat with minimally to non-compressive disk material and/or hemorrhage (12). All dogs had only a single abnormal nucleus propulsus signal in the affected region. The areas involved were C5–6 (1), T11–12 (1), T12–13 (6), T13–L1 (6), L1–2 (5), and L2–3 (1). The intramedullary hyperintensity was both focal (16) and diffuse (greater than one vertebral segment) (4) over the affected disk. Asymmetric intramedullary hyperintensity was seen in 16 dogs.

One dog had a hemilaminectomy performed over the affected disk. Hemorrhage and fragmented annulus were confirmed in surgery. The other 19 dogs did not have surgery. Follow up was obtained in 15 dogs. Fourteen dogs neurologically improved and was ambulatory by 6 weeks. The dog with no deep pain had no neurologic improvement at 16 week follow up. The clinical significance of these MR abnormalities will be discussed.

ABSTRACT #264

POSITIONAL MAGNETIC RESONANCE IMAGING OF THE LUMBOSACRAL REGION IN DOGS WITH DEGENERATIVE LUMBOSACRAL STENOSIS. DH Hicks, RS Bagley, AV Chen, SP Holmes, RL Tucker, PR Gavin. Washington State University, Pullman, WA.

Twelve dogs with degenerative lumbosacral stenosis (DLSS) were identified with a presumptive diagnosis of DLSS based on medical history, physical examination, and neurological examination. Neurological examination abnormalities included lumbosacral pain (8), paraparesis (5), pelvic limb ataxia (2), and pelvic limb muscle atrophy (3). Two dogs had decreased anal tone or tail tone. Urinary incontinence was the presenting complaint in two dogs; one of which also had a history of fecal incontinence. Diagnostic workup included complete blood count, serum biochemistry panel, urinalysis, and magnetic resonance (MR) imaging of the lumbar and sacral spinal region. Seven dogs had lumbar cerebrospinal fluid (CSF) collection. Seven dogs had electromyography (EMG) performed. Three dogs had spinal radiographs taken.

MR imaging was performed on each dog with the lumbosacral (LS) joint in neutral position and with the LS joint in a flexed position. Four dogs were also imaged with the LS joint in the extended position. For neutral positioning dogs were placed in an MR scanner (1.0 T) on a spine-receiving coil in dorsal recumbency. The pelvic limbs were allowed to remain in a neutral position without restraints (frog-legged position). For flexed joint positioning, the pelvic limbs were secured cranially with the stifle and tarsal joints in extension, fully flexing both coxofemoral joints and causing ventral spinal curvature in the caudal lumbar spine. A circular surface coil (20 cm) was positioned over the LS region and secured. For extended joint positioning, the pelvic limbs were secured caudally, fully extending both coxofemoral joints and resulting in dorsal curvature in the caudal lumbar spine. To accentuate extension of the LS joint, a cylindrical pad was placed between the dog and the MR scanner bed to act as a fulcrum. A circular surface coil (20 cm) was positioned between the pad and the dog. Multiplanar T2-weighted images were obtained to anatomically assess the caudal spine.

All dogs had abnormal intervertebral disk (IVD) protrusion at the L7-S1 space identified on MR imaging in the neutral position resulting in ventral spinal compression of the terminal neural elements. Additionally, 2 dogs had compressive IVD disease at L6–L7. Intervertebral disk disease was characterized by loss of intervertebral disk hyperintensity on T2-weighted images, protrusion of the dorsal annulus fibrosis, and mild to marked reduction of the spinal canal diameter. Imaging with the LS joint in a flexed position subjectively improved terminal spinal element compression compared to neutral positioning in all dogs. Four dogs were imaged in an extended position and each of these dogs, subjectively, had exacerbation of terminal neural element compression compared to neutral positioning. These results suggest that LS neural element compression may be dynamic relative to anatomical position of this joint. Additionally, the position of the animal in the MR gantry during imaging can affect the observable extent and degree of compression in this region.

ABSTRACT #265

RETROSPECTIVE STUDY OF 22 CASES OF CEREBELLAR INFARCTION IN DOGS: NEUROLOGIC AND CLINICOPATHOLOGIC FINDINGS. EW Darrin, SA Steinberg, C Vite. University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Cerebellar infarcts represent a significant fraction of vascular brain disease in dogs; however, underlying causes are not commonly found. The purpose of this study is to describe the clinical course and findings in dogs that were presented to our hospital with cerebellar infarction or developed cerebellar infarction while hospitalized.

A search of medical and necropsy records produced 22 instances of cerebellar infarction. Cases were included if they exhibited acute neurologic signs consistent with cerebellar disease and had either histopathologic confirmation of infarction or magnetic resonance imaging findings consistent with infarction. Cases were excluded if primary central nervous system neoplastic, infectious, or inflammatory disease was diagnosed. Neurologic signs, concurrent systemic diseases, infarct location, clinical laboratory results, endocrine testing, blood pressure, infectious disease titers, cerebrospinal fluid analysis, and medications were recorded when available.

The most common neurologic signs were gait and postural abnormalities and vestibular signs; cases were most commonly characterized as having a paradoxical vestibular syndrome. The most common signs were as follows: 17 of 22 dogs exhibited postural deficits during lateral hopping, and 10 of these dogs had deficits that were worse on the side ipsilateral to the cerebellar lesion. 16 of 22 dogs had a head tilt contralateral to the lesion. 12 of 22 dogs were ataxic, and 7 were unable to walk at the time of first examination. 10 of 22 dogs exhibited resting or positional nystagmus, and 6 of these had horizontal or rotary nystagmus with the fast phase directed to the side of the lesion. The most common laboratory abnormalities found were elevated liver enzymes (18/22), lymphopenia (14/22), and neutrophilia (10/22). Hypertension was noted in 7 of 16 dogs in which blood pressure was measured at the onset of signs. Most dogs had not received medication that could explain their laboratory abnormalities. Adrenal and thyroid function testing were performed in 10 dogs, 3 of which were confirmed to have hyperadrenocorticism, and one of which had hypothyroidism. Cerebrospinal fluid was collected in 9 dogs, 7 of which exhibited a mild neutrophilic pleocytosis.

Of 12 dogs that died, 8 died in the hospital and were diagnosed with severe systemic disease that may have been related to the formation of the infarct. Four dogs recovered from their initial neurologic signs but later died of other causes. Of the dogs that were not diagnosed with concurrent disease, all showed significant recovery when follow-up was available.

Based on these findings, the authors conclude that cerebellar infarction should be considered as a major differential diagnosis for acute paradoxical vestibular signs in dogs. Prognosis for recovery is good if there is no concurrent systemic disease. Prospective study is warranted to determine the prevalence of endocrine disease in dogs with cerebellar infarction and to gain insight into the cause and pathogenesis of this lesion.

ABSTRACT #266

CLINICAL PRESENTATION AND OUTCOME IN DOGS WITH HISTOLOGICALLY CONFIRMED CHOROID PLEXUS PAPILLOMAS. K Thankey[1], D Faissler[1], A Kavirayani[1], JH Keating[1], JJ McDonnell[2]. Tufts Cummings School of Veterinary Medicine (TCSVM), North Grafton, MA. Dogs and Cats Veterinary Referral, Bowie, MD.

Choroid plexus papillomas in dogs are rare, slow growing tumors of neuroectodermal origin. The biological behavior is not well documented. The purpose of this retrospective study was to characterize the clinical presentation and to determine outcome in dogs undergoing treatment. Criteria for inclusion in the study were availability of complete information regarding signalment, history, examination, radiographs, magnet resonance imaging (MRI), computed tomography (CT), treatment protocols, survival time, and histopathological analysis of the neoplastic tissue.

Medical records of 10 dogs were identified with confirmed choroid plexus tumors evaluated at TCSVM between 1996 and 2004. The most commonly affected breeds were golden (2/10) and Labrador retrievers (2/10). Genders included 1 female, 4 spayed female, 2 male and 3 castrated male dogs. Median age at diagnosis was 77 months (range 43–111). Average body weight was 34 ± 8 kg. Signs were present for a median time of 8 days (range 2–96) prior to presentation. One dog had a 4 months history of seizures. Upon physical examination, cervical pain was a frequent finding (6/10). Three/10 dogs presented with signs compatible with pneumonia. Abnormal findings in the neurological examination included depression (7/10), stupor (1/10), head tilt (5/10), head tremor (2/10), severe tetraparesis or tetraplegia (4/10), vestibular ataxia (4/10), cranial nerve dysfunction (6/10), proprioceptive deficits (7/10) and reduced withdrawal reflexes in the front legs (3/10). The lesions were localized to the central vestibular system (5/10),

brain stem (2/10), forebrain (2/10) or to the lower motor neuron system (1/10).

Thoracic radiographs at initial evaluation showed megaesophagus (4/10) with concurrent aspiration pneumonia (2/10). Definitive diagnosis and localization of the brain mass was established with MRI (6/10), CT (2/10) and autopsy (2/10). The tumor was found in one of the lateral ventricles (6/10) or the third (2/10) or the fourth ventricle (2/10).

Two dogs were euthanized after the diagnosis of a brain mass. Six dogs received supportive care and two dogs had surgical debulking. Complications after craniectomy included bleeding into the brain parenchyma (1/2), megaesophagus (1/2), and aspiration pneumonia (2/2). Median survival time for the 8 treated dogs was 8 days (range 1–69). Histopathology of the abnormal tissue revealed either a benign choroid plexus papilloma (8/10) or a locally invasive choroid plexus carcinoma (2/10).

Based on this retrospective data dogs with choroid plexus papillomas or carcinomas have a very guarded prognosis. A complicating factor is the surprisingly high number of dogs with megaesopahagus (4/10) and aspiration pneumonia (2/10) at initial diagnosis. The underlying pathophysiologic mechanism for this condition remains poorly understood.

ABSTRACT #267

FORAMEN MAGNUM DECOMPRESSION WITH CRANIOPLASTY FOR TREATMENT OF CAUDAL OCCIPITAL MALFORMATION SYNDROME IN DOGS. CW Dewey[1], KS Bailey[1], DJ Marino[1], G Barone[1], P Bolognese[2], TH Milhorat[2], DJ Poppe[2]. [1]Long Island Veterinary Specialists, Plainview, NY; [2]The Chiari Institute, Great Neck, NY.

Foramen magnum decompression (FMD) is the preferred mode of therapy for Chiari type I malformation of people. Surgery is often successful, but 8–30% of patients require re-operation, usually due to excessive scar tissue formation at the FMD site. This scar tissue causes compression at the FMD site, re-creating the original disease state. A FMD procedure for canine caudal occipital malformation syndrome (COMS) has recently been described; the success rate of this procedure is approximately 81%. Similar to humans, about 25% of canine patients require re-operation due to excessive post-operative scar tissue formation. In our experience, clinical signs of worsening associated with scar tissue impingement typically occur within 3 mos of surgery. A cranioplasty procedure developed for Chiari type I patients at the Chiari Institute has essentially eliminated the problem of post-operative scar tissue compression at the FMD site. The purpose of this report is to describe a modification of that procedure for dogs with COMS. Six dogs with MRI confirmed COMS underwent FMD with cranioplasty. Five of the 6 dogs were Cavalier King Charles spaniels, 1 was a Pug dog. There were 2 male castrated dogs, and 4 female spayed dogs. In one dog, the surgery was a re-operation (excessive scar tissue following standard FMD). Mean age was 3.14 yrs (range, 14.5 mos-5 yrs, 9.5 mos). Following the FMD procedure previously described, guide holes for either 1.5 mm diameter (1.1 mm drill bit) or 2.0 mm (1.5 mm drill bit) titanium screws were made in the occipital bone, around the edge of the FMD defect. Self-tapping titanium screws were inserted into the holes for an approximate depth of 2–3 mm. The head was then released from the ventroflexed position. A skull plate was fashioned using titanium mesh and polymethylmethacrylate (PMMA), and fixed to the back of the skull, using the titanium screw heads as anchor posts for the PMMA. The caudal aspect of the plate extended slightly over the dorsal defect of C1; this tail aspect of the plate was curved dorsally, to avoid impinging on the medulla or cranial cervical spinal cord. Closure was routine. There were no intraoperative complications. Postoperative complications included a mild head tilt in one dog (re-operated case), and the need for narcotic pain medications for about 1 wk in another dog (persistent neck pain). All of the dogs experienced clinical improvement following the procedure, and none has required repeat surgery. Mean follow-up time was 2.3 mos (range, 1 to 5 mos). Pre-operative drugs included prednisone (4 dogs), gabapentin (3 dogs), tramadol (2 dogs) and carprofen (2 dogs). Post-operatively, 3 dogs continued to receive gabapentin, and 1 dog occasionally received carprofen. Cranioplasty with titanium screws and titanium/PMMA plates appears to be well tolerated in dogs with COMS. Although no dogs required re-operation in this small case series, additional cases with more follow-up information will be needed to ascertain to what level, if any, the cranioplasty procedure mitigates compressive scar tissue compression at the FMD site.

ABSTRACT #268

ULTRASOUND-GUIDED CEREBROSPINAL FLUID COLLECTION FROM THE CEREBELLOMEDULLARY CISTERN IN THE CANINE. William Lee, Margaret R. Kern, Erica Baravik, Andrew Shores, Armando Garma, H. Dan Cantwell, Carolyn Boyle. Mississippi State University, Starkville, MS.

Cisternal CSF collection and analysis is an important diagnostic tool for a variety of neurologic disorders. The objectives of this study were to describe a technique for ultrasound (U/S)-guided CSF collection in the canine and to compare this technique to the traditional (or blind) method of collection. Variables measured were the number of attempts required for successful retrieval of an adequate volume of CSF to perform a complete CSF analysis, time required from skin puncture to collection of the first drop of CSF, and the number of RBCs present in the CSF.

Twenty-four healthy adult dogs were anesthetized and CSF was collected with U/S guidance. All dogs were clipped and aseptically prepped. A 7.5 MHz microconvex linear probe was positioned longitudinally on the dorsal cervical midline at the level of the atlanto-occipital joint. This positioning provided a longitudinal image of the atlanto-occipital space including the occipital bone and the first cervical vertebra, dura mater, subarachnoid space, and spinal cord. CSF was obtained without U/S guidance from 17 dogs that presented to the Animal Health Center at Mississippi State for various neurologic conditions.

Collection methods were compared using the exact Wilcoxon-Mann-Whitney test. There was no significant difference ($p > 0.05$) between methods in number of attempts or time required. The median number of attempts was 1 for both techniques, with a maximum of 3 and 5 attempts needed for U/S-guided and blind techniques, respectively. The mean difference between methods was −0.63 attempts with a 95% confidence interval of (−1.25 attempts, 0.01 attempts). The median time required with U/S guidance was 26.5 seconds (sec) with a minimum of 5 sec and maximum of 260 sec. The median time required using the blind technique was 39 sec, with a minimum of 10 sec and a maximum of 370 sec. The mean difference between methods was −38.8 sec with a 95% confidence interval of (−95.0 sec, 17.4 sec). There was a significant difference in RBCs ($p = 0.023$), with a median number of 8 RBCs/uL using U/S and 1 RBC/uL with the blind method. Minimum and maximum RBCs were 8 and 830 RBCs/uL, and 1 and 266 RBCs/uL for U/S-guided and blind techniques, respectively. The mean difference in RBCs between methods was 73.59 RBCs/uL with a 95% confidence interval of (−74.8 RBCs/uL, 221.9 RBCs/uL).

This study described the anatomical structures seen via ultrasonography of the atlanto-occipital space and demonstrated visualization of spinal needle placement into the subarachnoid space. CSF was successfully collected with the U/S-guided technique. Although the sample size in this study was small, the results suggest that the U/S-guided technique may be a valid alternative to the traditional method of CSF collection.

ABSTRACT #269

USE OF INTRAOPERATIVE ULTRASONOGRAPHY IN THE ASSESSMENT AND MANAGEMENT OF CANINE SPINAL CORD LESIONS. B Nanai, R Lyman. Animal Emergency and Referral Center, Fort Pierce, FL.

Visualization of the spinal cord and the surrounding tissues is limited during spinal surgeries. It is desirable to obtain more information on the surgical site intraoperatively, in order to evaluate the extent of the lesion, to guide further diagnostic procedures (such as fine needle aspirations or biopsies) and to assess the immediate degree of decompression. The purpose of the study was to determine 1) whether diagnostic quality ultrasonographic images of the spinal cord and the surrounding tissue structures can be obtained intraoperatively, 2) how intraoperative ultrasonography benefits the clinician in the evaluation and management of various spinal cord lesions.

Thirteen dogs undergoing spinal surgery received intraoperative ultrasound examination. Their age was between 6 and 13 years and weight between 8 and 50 kg. Presenting neurological signs included spinal pain, ataxia, paraparesis, paraplegia, tetraparesis and root signature. The surgical procedures included continuous dorsal laminectomies and hemilaminectomies. Final neurological diagnoses were Hansen type II discs (six dogs), Hansen type I disc (one dog), caudal cervical vertebral instability and malformation (three dogs), spinal cord neoplasia (astrocytoma in one dog and anaplastic neoplasia in another dog), discospondylitis (one dog), and granulomatous meningoencephalitis (one dog). A Phillips® 5000 HDI ultrasound unit (Ultrasource®, Grand Rapids, Michigan) was used with a linear 12–5 MHz broad band transducer. The transducer and the cable were covered with a sterile transducer cover (Latex Free Transducer Cover #610–575, CIVCO Medical Industry, Kalona, Iowa). The probe was merged into the saline filled surgical site following laminectomy and held approximately 0.5–1.5 cm from the spinal cord. Longitudinal and transverse images were obtained.

In all 13 dogs (100%) it was possible to image the borders of the spinal cord, the central canal and the spinal parenchyma. Power Doppler imaging technique enabled visualization of the spinal cord microcirculation in all

patients. Measurements were obtained of the diameter of the spinal cord and the central canal. Ultrasound guided aspiration of the spinal cord was performed in one dog with a focal enlargement and hyperechoic parenchymal cervical spinal cord lesion. Cytologic evaluation of the sample revealed granulomatous inflammation. A spinal tumor (astrocytoma) was imaged in another dog. Extradural compressive disc material, articular facets, and degree of decompression were imaged in the other patients.

This study suggests that intraoperative spinal cord imaging by ultrasound is a useful and viable technique in the diagnosis and management of spinal cord lesions. Further studies are currently underway to establish whether microcirculation integrity is useful in the prognosis of compressive spinal cord lesions.

ABSTRACT #270

BRAIN STEM AUDITORY EVOKED RESPONSE (BAER) TESTING IN CAVALIER KING CHARLES SPANIELS WITH CAUDAL OCCIPITAL MALFORMATION SYNDROME. CW Dewey, KS Bailey, G Barone, J Stefanacci. Long Island Veterinary Specialists, Plainview, NY.

Caudal occipital malformation syndrome (COMS) is a common neurologic disorder in the Cavalier King Charles spaniel (CKCS) breed. The only reliable diagnostic test to confirm the diagnosis of COMS in dogs is magnetic resonance imaging (MRI). An MRI diagnosis of COMS is based on demonstrating the combination of rostral compression of the caudal cerebellum by the caudal occiput and attenuation/obliteration of the dorsal subarachnoid space (CSF signal) at the cervicomedullary junction. There is a need for widespread screening of CKCS litters for the presence or absence of COMS, in order to adopt breeding programs aimed at decreasing the incidence of the disorder in this breed. However, when screening litters of puppies for COMS, the cost of MR imaging is often prohibitive. The brain stem auditory evoked response (BAER) test is a clinical electrodiagnostic tool used to evaluate hearing ability as well as the functional integrity of the brain stem. In dogs and people with intact hearing ability, the BAER test has been shown to be a sensitive indicator of the presence or absence of brainstem dysfunction.

Brain stem auditory evoked response testing was performed on 10 clinically affected CKCS dogs with MRI-confirmed COMS and 2 CKCS dogs without MRI evidence or clinical signs indicative of COMS. Each dog was an individual patient (i.e., not littermates), and all had reached skeletal maturity (age range: 10–60 mos, mean = 34 mos). There were 4 FS, 6 MC, and 2 MI dogs. All dogs were evaluated using an alternating rarefaction-condensation stimulus at 80 dB, 100 dB or both, with a vertex to mastoid (VM) recording arrangement. A minimum of 500 measurements was averaged for each patient. Both I–III and III–V peak-to-peak latencies were determined for left and right side stimulation in each dog. A latency difference (I–III, III–V, or both) between left and right sides greater than 0.20 ms was considered abnormal. All dogs with COMS evident on MRI had abnormal BAER recordings. Three dogs had either I–III (1 dog) or III–V (1 dog) differences exceeding 0.20 ms; 7 dogs had differences for both I–III and III–V intervals. The mean latency difference for the I–III interval was 0.37 ms (range, 0.21–0.81 ms) and for III–V was 0.35 ms (range, 0.21–0.90 ms). Both dogs without MRI evidence of COMS had normal BAER examinations, with mean I–III and III–V interval differences of 0.08 ms and 0.11 ms, respectively.

Results of this pilot investigation suggest that BAER testing may be a useful screening tool for identifying adult CKCS dogs with COMS. The potential utility of BAER as a screening method needs to be evaluated in CKCS puppies with MRI-confirmed COMS, along with a reasonable number of anatomically normal CKCS puppies to serve as controls.

ABSTRACT #271

TRANSFRONTAL CRANIECTOMY, RADIATION THERAPY, AND/OR CHEMOTHERAPY IN THE TREATMENT OF CANINE MENINGIOMAS. A Bilderback[1], D Faissler[1], AF Sato[1], JH Keating[1], JJ McDonnell[2]. [1]Tufts Cummings School of Veterinary Medicine (TCSVM), North Grafton, MA. [2]Dogs and Cats Veterinary Referral, Bowie, MD.

Treatment of meningioma includes palliative therapy, such as corticosteroids and/or anticonvulsants, and primary therapy which is comprised of surgical excision and/or radiation therapy. Chemotherapy has not been shown to be efficacious. The purpose of this retrospective study was to investigate complications and survival times in dogs with olfactory and/or frontal lobe meningiomas treated with surgical debulking via modified bilateral transfrontal craniectomy alone or in combination with either radiation therapy (RT) or chemotherapy.

Medical records of 21 dogs with olfactory and/or frontal lobe meningiomas evaluated at TCSVM between 1999 and 2005 were identified. Information obtained includes signalment, history, neurologic evaluation, tumor location, imaging, treatment protocols, histopathology, postoperative complications, follow-up, death and necropsy. The control group (n = 7) was restricted to dogs with palliative therapy and diagnosis via necropsy. The treatment group (n = 14) was restricted to dogs who had magnetic resonance imaging (MRI) prior to surgery, diagnosis via biopsy, and surgical debulking via modified bilateral transfrontal craniectomy alone (n = 5) or in combination with either RT (n = 5), 16 treatments of 3 gray each, or chemotherapy, lomustine (n = 3) 80–100 mg/m^2 by mouth every 4 weeks or hydroxyurea (n = 1) 20–30 mg/kg by mouth once daily.

Seizures were the most common clinical sign (7/7 controls, 13/14 treated). Median age of onset was 10.9 years (range: 7.4–13.7 years). Transient (less than 1 month) postoperative complications included epistaxis (4/14), nasal discharge (3/14), and subcutaneous emphysema (3/14). Long term (longer than 1 month) complications included nasal discharge (2/14), fungal infection (2/14), and epistaxis (1/14). Life threatening complications included aspiration pneumonia (2/14), pneumocephalus (1/14), and cardiopulmonary arrest 3 days after surgery (1/14). Survival percentages and survival times for the control and treatment groups are listed in the table below.

	Sample Size	Percentage Alive at 6 months	Percentage Alive at 12 months	Percentage Alive at 18 months	Percentage Alive at 24 months	Mean Survival (months)	Median Survival (months)	Range Survival (months)
Control	7	28	14	14	14	6.5	3.8	0.5 – 25.1
Surgery	5	60	40	0	0	8.3	6.7	0.1 – 16.1
Surgery + Chemo	4	75	75	50	50	18.3	18.4	5.9 – 30.5
Surgery + RT	5	80	80	40	40	18.7	16.9	3.9 – 29.1

Based on this study, modified bilateral transfrontal craniectomy is a safe procedure with a low mortality rate (1/14). Survival times for the control, surgery alone, and surgery with RT groups are similar to that previously described in the literature (Axlund, McGlasson, et. al., 2002; Adamo, Forrest, et. al., 2004). However, this study suggests that surgery followed by chemotherapy may have survival data similar to that of surgery followed by RT. Further investigation of the use of chemotherapy in conjunction with surgery for the treatment of meningiomas in dogs is warranted.

ABSTRACT #272

THE EFFECT OF CHLORAMPHENICOL OPHTHALMIC OINTMENT ADMINISTRATION ON SERUM PHENOBARITAL CONCENTRATIONS OF HEALTHY DOGS. LK Pearce,[1] MG Papich,[2] MR Lappin.[1] Colorado State University,[1] Fort Collins, CO and North Carolina State University,[2] Raleigh, NC.

Administration of chloramphenicol orally or parenterally is known to prolong pentobarbital anesthesia. Presumably the mechanism is via inhibition of drug metabolizing enzymes resulting in increased phenobarbital (PB) concentrations in plasma. However, specific mechanisms have not been determined. Clinically, we have observed that the administration of chloramphenicol (CAP) ophthalmic ointment to dogs on long term PB therapy may be associated with decreased severity and numbers of seizures when applied during acute clustering. The purpose of this study was to determine whether conjunctival administration of CAP leads to detectable concentrations of CAP in serum and whether PB concentrations increase in healthy dogs on chronic oral PB therapy.

Age-matched, young adult, mixed sex beagles (n = 12) were purchased and shown to be normal based on results of physical examination and serum biochemical profile. After PB administration at 3.5–5.0 mg/kg q12h orally, all beagles were shown to have PB concentrations of >15 μg/mL by day 30. On day 36, PB was administered PO to all dogs, 5 hours before administration of 5 mg CAP ophthalmic ointment into both conjunctival sacs of 6 randomly selected dogs and 9 hours after the administration of CAP. Blood for serum collection was collected from all 12 dogs prior to and then 15 min, 30 min, 1 hr, 1.5 hr, 2 hr, 3 hr, 6 hr, 9 hr, 12 hr, and 24 hr after the administration of CAP. PB concentrations were measured in all sera (Immulite Diagnostic Products Corp., Los Angeles, CA); CAP concentrations were measured by high pressure liquid chromatography in the research laboratory of one of the investigators (MP) on sera collected at 1 hr, 3 hr, and 6 hr.

CAP was detected in the serum of all dogs at 1 hr (mean = 16.4 μg/ml; SD = 3.0), 3 hr (mean = 16.4 μg/ml; SD = 2.4), and 6 hr (mean = 16.1 μg/ml; SD = 2.3) after administration. PB concentrations did not increase in sera after CAP administration (Pre-treatment sample mean = 20.9 μg/ml; SD = 2.7; 9 hr sample mean = 17.8 μg/ml; SD = 2.9). In addition, PB concentrations in CAP treated dogs (mean = 17.8 μg/ml; SD = 2.9) were not significantly higher than control dogs (mean = 16.3 μg/ml; SD = 1.9) at the 9 hr post-CAP treatment time.

Even though CAP was absorbed systemically after ophthalmic administration, failure to detect increased serum PB concentrations suggests the observed clinical effect of lessening number and severity of seizures may be

attributable to another mechanism, which may include increased CNS penetration via inhibition of functional enzymes in the blood-brain barrier. It is possible that CAP inhibits P-glycoprotein and increases CNS concentrations of PB without changes in the systemic concentrations. Further studies will include measurement of PB and CAP in CSF.

ABSTRACT #273

IS RENAL FUNCTION GENETICALLY DETERMINED? RESULTS OF A TWIN CATTLE STUDY. MR Faucher, A Bonnet, V Laroute, HP Lefebvre. UMR 181 INRA-ENVT Experimental Physiopathology and Toxicology, National Veterinary School of Toulouse, France.

The relative contributions of genetic and environmental influences on renal function has never been documented in a domestic animal species to our knowledge. The aim of this study was to assess renal function in monozygotic twin cattle. If renal function is genetically determined, the variability within a twin pair should be lower than that observed in individuals which are not genetically linked

Six pairs of healthy adult monozygotic twin cattle obtained by micromanipulation were bred under strictly similar environmental conditions. Iohexol (64.7 mg/kg) and para-aminohippuric acid (PAH) (10 mg/kg) were used as markers of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). They were administered intravenously, and repeated blood sampling was performed. Iohexol and PAH were assayed by HPLC. Pharmacokinetic analysis was performed by a non-compartmental approach. Within- and between-pair coefficients of variation (CVs) of each pharmacokinetic parameter were calculated.

Mean ± SD values of the exo-iohexol and PAH clearances were 0.9 ± 0.14, and 7.6–p0.53 mL/kg/min, respectively. Within- and between-pair CVs were, respectively, 8.4 and 19.9% for exo-iohexol ($P < 0.05$), 9.0 and 20.3% for endo-iohexol ($P < 0.05$), and 6.6 and 7.3% for PAH ($P > 0.05$).

These results indicate that GFR is genetically determined in cattle, as recently suggested in humans. Inversely, ERPF may differ between two individuals with the same genome indicating that this variable depends more on the environmental conditions.

ABSTRACT #274

OXIDATIVE STRESS EVALUATION IN CATS WITH CHRONIC RENAL FAILURE AND ANEMIA. MD dos Santos, MD Lustoza, CS Mori, A Reche Jr, MM Kogika. School of Veterinary Medicine, University of São Paulo, Brazil.

In order to investigate the contribution of oxidative stress to the anemia of chronic renal failure (CRF) in cats, erythrocyte reduced glutathione (GSH), as well as erythrocyte antioxidant enzymes – glutathione peroxidase (GPX), glutathione reductase (GR) and superoxide dismutase (SOD) were measured in 22 healthy cats and in 25 cats with CRF and anemia (PCV up to 30%). Further, in both groups, plasma concentrations of thiobarbituric acid reactive substances (TBARS), an oxidative stress marker, was also measured.

The activities of antioxidant enzymes - GR, GPX and SOD were measured by automatic analyzer. Plasma TBARS and erythrocyte GSH were determined by thiobarbituric acid colorimetric assay and ditionitrobenzoic acid method respectively.

No differences were observed in erythrocyte concentrations of GSH and in activity of GPX between groups. However, erythrocyte activity of GR was significantly higher ($P = 0.006$) in cats with CRF and anemia (25.45 ± 9.40 U/g Hb; mean ± standard deviation) than in healthy ones (18.21 ± 5.69 U/g Hb), suggesting an adaptive response probably due to the increase of oxidized glutathione formation. Erythrocyte activity of SOD was significantly lower ($P = 0.022$) in cats with CRF and anemia (2,471 ± 534 U/g) when compared to healthy ones (2,797 ± 799 U/g Hb) indicating a partial impairment of erythrocyte antioxidant system. Furthermore, cats with CRF and anemia presented higher plasma concentrations of TBARS (0.82 ± 0.36 µmol/L) when compared to the healthy controls (0.39 ± 0.09 µmol/L) ($P = 0.0001$) suggesting an increased plasma oxygen reactive species (ROS) production and plasma lipid peroxidation. Severity of renal dysfunction seems to be related to oxidative stress in cats, as detected by correlation between serum creatinine and plasma TBARS concentrations ($r = 0.712$; $P = 0.0001$), as well as between PCV and plasma TBARS ($r = -0.817$; $P = 0.0001$).

Thus, the results point the presence of oxidative stress in cats with CRF, most likely triggered by increased ROS production, which probably may have occurred in consequence of the disease as well as of the hypoxic condition and leucocytes activation due to anemia. Increased GR activity and maintenance of erythrocyte GSH concentration in cats with CRF and anemia suggest that antioxidant compensatory mechanisms are partially able to keep erythrocytes protected from deleterious effects of ROS despite of higher plasma lipid peroxidation.

ABSTRACT #275

INFLUENCE OF PREDNISOLONE ON URINARY CALCIUM OXALATE RELATIVE SUPERSATURATION IN HEALTHY YOUNG ADULT CATS. N Geyer, J Bartges, C Kirk, S Cox, A Hezel, T Moyers, J Hayes, The University of Tennessee, Knoxville, TN.

Glucocorticoids are recommended to treat cats that have idiopathic hypercalcemia and calcium oxalate urolithiasis. The purpose of this study was to evaluate daily administration of prednisolone to healthy cats on urinary saturation for calcium oxalate.

Five healthy, intact female cats, aged 10 to 12 months, and weighing 2.27 to 4.01 kg, were evaluated. Cats received either prednisolone suspension (10 mg PO q24hr) or an equivalent volume of a similarly colored placebo suspension in a blinded, cross-over controlled study. Treatments were administered for 2 weeks with a 1 week washout period, and a 24-hour urine sample was collected using a modified litter box at the end of each treatment period. A dry, adult maintenance food (SportMix, Midwestern Pet Foods) was fed to maintain body weight and condition. Twenty-four hour urine samples were mixed, the volume recorded, and pH, sodium, potassium, chloride, calcium, magnesium, phosphorous, citrate, oxalate, creatinine, and ammonia concentrations were determined. Molar concentrations of these analytes were entered into a computer program (EQUIL 89d, University of Florida) for determination of relative supersaturation for calcium oxalate monohydrate (RSScom and dihydrate (RSScod). Data were analyzed using 2-tailed, paired t-tests with $p \leq 0.05$ considered significant.

Body weight did not change between study periods ($p = 0.9$). Significant differences were not found for 24-hour urinary volume, or excretions of sodium, calcium, ammonia, oxalate, citrate, or chloride. Significant differences were found in 24-hour urine pH (placebo: 6.81 ± 0.4, prednisolone: 6.08 ± 0.1; $p = 0.02$), potassium (mEq/kg/24hr; placebo: 3.89 ± 0.4, prednisolone: 4.56 ± 0.7; $p = 0.045$), magnesium (mg/kg/24hr; placebo: 1.43 ± 0.4, prednisolone: 3.67 ± 1.5; $p = 0.01$), phosphorous (mg/kg/24hr; placebo: 61.4 ± 6.5, prednisolone: 78.0 ± 6.3; $p = 0.005$), and creatinine (mg/kg/24hr; placebo: 44.7 ± 3.3, prednisolone: 59.3 ± 7.8; $p = 0.03$). No significant difference was found for RSScom (placebo: 0.363 ± 0.23, prednisolone: 0.617 ± 0.42; $p = 0.3$) or RSScod (0.472 ± 0.38, prednisolone: 0.488 ± 0.40; $p = 0.89$).

Prednisolone administration did not induce a diuresis, nor was it associated with increased calcium excretion or urinary saturation for calcium oxalate. Prednisolone administration did increase potassium, magnesium, and phosphorous excretion, which may inhibit calcium oxalate formation despite the aciduria: however, long term administration could induce whole body depletion of electrolytes. Increased creatinine excretion may represent catabolism induced by prednisolone. Prednisolone administration, therefore, may not increase risk of calcium oxalate urolithiasis in young adult cats. Further studies in older cats and cats that have idiopathic hypercalcemia and calcium oxalate urolithiasis are warranted.

ABSTRACT #276

TRENDS IN FELINE UROLITHIASIS: 1985–2004. A.B. Cannon, J.L. Westropp, P.H. Kass, A.L. Ruby, D.L. Johnson, G.V. Ling. Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA.

Urolithiasis occurs in 15–21% of cats presenting with lower urinary tract signs (LUTS). The objective of this study was to characterize the types of calculi, as well as the age, breed, gender, and other risk factors for cats with urolithiasis whose 5363 stones were analyzed at the University of California, Davis (UCD) Urinary Stone Analysis Laboratory from July 1, 1985 through December 31, 2004. Struvite stones were the predominant stone type evaluated until 1993. Over the past 15 years, the ratio of CaOx stones to struvite stones significantly increased ($p = 0.0003$). When evaluating only the last three years, however, the percentage of struvite stones (44%) was higher than the percentage of CaOx stones (40%). Himalayan and Persian cats had a higher risk ratio (RR) for both CaOx (RR = 4.39 and 2.82 respectively) and struvite (RR = 2.30 and 2.14, respectively) stones compared to their expected breed frequency based on UCD Veterinary Medical Teaching Hospital data. The percentage of struvite stones submitted from females over the last 15 years significantly decreased ($p < 0.0001$). During this time period, the mean percentage of stones analyzed each year

containing uric acid was 10% (range 7–18%); no significant differences were found between genders (49% female, 51% males). The increasing proportion of CaOx uroliths submitted over time is reinforced by other studies and could be due to contemporaneous alterations in feline diets. However, the decrease in the percentage of CaOx calculi and the increase in the percentage of struvite calculi over the last three years portends a possible change in this trend.

ABSTRACT #277

RELIABILTY AND ACCURACY OF PORTABLE PH METERS TO MEASURE URINE PH. KY Johnson, JP Lulich, CA Osborne. University of Minnesota College of Veterinary Medicine, St. Paul MN.

Reagent strips are the primary clinical tool for measuring urine pH. However, reagent strips lack accuracy and may lead to errors in diagnosis and management of some diseases. Therefore, portable pH meters have recently been recommended as an alternative. The objective of this study was to compare the reliability and accuracy of four portable pH meters (Checker 1, Oakton Waterproof pH testr BNC, Extech Waterproof Palm pH Meter, Omega PHH-5012) and two reagent strips (Multistix, pHydrion Vivid 1–11) against a Corning 430 benchtop meter (reference) for measuring pH in canine urine.

To determine reliability, pH was measured repeatedly in four urine samples: each sample was divided into 70 two-milliliter aliquots and distributed equally among the seven devices. To evaluate accuracy, pH was measured in 201 urine samples; each sample was divided into 7 two-milliliter aliquots, and distributed equally among the seven devices. The evaluator was masked as to the origin of each aliquot. Reliability of each device was compared using coefficient of variation. Accuracy was compared using Concordance Correlation Coefficient.

Coefficient of variation for all portable pH meters was less than 0.425% confirming repeatability of measurement. The average coefficient of variation for reagent strips was approximately nine times greater. Portable pH meters had high Concordance Correlation Coefficients indicating close agreement with reference measurements. Reagent strips had the lowest Concordance Correlation Coefficients. These results indicate that reagent strips are suitable for routine urinalysis and screening; however, portable pH meters should be used when consistent and accurate pH measurements are crucial.

ABSTRACT #278

ASSOCIATION OF MICROALBUMINURIA AND THE URINE ALBUMIN:CREATININE RATIO WITH SYSTEMIC DISEASE IN CATS. JC Whittemore, WA Jensen, SV Radecki, MR Lappin. From the Department of Clinical Sciences, Colorado State University, Fort Collins, CO and Heska Corporation, Fort Collins CO.

Previous studies have shown microalbuminuria (MALB) and the urine albumin:creatinine ratio (UAC) to be predictors of morbidity and mortality in cats. It is unknown whether the presence of MALB can be utilized to identify underlying diseases or influence outcome. The objectives of this study were to determine the prevalence of systemic disease in cats with and without MALB and to determine the diagnostic utility of a semi-quantitative MALB kit ($MALB_E$, E.R.D.-HealthScreen® Urine Test), quantitative MALB assay ($MALB_Q$, ERD™ Test), UAC and urine protein : creatinine ratio (UPC) for systemic disease in cats.

Urine samples from 441 of 611 cats presented to Colorado State University (CSU) met inclusion and exclusion criteria. Urinalyses were performed at the CSU Clinical Pathology Laboratory. Urine dipstick (DpP; cutoff of trace), UPC (cutoffs of 0.4 and 0.1), $MALB_Q$ and $MALB_E$ (cutoffs of 1 mg/dl), and UAC values (cutoffs of 100 and 200 mg/gm) were determined. Clinical diagnoses recorded within 3 months of urine collection were grouped as: healthy, neoplasia, infection/inflammation/immune-mediated, urinary, endocrine, and other disease. The influence of clinical diagnosis, gender, age, BUN, creatinine, blood pressure, urine culture results, temperature, pyuria, hematuria and bacteriuria, on $MALB_Q$, $MALB_E$, or DpP was evaluated by logistic regression. P values less than 0.05 were considered significant.

All cats were positive using $UPC_{0.1}$ cutoff so this test could not be further evaluated. The small number of cats positive by $UPC_{0.4}$, UAC100 and UAC200 precluded any statistical evaluation of these tests. Factors significantly associated with a positive $MALB_Q$ were health status, presence of urinary disease, gender, BUN, pyuria, and hematuria. Factors significantly associated with a positive $MALB_E$ were presence of urinary disease, age, BUN, creatinine, pyuria, and hematuria. The only factor significantly associated with DpP status was hematuria.

Based on this study, UAC ratios were not useful for identifying underlying disease due to their poor sensitivity. In contrast, MALB is associated with the presence of disease. Further prospective studies are necessary to quantify the diagnostic utility of MALB tests for detection of occult disease in cats.

ABSTRACT #279

PARATHYROID HORMONE EVALUATION IN CATS WITH CHRONIC RENAL FAILURE. MM Kogika, LH Giovaninni, MD Lustoza, MY Hasegawa, D Pereira, VABF Wirthl, A Reche Jr. School of Veterinary Medicine – University of São Paulo, Brazil.

The aim of the present study was to evaluate serum parathyroid hormone (PTH) and biochemical parameters related to calcium metabolism in healthy cats and in cats with chronic renal failure (CRF), in order to verify the prevalence of renal secondary hyperparathyroidism (RHPTH) in CRF animals.

Chronic renal failure can cause many metabolic abnormalities, and calcium and phosphate metabolism disturbance is one of the most important that may play a role in the progression of the disease. Hyperphosphatemia occurs in consequence of decreased glomerular filtration rate, causing reciprocal decrease of biological active form of calcium (ionized calcium), which stimulates parathyroid hormone (PTH) secretion, and in consequence the development of RHPTH; all that process may cause soft tissue calcification as well as PTH itself may act as uremic toxin.

Fifty-five clinically normal cats (control group), aging from 8 to 136 months and forty cats with CRF in stages 3 and 4 (study group), aging from 21 to 252 months were evaluated; for the study group, the presence of renal azotemia was considered (urea $>$ 80 mg/dL and creatinine $>$ 2.0 mg/dL) and also the cats did not show any concomitant disease or submitted to drug therapy that could cause alteration of mineral metabolism. Serum ionized calcium (measured by ion-sensitive electrode method), total calcium, phosphate and intact PTH (determined by immunoradiometric assay) were performed.

No difference (Student t test) between control group and cats with CRF concerning total calcium (control = 9.7 ± 0.1 mg/dL; CRF = 9.8 ± 0.21; mean ± standard error mean) and ionized calcium (control = 5.3 ± 0.02; CRF = 5.3 ± 0.10) were observed. However, difference (Mann-Whitney) between both groups related to phosphate (P = $<$0.001) and intact PTH (P = $<$0.001) was detected (PTH = 207.0 ± 40.86 pg/mL in CRF group and 43.6 ± 7.49 pg/mL in control group; Phosphate = 6.6 ± 0.56 mg/dL in CRF cats and 4.4 ± 0.11 mg/dL in control group). Hyperphosphatemia was observed in 60% and intact PTH increased in 65% of cats with CRF. A positive correlation was detected between intact PTH and phosphorus (r = 0.473; P = 0.000001); from all cats with CRF that presented hyperphosphatemia (24 of 40), 19 showed concomitantly increased serum intact PTH. No correlation between ionized calcium and PTH, as well as between ionized calcium and phosphate was observed. Positive correlation was detected between total calcium and ionized calcium. In conclusion, the prevalence of RHPTH was observed in 65% of the CRF cats, and hypocalcemia did not seem to be essential for the stimulation for PTH secretion, and hyperphosphatemia showed to demonstrate, indirectly, the presence of RHPTH in CRF cats; however the determination of PTH would be more accurate.

ABSTRACT #280

UROLITH RECURRENCE IN CATS. H Albasan, CA Osborne, JP Lulich, L Koehler, K Carpenter, L Ulrich, L Swanson, L Pederson, M Buettner. University of Minnesota, Minnesota Urolith Center, St. Paul, MN.

Urolith recurrence in cats is considered common. However, systematic evaluations of urolith recurrence have not been published. To determine the frequency of calcium oxalate, struvite, and urate urolith recurrence, urolith submissions to Minnesota Urolith Center (MUC) were reviewed. In 1998, uroliths from 4760 cats were submitted for analysis. To identify recurrence, the MUC database was searched for urolith resubmissions from the same patients between 1998 and 2003.

In 1998, 2393 cats were diagnosed with calcium oxalate uroliths. Recurrence (subsequent urolith submission) was detected in 169 cats (7.1%), 15 (0.6%) had a second recurrence, and 2 (0.1%) had a third recurrence; mean recurrence times were 23, 38, and 48 months, respectively. Urolith recurrence rate was not different between females and males.

In 1998, 1821 cats were diagnosed with Struvite uroliths. Recurrence was detected in 49 cats (2.7%), and 3 (0.2%) had a second recurrence; mean recurrence times were 27 and 40 months, respectively. Urolith recurrence rate was 1.6 times higher in females than males.

In 1998, 221 cats were diagnosed with urate uroliths. Recurrence was detected in 24 cats (10.9%), and 5 (2.3%) had a second recurrence; mean recurrence times were 20 and 41 months, respectively. Urolith recurrence rate was 1.8 times higher in females than males.

These results provide insight into the rate and frequency of urolith recurrence in cats. However, because some stones associated with recurrent episodes may not have been submitted to the MUC, results likely represent an underestimate of the actual recurrence rate.

ABSTRACT #281

SURVIVAL OF DOGS WITH GLOMERULONEPHRITIS: 40 CASES. CD Koirala[1], MA Labato[1], LA Ross[1], MJ Acierno[2], LM Freeman[1], [1]Cummings School of Veterinary Medicine, N. Grafton, MA, [2]Louisiana State University,Baton Rouge, LA.

It has been reported that dogs with protein-losing glomerular disease have a poor prognosis, with one study finding a median survival time of 28 days. Since that report, anecdotally there appears to be an increase in the number of dogs with glomerulonephritis in some areas. New treatment modalities have also become the standard of care for these dogs. In our experience, some dogs have survival times longer than previously reported. In this retrospective study, medical records of 40 dogs with biopsy or necropsy confirmed diagnoses of glomerulonephritis were reviewed to determine if there were laboratory parameters or treatment effects that could be applied as prognostic indicators of survival.

Dogs were divided into two groups: long- term survivors (>6 months) or short- term survivors (<6 months). The parameters evaluated included age, sex, breed, presence of hypertension (systolic blood pressure > 150 mmHg), urine protein:creatinine ratio, urine specific gravity, presence of azotemia (BUN > 27 mg/dl, creatinine > 2.0 mg/dl), platelet count, hematocrit, antithrombin level, presence of urinary tract infection, evidence of serological exposure to infectious diseases (leptospirosis, borreliosis, Rocky mountain spotted fever, Ehrlichia species), ANA, treatment regimens (intravenous fluids, antibiotics, antihypertensives, diuretics, immunosuppressives).

The median survival for all dogs was 173 days (range 2–1412 days). Nineteen dogs survived longer than 6 months with a median survival of 461 days (range 191–1412 days). Twenty-one dogs survived for less than 6 months with a median of 7 days (range 2–173 days). The only statistically significant parameters that predicted survival were BUN, creatinine and hematocrit values. Higher values of BUN and/or creatinine, and a low hematocrit as measured on presentation, were associated with a worse clinical outcome. None of the treatments correlated with survival except the administration of furosemide, which was associated with shorter survival. There was no predictive value for seropositivity to Borrelia.

There appears to be a population of dogs with glomerulonephritis that are long -term survivors. The poor prognosis reported in earlier studies may not be accurate for all dogs. Progression of glomerular disease is unpredictable, with no apparent correlation between survival time and parameters at presentation. Other biological markers may need to be assessed. Those dogs living greater than 6 months seem to have a fair to good prognosis.

ABSTRACT #282

APPLICATION OF CONTINUOUS RENAL REPLACEMENT THERAPY (CRRT) FOR ACUTE RENAL FAILURE IN DOGS. Jeong-Hyoun Park, Hwa-Yong Youn, Seong-Jun Park[1] and Cheol-Yong Hwang. College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; [1]College of Veterinary Medicine, Chungnam National University, Daejeon, Republic of Korea.

Continuous renal replacement therapy (CRRT) in human medicine is becoming increasingly popular for the support of critically ill patients with acute renal failure (ARF). The development of pump-driven volumetric-control CRRT machines with small extracorporeal volumes has lead to the widespread use of CRRT further into the pediatric field. However, the experiences are limited for small animal CRRT. Therefore, in this study, the established methods of CRRT were applied to canine ARF patients and its safety and the efficacy were evaluated.

CVVHDF (Continuous venovenous hemodiafiltration) modality was applied in one naturally and four artificially induced ARF dogs when the status met for the indication of hemodialysis. During the 24 hours period of CRRT, blood samples were collected every 6 hours and measured for CBC, serum chemistry (BUN, CREA, TP, Alb, Ca, P, Glucose, electrolytes, calculated Osmolarity) and venous blood gas analysis parameters (HCO^{3-}, pH, Ca^{2+}). Blood Pressure, temperature, pulse, respiratory rates were also monitored and clinical signs and complications that could occur in intradialysis were recorded in detail.

Serum blood urea nitrogen and creatinine levels were decreased and serum potassium and phosphorus concentrations were normalized during CRRT. Blood pH, bicarbonates were also normalized. From the little changes of albumin and total protein, it could be ascertained that large molecular weight substances like proteins were not filtered but were maintained. Intradialysis adverse events described on intermittent hemodialysis did not observed in any dogs and blood coagulopathy with long-term heparin anticoagulation was detected but this was not occurred in the 2 dogs treated with regional citrate anticoagulation.

This study shows that CRRT is feasible, sustainable, and useful in dogs with ARF. Considering the gentle nature and the consequence and other important advantages of CRRT, CRRT may soon become the ''gold standard'' for renal replacement therapy in the intensive care unit management of companion animal ARF.

ABSTRACT #283

BIOCHEMICAL RAMIFICATIONS OF FELINE URETERAL REPLACEMENT WITH AN AUTOGENOUS ILEAL GRAFT. SB Reimer, PJ Lotsikas, D Gietzen, MD Winter, DM Merkley. Iowa State University, Veterinary Teaching Hospital, Ames, IA.

Feline ureteral obstruction as a result of ureteroliths is an emerging challenge facing veterinary medicine. A recent study identified a 10-fold increase in the frequency of feline upper tract uroliths over the past twenty years. Surgical removal of ureteral calculi is currently the treatment of choice for this disease. Maintaining patency of the ureteral lumen is difficult owing to the small size of the feline ureter, which is approximately 0.4 mm in diameter. Underlying renal disease is often present in cats afflicted with uroliths, necessitating preservation of both kidneys. In human medicine when nephrectomy is not an option, ureteral replacement with a segment of ileum is commonly employed as a salvage procedure. The ileum possesses desirable characteristics that make it an acceptable replacement for the ureter. It is capable of producing peristalsis to propel urine from the kidney to the urinary bladder and has fewer biochemical complications when compared to other segments of the intestine. The purpose of this study was to develop the optimal surgical technique for interpolation of a segment of autogenous ileum into the feline urinary system.

Eight healthy, adult, male mixed breed cats underwent an ureteroneoileostomy. Normal renal function and anatomy was confirmed by a complete blood count, urinalysis, serum biochemical analysis, abdominal ultrasound, and an excretory urogram prior to surgery. A unilateral nephrectomy of the right kidney was performed to assure accurate measurement of remaining renal function. A section of ileum was isolated from the small intestine followed by anastomosis of the remaining small intestine. Three procedures of ureteroneoileostomy were investigated using the following techniques: 1) Extraluminal mucosal appositional technique, 2) Traditional intraluminal technique, 3) Intraluminal technique with regional mucosal denuding of the ileal segment. Renal profiles were collected daily for one week and then weekly for one month following surgery. Euthanasia was performed on any cat with a serum creatinine level greater than 6 mg/dl or a serum blood urea nitrogen level greater than 110 mg/dl following surgery.

During this preliminary study, the intraluminal ureteroneoileostomy technique with mucosal denuding was identified as the optimal surgical technique for ureteral replacement in the cat. The procedure was performed in six cats. Three cats died or were euthanized within 3 days of surgery. One died from aspiration pneumonia, one cat was died due to hemoabdomen, and one cat was euthanized for elevated renal values associated with ureteral obstruction secondary to swelling. The three surviving cats had BUN and creatinine levels within an acceptable range of uninephrectomized cats one month following surgery (BUN: mean 38.3, reference range 15–35, Creatinine: mean 2.1, reference range 0.1–1.6).

Unusual complications not directly associated with renal function were experienced in two cats in this study. Given the overall outcome of the surviving cats, the intraluminal ureteroneoileostomy technique with mucosal denuding is a novel procedure that is a feasible surgical option for ureteral obstruction. This technique will be employed in a larger scale investigation to determine the long-term effects of this procedure in the cat.

ABSTRACT #284

GALLBLADDER DISEASE IN 36 SHETLAND SHEEPDOGS. AL Aguirre[1], SA Center[1], AE Yeager[1], JF Randolph[1], HN Erb[2]. Departments of Clinical Sciences[1] and Population Medicine & Diagnostic Sciences[2], College of Veterinary Medicine, Cornell University, Ithaca, NY.

During the past 10 years, we have suspected that Shetland Sheepdogs (SS) are uniquely predisposed to gallbladder disease (GBD), particularly biliary mucoceles. To explore this impression, we completed a retrospective study over a 10 year interval (1995 to 2005), identifying dogs with extrahepatic biliary disorders, and deriving the population of SS. Eleven additional cases of SS with GBD were acquired from consulting veterinarians. Study inclusion required: 1) routine clinicopathologic and coagulation test results, 2) abdominal ultrasound images/reports, 3) hepatic and gallbladder (GB) histopathology, 4) description of biliary structures at surgery or necropsy, and 5) information on case outcome. To determine SS proclivity for GBD, ill dogs from the general hospital population and ill SS lacking GBD were enumerated. Gender and reproductive status of ill dogs and ill SS lacking GBD were compared to SS with GBD. The Wilcoxon Rank Sum test detected differences in clinicopathologic features of SS with GBD between survivors and nonsurvivors. A Chi-square goodness-of-fit test examined whether SS were predisposed to GBD and inspected for gender predisposition. An alpha = 0.05 using two-tailed p-value was applied.

A lower percentage of SS (1.6%) was found in the ill hospital population lacking GBD compared to dogs with GBD (10.2%); $p < 0.0001$. SS had an odds ratio and relative risk for GBD (7.2 and 6.6, respectively) supporting breed predilection. There were no differences in gender or reproductive status between SS with GBD and SS with other illnesses or ill dogs of other breeds (all $p > 0.1$). Seven dogs had a GB mucocele discovered during diagnostics for other health problems. Of 36 SS with GBD, 31 had a biliary mucocele and12/31 had concurrent disorders that might promote mucocele formation: pancreatitis (n = 4) and 2 each with hyperadrenocorticism, GB hypokinesis, protein losing nephropathy, and hypothyroidism. All dogs with mucoceles had cystic mucosal hyperplasia. A 72% survival rate was documented for SS with GBD. Of non-survivors (8/36), 50% had a ruptured GB. Nonsurvivors had clinicopathologic markers common to severe illness including a significantly lower PCV (39 vs 45%), higher WBC (27.0 vs 12.2 thou/uL), neutrophil (23.5 vs 9.0 thou/uL) and band (2 vs 0 thou/uL) counts, lower potassium (3.8 vs 4.7 mEq/L), calcium (9.1 vs 10.6 mg/dL), total protein (5.5 vs 6.3 gm/dL) and albumin (2.8 vs 3.4 gm/dL) concentrations and higher BUN (44 vs 15 mg/dL) concentration and AST (128 vs 42 U/L) activity; all $p < 0.04$. Most SS with GB disease were hyperbilirubinemic and hypercholesterolemic and it was common to find a history of hyperlipidemia. This study confirms a SS predisposition to GBD, specifically GB mucocele. Conditions fostering hyperlipidemia or hypercholesterolemia may predispose SS to biliary mucocele formation perhaps related to this breed's tendency for hyperlipidemia.

ABSTRACT #285

INVESTIGATION OF IDIOPATHIC VACUOLAR HEPATOPATHY IN SCOTTISH TERRIERS AND THE ROLE OF PROGESTERONE STEROIDS IN THE ETIOLOGY. AT Gary, CB Webb, DC Twedt. Colorado State University, Ft. Collins, CO.

Idiopathic vacuolar hepatopathy (IVH), a condition characterized by hydropic degeneration and accumulation of glycogen in hepatocytes, is frequently documented in Scottish terriers having elevations in serum alkaline phosphatase (ALP). IVH is indistinguishable histologically from steroid hepatopathy, which occurs secondary to elevated levels of exogenous or endogenous glucocorticoids. Histologic changes in dogs consistent with a steroid hepatopathy have also been reported after administration of progestins which have glucocorticoid-like properties. It is postulated that IVH may also occur secondary to endogenous elevations of specific non-glucocorticoid hormones such as progesterone. The purpose of this study is to determine if there is an association between elevated serum ALP and IVH with abnormal adrenal steroid hormone precursors in Scottish terriers.

Two populations of Scottish terriers were identified: healthy dogs with normal liver enzymes (Grp A) and asymptomatic dogs having elevations in ALP of unknown cause (Grp B). In each dog a thorough drug history, complete physical examination, CBC, serum chemistry, and urinalysis was performed. An endogenous ACTH level and an adrenal panel assayed before and after ACTH stimulation was also obtained. Grp B dogs had additional diagnostics including serum ALP steroid isoenzyme activity (cALP), liver and adrenal ultrasound and fine-needle aspiration or biopsy of the liver. Liver histology and cytology samples were stained using PAS for glycogen.

12 Scottish terriers were evaluated: 6 normal dogs in Grp A and 6 with elevations in ALP in Grp B. Grp A consisted of 2 intact males, 1 male castrated (MC), 1 intact female, and 2 female spayed (FS) dogs with an average age of 4.8 years. Grp B consisted of 3 FS and 3 MC dogs with an average age of 8.7 years. ALP values for Grp B ranged from 605 to 6147 (normal 20–142 IU/L) with cALP ranging from 48–84% (598–4760 IU/L) of the total ALP. Imaging studies revealed nonspecific findings of hepatomegaly and/or hyperechogenicity in all dogs in Grp B; ill-defined hypoechoic liver nodules were also present in 1 dog. Liver biopsy (1/6) and fine-needle aspiration (5/6) revealed hepatocellular vacuolization with glycogen accumulation.

Abnormal adrenal steroids were present in 5/6 dogs in Grp A with mildly elevated basal levels of androsteindione, cortisol, estradiol, progesterone (PRG), and 17-hydroxyprogesterone (17 OH). Moderate to marked post-ACTH elevations in PRG and 17 OH were present in Grp A as well as mild elevations in androsteindione, cortisol, and estradiol. Abnormal adrenal steroids were present in 4/6 dogs in Grp B including mild elevations in post-ACTH cortisol, estradiol, PRG, and 17 OH. There was no statistical association between elevated ALP and PRG.

The results of this study do not provide conclusive evidence to support an association between IVH and elevations in selected adrenal hormones in this group of Scottish Terriers. Further study is needed to elucidate the underlying pathophysiologic mechanisms associated with IVH.

ABSTRACT #286

DEVELOPMENT OF A CLINICAL SCORING INDEX FOR DISEASE ACTIVITY IN FELINE INFLAMMATORY BOWEL DISEASE. JM Crandell[1], AE Jergens[1], JA Morrison[1], MA Pressel[1], KG Miles[1], E Portillo[1], KF Burke[2], JM Steiner[2], R Evans[1]. College of Veterinary Medicine, Iowa State University[1], Ames, IA and the GI Laboratory, Texas A&M University[2], College Station, TX.

Feline inflammatory bowel disease (FIBD) is an idiopathic gastrointestinal (GI) disorder characterized by persistent gastrointestinal signs, histologic evidence of mucosal inflammation, and general responsiveness to immunotherapeutic intervention. Uncontrolled studies and anecdotal reports from many clinicians and institutions report a favorable response of FIBD to many forms of medical therapy. Critically important to the design of any therapeutic trial is a method of assessing the response or lack thereof to a given treatment modality. The objective of this study was to develop a clinical scoring index for measurement of disease activity and therapeutic effect in cats with IBD.

A retrospective study covered data on 62 cats diagnosed with FIBD over a 10 year period (1993–2003). FIBD was diagnosed on the basis of established clinical criteria and histologic lesions of mucosal inflammation. Following review of these medical records, nine independent variables were identified and correlated to histology scores using multiple regression analysis. Subsequent computations reduced the number of independent variables to six (histology, GI signs, serum total protein and phosphorous concentrations, serum ALP, and endoscopic lesions) which correlated best to inflammation and comprised the FIBD activity index. This FIBD index was next evaluated prospectively in 23 cats presented to ISU with histories of chronic GI disease. A final diagnosis of FIBD was made in 17 cats while 6 cats were diagnosed with food-responsive GI disease. Cats with IBD were fed an elimination diet and prescribed oral prednisolone (1–3 mg/kg) for 14–21 days. In these 17 cats, the FIBD index was determined before and during therapeutic intervention.

Chronic signs of anorexia, vomiting, diarrhea, and weight loss predominated in cats with IBD. Salient serologic abnormalities of FIBD included hyperproteinemia (3/17), increased ALP/ALT (3/17), hypocobalaminemia (3/17), hypophosphatemia (8/17), and increased serum fPLI concentrations (3/17). Endoscopic examination demonstrated lesions of granularity, friability, and/or erosions in 16/17 cats. All FIBD cats had lymphocytic-plasmacytic mucosal inflammation. Complete response to medical therapy was observed in 15/17 FIBD cats, while partial remission was seen in the other 2 cats. Alterations in clinical scoring indices were observed in all FIBD cats as a consequence of medical therapy. Pre-treatment FIBD index scores (mean score = 7.3) were markedly reduced during the 14–21 day treatment period (mean post-treatment IBD score = 0.3).

The concentrations of select acute phase proteins (serum acid glycoprotein, haptoglobin) showed heterogeneous responses to treatment in FIBD cats. We conclude that the FIBD index is a useful measure of inflammatory activity in cats with IBD and is suitable for clinical evaluation of the therapeutic effect in these patients.

ABSTRACT #287

EVALUATION OF FECAL BACTERIAL DIVERSITY IN HEALTHY CATS AND IN CATS WITH INFLAMMATORY BOWEL DISEASE OR GASTROINTESTINAL NEOPLASIA. KF Burke[1], JM Steiner[1], JD Broussard[2], M Alvarez[2], and JS Suchodolski[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX and [2]The Animal Medical Center, New York, NY.

Idiopathic inflammatory bowel disease (IBD) is a common, yet poorly understood, disorder in cats. Studies in human medicine have suggested that the manifestation of intestinal inflammation is critically dependent on the

presence and composition of the intestinal bacterial microflora. The role of the intestinal microflora in cats with IBD has not been well defined. Recently, the amplification of bacterial 16S rDNA with subsequent separation by denaturing gradient gel electrophoresis (DGGE) has been described as an approach for the assessment of bacterial diversity in biological samples in humans and dogs. The aim of this study was to evaluate the bacterial diversity in fecal samples from healthy cats and cats with IBD or gastrointestinal neoplasia by use of DGGE fingerprinting.

One gram of fresh feces was collected from 3 groups of cats: 1) healthy cats (n = 10), 2) cats with IBD (including cats with mild to moderate intestinal inflammation (n = 6) and those with severe intestinal inflammation (n = 3)), and 3) cats with gastrointestinal neoplasia (lymphosarcoma, n = 4; adenocarcinoma, n = 2). Cats received no antibiotic therapy at the time of fecal collection. Bacterial DNA was purified by phenol:-chloroform:iso-amylalcohol extraction, the variable V6–V8 region of 16S rDNA was amplified using universal bacterial primers (F-968-GC; R-1401), and PCR amplicons were separated by DGGE. The separation of amplicons by DGGE was evaluated by cluster analysis and similarity scores (Dice coefficient; 100% represents complete identity) within and between samples of all 3 groups using commercial gel analysis software (Bionumerics 3.0, Applied Maths). Dendrograms, showing clustering according to the similarity of banding patterns of individual samples, were constructed by the unweighted pair group method using arithmetic averages. Data were analyzed with a statistical software package (GraphPad Prism 4.0).

Constructed dendrograms revealed that 6 of 10 healthy cats formed a significant cluster based on the similarity of their DGGE profiles. A significant cluster was also present for 5 of 6 cats with gastrointestinal neoplasia, while the DGGE profiles of the cats with IBD showed mixed clusters. The mean ± SD number of bands on the DGGE profile was 13.1 ± 3.9 for healthy cats and significantly greater than the number of bands of the cats with IBD (8.8 ± 4.1; $p = 0.0322$, *t*-test). The number of bands of the healthy cats was not significantly different to the number of bands of the cats with GI neoplasia (median: 9.5, $p = 0.1179$, Mann-Whitney U test).

In this study, the majority of healthy cats and also cats with GI neoplasia showed clustering of their DGGE profiles within each group. Further, the results of this study suggest that bacterial diversity may be decreased in cats with IBD when compared to healthy control cats.

ABSTRACT #288

EFFECT OF TYLOSIN ON THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE JEJUNAL MICROFLORA. JS Suchodolski[1], JA Harmoinen[2], E Westermarck[2], DA Williams[1], T Spillmann[2], and JM Steiner[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Department of Clinical Veterinary Sciences, Helsinki University, Helsinki, Finland.

The composition of the intestinal microflora has a significant impact on the health status of an individual and derangements of the small intestinal microflora have been implicated as a cause or risk factor for small intestinal disease. Tylosin, a member of the macrolide class of antibiotics, is commonly recommended for the treatment of chronic enteropathies in dogs. However, there are only few data available about the influence of tylosin on the qualitative and quantitative composition of the small intestinal microflora. The aim of this study was to evaluate the dynamics of the jejunal microflora after administration of tylosin.

Five healthy dogs, each with a pre-existing jejunal fistula inserted approximately 60 cm distal to the pylorus were used in this study. Tylosin was administered at a dose of 20–22 mg/kg/day for a period of 14 consecutive days. Samples of jejunal juice were collected through the fistula on day 0 (before tylosin administration), day 14, and day 28 (14 days after withdrawal of tylosin). For evaluation of qualitative changes in the jejunal microflora between the sampling periods the variable V6–V8 region of 16S rDNA was amplified using universal bacterial primers, and PCR amplicons were subsequently separated by denaturing gradient gel electrophoresis (DGGE) followed by comparison of similarity indices (Dice coefficient; 100% represents complete identity) of the resulting DGGE profiles. Dendrograms, showing clustering according to the similarity of banding patterns of individual samples, were constructed by the unweighted pair group method using arithmetic averages. For evaluation of quantitative changes in bacterial DNA between different sampling periods, the variable V3 region of 16S rDNA was amplified by quantitative real time PCR. The effect of tylosin on the jejunal microflora was determined by repeated measures 1-way ANOVA.

Administration of tylosin led to a significant decrease in the similarity of DGGE profiles compared to baseline (mean ± SD: 46.8 ± 28.8%; $p = 0.03$). After withdrawal of tylosin, DGGE banding patterns were not significantly different from baseline (mean ± SD: 71.3 ± 11.2%; $p = 0.53$). Constructed dendrograms based on DGGE profiles showed a high degree of inter-individual variation between the different time-points. There were no significant changes for band numbers ($p = 0.45$) or total bacterial DNA ($p = 0.94$) as measured by quantitative real time PCR between the different time-points.

These results indicate that administration of tylosin leads to significant but transient changes in the qualitative composition of the jejunal microflora. These changes vary between individual dogs. In this study, tylosin did not lead to significant quantitative changes in the jejunal microflora.

ABSTRACT #289

PURIFICATION AND PARTIAL CHARACTERIZATION OF CANINE NEUTROPHIL ELASTASE. A Stoll, CG Ruaux, JS Suchodolski, DA Williams, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Canine neutrophil elastase (cNE) belongs to the group of serine proteinases and is a constituent of azurophil granules within neutrophils. It has previously been reported that the concentration of neutrophil-derived proteins in feces is a promising marker for intestinal inflammation in humans. The aim of this study was to develop a rapid and reproducible protocol for the purification of cNE from dog blood and to partially characterize cNE as a prelude to the development of an immunoassay for the measurement of this protein in fecal samples.

Blood was collected from healthy dogs euthanized for unrelated research projects. First, leucocytes were separated by dextran sedimentation. Canine NE was separated from the cells by high salt extraction, followed by three freeze (−20°C)-thaw-sonication cycles. The protein was further purified by strong cation-exchange column chromatography and continuous-elution electrophoresis. Purified cNE was partially characterized by determination of molecular mass using an Agilent 2100 bioanalyzer with the Protein 50 assay kit, estimation of the isoelectric point by polyacrylamide gel electrophoresis, and N-terminal amino acid sequencing by Edman degradation. Finally, the specific absorbance was determined by the bicinchoninic acid protein assay.

The molecular mass of cNE was estimated at 26,500 Dalton based on comparison to known molecular weight standards using the Agilent Protein 50 assay kit. Isoelectric focusing showed an isoelectric point of 8.5. The N-terminal amino acid sequence of the first 25 residues was Ile-Val-Gly-Gly-Arg-Pro-Ala-Gln-Pro-His-Ala-Trp-Pro-Phe-Met-Val-Ser-Leu-Gln-Arg-Arg-Gly-Gly-His-Phe. The sequence showed 100% homology to the protein sequence predicted by the nucleotide sequence available through the canine genome project. The approximate specific absorbance of cNE at 280 nm was determined to be 0.85 for a 1 mg/ml solution.

We conclude that canine neutrophil elastase can be successfully purified from dog blood using this method. The results of this study will facilitate future development of an immunoassay for measurement of fecal cNE as a potential non-invasive diagnostic tool for the assessment of intestinal inflammation in canine patients.

ABSTRACT #290

FLUORESCENCE *IN SITU* HYBRIDIZATION CONFIRMS ERADICATION OF NATURALLY-ACQUIRED *HELICOBACTER* GASTRITIS IN THE DOG AND CAT. MA Pressel[1], JM Crandell[1], AE Jergens[1], JA Morrison[1], M Baumgart[2], KM Simpson[2]. 1. College of Veterinary Medicine, Iowa State University, Ames, IA; 2. College of Veterinary Medicine, Cornell University, Ithaca, NY.

Recent studies suggesting a pathogenic role for *Helicobacter* species in canine and feline gastritis are inconclusive. Furthermore, the effectiveness of medical therapy for naturally acquired *Helicobacter* gastritis has not been thoroughly evaluated to date. The aims of this report were to document 4 cases of naturally-occurring *Helicobacter* gastritis in the dog and cat and to confirm eradication of infection following combination drug therapy using histology and fluorescence *in situ* hybridization (FISH) with *Helicobacter*-specific probes.

Two cats and 2 dogs having chronic gastrointestinal signs of vomiting (1 cat, 1 dog) and small bowel diarrhea (1 cat, 1 dog) were referred to the ISU VTH for diagnostic evaluation and therapeutic management. All animals had been unsuccessfully treated with a variety of dietary trials, anthelmintics, and metronidazole. Physical examination in all patients was normal. Admission laboratory testing failed to demonstrate consistent abnormalities between animals; one cat showed moderate elevation of hepatic (e.g., ALT and ALP) enzymes. Diagnostic endoscopic (2 dogs, 1 cat) or surgical biopsies (1 cat) of gastric and duodenal mucosa revealed multifocal-to-diffuse lymphocytic +/− plasmacytic gastritis with abundant spiral bacteria (spirochetes visualized via H&E and/or Warthin-Starry stains) present

within gastric glands. A definitive diagnosis of *Helicobacter* gastritis was confirmed in each animal using fluorescent-labeled 16s rRNA-targeted oligonucleotide probes specific for *Helicobacter* species.

A treatment regimen consisting of oral metronidazole, ampicillin, and bismuth subsalicylate was administered to each patient for 21 days. Rapid resolution of clinical signs was observed in 3/4 patients while one cat continued to exhibit small bowel diarrhea. Upper GI endoscopy for biopsy collection following drug therapy showed eradication of gastric *Helicobacter* spp. infection via histopathology (gastric spiral bacteria were not visualized on tissue sections stained with H&E and Warthin-Starry stains) and negative FISH analysis in each animal. The cat with persistent small bowel diarrhea was subsequently diagnosed with lymphocytic-plasmacytic enteritis.

We conclude that fluorescence *in situ* hybridization (FISH) of routine formalin fixed biopsy specimens enabled rapid and specific molecular identification and localization of *Helicobacter* spp. within the mucosa and that triple-combination drug therapy is useful in the resolution of clinical signs, clearance of gastric mucosal spirochetes and amelioration of histopathologic lesions caused by gastric *Helicobacter* infections.

ABSTRACT #291

DOGS WITH PROTEIN LOSING ENTEROPATHY ARE IN A HYPERCOAGULABLE STATE. SM Lahmers[1], R Sellon[1], P Peterson[2]. [1]Veterinary Clinical Sciences, Washington State University, Pullman, WA; [2]Veterinary Specialty Center, Lynnwood, WA.

Thromboembolism (TE) develops from an imbalance between pro- and anticoagulant factors. Protein-losing enteropathy (PLE) is a possible risk factor for TE but the contributing mechanisms are unknown. Our hypothesis was that dogs with PLE are in a hypercoagulable state that reflects a loss of anticoagulant factors and excess production of select procoagulant factors. Sixteen dogs with histologically confirmed PLE and no other identifiable risk factors for TE and 17 normal control dogs were prospectively evaluated. Results of a CBC, biochemical panel, urinalysis, D-dimer, antithrombin (AT), fibrinogen, Factor VIII:C activity and thrombin-antithrombin complex (TAT, a measurement of thrombin formation) were compared between the two groups. AT values were decreased in PLE dogs ($p = 0.001$); however, in only 2 of the PLE dogs were AT values below the reference range for normal dogs. Fibrinogen concentration was greater in PLE dogs ($p = 0.007$). There were no differences between groups in Factor VIII:C activity. Platelet numbers were higher in PLE dogs ($p > 0.0001$) with 75% of the PLE dogs having thrombocytosis while none of the control dogs did. Increased TAT formation was identified in the PLE group ($p = 0.04$) and a platelet/albumin (P/A) ratio provided the best correlation with elevated TAT ($r = 0.57$). A P/A >240,000 identified all PLE dogs with increased TAT formation. We conclude that PLE dogs are hypercoagulable in part due to increased procoagulant factors (thrombocytosis) and decreased anticoagulant factors (AT) and that a P/A ratio may be a clinically useful parameter for identifying PLE dogs with increased TAT formation.

ABSTRACT #292

ASSESSMENT OF STABILITY AND DETERMINATION OF A REFERENCE RANGE FOR CANINE C-REACTIVE PROTEIN IN SERUM. N Berghoff, JS Suchodolski, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

C-Reactive Protein (CRP) is an acute phase reactant and has been proposed as a marker for inflammatory disease. It is recommended by the manufacturer of a commercial test kit (TriDelta Diagnostics) that serum samples for measurement of CRP should be frozen within 24 hours of blood collection, assuming that a loss of CRP may occur when stored at higher temperatures. A reference range for this test kit is not provided by the manufacturer. The purpose of this study was to determine the stability of canine CRP in serum when the samples are stored at different temperatures, and to establish a reference range for CRP in canine serum samples.

CRP was measured in all samples using the TriDelta PHASE™RANGE Canine C-Reactive Protein Assay, a solid phase sandwich immunoassay. A total of 12 canine serum samples with known CRP concentrations (between 10 and >120 mg/L) were used to establish stability of CRP. Each sample was divided into 5 aliquots on day 0. Aliquot (1) was kept frozen at −20°C, (2) was stored at 4°C for 1 week, (3) was stored at 4°C for 4 weeks, (4) was stored at room temperature (RT; approximately 23°C) for 1 week, and (5) was stored at RT for 4 weeks. All aliquots, including those to be stored at higher temperatures, were refrozen immediately after aliquoting to ensure consistency among the samples regarding possible freeze-thaw damage. On day 1, aliquot groups (3) and (5) were thawed and stored at 4°C and RT, respectively. Aliquot groups (2) and (4) were thawed on day 22 and also stored at 4°C and RT, respectively. On day 29, aliquots from group (1) were thawed and all 5 aliquots from each sample were tested on the same ELISA plate to avoid inter-assay variation. CRP concentrations were compared between the 5 groups of aliquots using a Friedman test with Dunn's post test. A reference range for CRP was determined by measuring CRP in serum samples from 51 healthy dogs and was calculated using the lower 97.5th percentile.

The Friedman test found statistically significant differences within the stability test samples ($p = 0.0004$). Dunn's post test showed significant differences between groups (1) and (5) (medians 13.56 vs. 10.16; $p < 0.001$), as well as groups (2) and (5) (medians 13.61 vs. 10.16; $p < 0.01$). There was no significant difference between any other groups. The reference range for CRP was 0–7.6 mg/L.

These data show that only prolonged storage of serum samples at RT leads to a significant decrease in canine CRP concentrations. This suggests that CRP in serum samples is more stable than previously assumed and that it may be safe to keep serum samples under refrigeration for short term storage (up to 4 weeks), eliminating the need to keep the sample frozen, which will also facilitate shipment of samples to diagnostic laboratories. The evaluation of 51 serum samples for the reference range shows that CRP is either not detectable or present only in very low concentrations in healthy dogs.

ABSTRACT #293

EFFECT OF SEEING AND SMELLING FOOD ON SERUM TOTAL BILE ACID CONCENTRATIONS IN HEALTHY DOGS. JM Steiner, JS Suchodolski, and KM Findlay. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Serum total bile acid concentrations are widely used for assessment of hepatic function in non-icteric dogs. Serum bile acids are assessed after withholding food for 12 hours and again 2 hours after feeding a small amount of a high-fat meal. In normal dogs serum bile acid concentrations rise slightly after feeding. However, there are also dogs that show paradoxical serum bile acid results with higher pre-prandial than post-prandial serum bile acid concentrations. One suggested explanation for this phenomenon is that seeing or smelling food during the fasting period may increase pre-prandial serum bile acid concentrations. Thus the goal of this study was to evaluate the effect of seeing and smelling food on serum total bile acid concentrations in healthy dogs.

Eight healthy dogs were enrolled into this study. The general health of the dogs was evaluated by physical examination, complete blood count, serum chemistry profile, and a heartworm antigen test. Dogs were admitted to the veterinary teaching hospital, fed their regular diet, and then held off food for at least 12 hours. Great care was taken not to expose these dogs to any sight or smell of food. After withholding food for at least 12 hours a baseline blood sample was collected and a bowl of food containing ¼ can of a high fat canned commercial pet food was placed in front of each cage. After 15 minutes the food was removed and further blood samples were collected at 30, 60, 120, 180, 240, 480, and 720 minutes after displaying the food. All serum samples were evaluated for total bile acid concentrations using an enzymatic colorimetric assay (Bile Acids-L_3K®, analyzed on a Roche Hitachi® 911 biochemistry analyzer).

Mean serum bile acid concentrations did not increase significantly after seeing and smelling food ($p = 0.115$; 1-way ANOVA). Serum bile acid concentrations were highest for the base-line blood sample for 4 of 8 dogs. Two of the remaining 4 dogs had negligible increases after seeing and smelling the food. One dog had an increase from 16.8 to 17.0 mg/dL (CV: 0.8%) 60 minutes after seeing and smelling food and another dog had an increase from 7.6 to 7.7 mg/dL (CV: 0.9%) 120 minutes after seeing and smelling food. These variations are within the range for intra-assay variability for commercial assays. Another dog had a sizeable increase from 9.0 to 17.3 mg/dL. However, this increase occurred 720 minutes after seeing and smelling food and thus was unlikely related to this event. Only one of the dogs had a sizeable increase in serum bile acid concentration from 10.8 to 23.6 mg/dL 120 minutes after seeing and smelling food. However, this dog showed a great degree of variability of serum bile acid concentrations over the length of the study, which may suggest that the increase in serum bile acid concentration observed was due to overall variability rather than seeing and smelling the food.

In conclusion, serum bile acid concentrations did not significantly increase in response to seeing and smelling food in healthy dogs. These results suggest that seeing or smelling food are unlikely reasons for paradoxical serum bile acid concentrations.

ABSTRACT #294

COMPARISON OF THE IN-HOUSE IDEXX SNAP® BILE ACID TEST KIT WITH A REFERENCE LABORATORY METHOD. JS Suchodolski, EM Morris, and JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Measurement of pre- and postprandial serum bile acid concentrations are commonly used for evaluation of hepatic function in non-icteric canine and feline patients. Recently, IDEXX Laboratories Inc. has released a new diagnostic kit for in-house measurement of serum total bile acid concentrations, the IDEXX SNAP® Bile Acid test kit. The aim of this study was to compare pre- and postprandial bile acid concentrations in dogs using the IDEXX SNAP® Bile Acid test kit with a laboratory reference method.

A total of 130 canine serum samples were analyzed for preprandial (n = 44) or postprandial (n = 86) total bile acid concentration. Fifty-five serum samples were from healthy dogs as evaluated by physical examination, complete blood count, and serum chemistry profile. Thirty-six serum samples were from dogs with suspected liver disease. From 39 animals, paired pre- and postprandial serum bile concentrations were analyzed. Serum samples were analyzed using two different methods: the IDEXX SNAP® Bile Acid test kit utilizing competitive immunoassay technology and a reference method utilizing an enzymatic and colorimetric assay (Bile Acids-L_3K® measured on a Roche Hitachi® 911 biochemistry analyzer). The results were analyzed for correlation using a statistical software package (GraphPad Prizm 4.0). Additionally, pre- and postprandial bile acid concentrations were classified according to their diagnostic category as normal or elevated using the recommended reference ranges of both methods.

There was a significant correlation between the two test methods (Spearman r = 0.7569; 95% confidence interval: 0.6695 to 0.8237; $p < 0.0001$). Seven samples had moderately to severely increased serum total bile acid concentrations (arbitrarily defined for the purpose of this study as concentrations of >60 μmol/L) when measured by the reference method. All 7 of these dogs had serum total bile acid concentrations above the upper limit of the working range of the assay (>30 μmol/L) when assessed by the IDEXX SNAP® assay, indicating that further evaluation using a reference method would be necessary. In 39 cases where pre- and postprandial serum samples were available, liver disease could be excluded in 36 patients (92.3%) using the IDEXX SNAP® Bile Acid test kit. In the 3 remaining cases postprandial bile acid concentrations measured on the IDEXX SNAP® Bile Acid test kit were close to the upper limit or above the working range of the assay necessitating evaluation of serum bile acid concentrations by a reference method.

Based on the results of this study, in dogs the IDEXX SNAP® Bile Acid test for measurement of serum total bile acid concentrations correlates well with the laboratory reference method used here and thus can be used instead of the reference method for ruling out liver disease in dogs. The assay is also useful to indicate the need for further analysis using a reference method in those dogs with serum bile acid concentrations in the higher end of the working range of the assay.

ABSTRACT #295

ANALYSIS OF LINKAGE OF MICROSATELLITE FH2608 WITH IgA DEFICIENCY IN THE GERMAN SHEPHERD DOG. U Tress, LA Clark, JS Suchodolski, DA Williams, JM Steiner. Gastrointestinal Laboratory, Texas A&M University, College Station, TX.

Chronic gastrointestinal disease is frequently observed in dogs. Immunoglobulin A deficiency (IgAD), an inherited primary immune deficiency in humans, has also been reported to occur in certain dog breeds including the German Shepherd Dog (GSD). It has been shown that IgAD in humans leads to an increased risk for the development of chronic gastrointestinal disease and may be associated with chronic gastrointestinal disease in GSDs as well (*J Vet Intern Med* 2005; 19, 473). Previously, mutations in the CARD15 (a.k.a. NOD2) gene in humans have been linked to chronic gastrointestinal disease. FH2608, a canine microsatellite marker on chromosome two, is located at a distance of about two mega bases (MB) of CARD15. The goal of this study was to investigate whether certain alleles of FH2608 are associated with IgAD in GSDs, suggesting CARD15 to be a possible candidate gene for this condition.

Four fecal samples and four buccal swabs each from 62 pure-bred GSDs were collected. IgA was extracted from all fecal samples and IgA concentrations were measured as described previously (*J Vet Intern Med* 2004; 18, 427–428). DNA was extracted from the buccal swabs using a commercial DNA extraction kit. PCR was performed in an Eppendorf Mastercycler to amplify FH2608. The PCR products were analyzed on an ABI 3130 genotyper. Nine of the 62 dogs were IgA deficient and the remaining 53 dogs had normal fecal IgA concentrations. A Fisher's exact test for linkage analysis was used to assess a possible association of different alleles with IgAD. Significance was defined as $p < 0.0001$, the standard p-value for linkage analysis.

The observed alleles for FH2608 were 220, 228, 236, 240, 244, 248, 252, 256, and 260 in the normal dogs and 244, 248, and 252 in the IgA deficient dogs. Allele 248 occurred most often in both IgA deficient and normal dogs with frequencies of 15 out of 18 possible alleles (83%) and 52 out of 106 (49%), respectively. There was no significant association of allele 248 with IgAD ($p = 0.0059$).

In conclusion, the microsatellite FH2608 does not appear to be significantly associated with IgAD in GSDs. However, since FH2608 is not within CARD15 but rather is proximal to the gene, the possibility remains that there are mutations in the CARD15 gene that contribute to the pathogenesis of IgAD and chronic gastrointestinal disease.

ABSTRACT #296

RESPONSE OF SERUM CHOLECYSTOKININ CONCENTRATIONS TO FEEDING IN HEALTHY DOGS. JM Steiner[1], JF Rehfeld[2], KM Findlay[1], JS Suchodolski[1], and SA Read[1]. [1]Gastrointestinal Laboratory, Texas A&M University, College Station, TX; [2]Copenhagen University Hospital, Copenhagen, Denmark.

Cholecystokinin (CCK), is one of the most important gastrointestinal hormones and is synthesized by enterochromaffin cells of the duodenum and jejunum. CCK leads to stimulation of pancreatic enzyme secretion, pancreatic growth, and gall bladder contraction. Serum total bile acid concentrations are widely used for assessment of hepatic function in non-icteric patients. Serum bile acids are assessed after withholding food for 12 hours and again 2 hours after feeding a small amount of a high-fat meal. In normal dogs serum bile acid concentrations rise slightly after feeding. However, there are dogs that show paradoxical serum bile acid results with elevated pre-prandial serum bile acid concentrations that are below the upper limit of the reference range for post-prandial serum bile acid concentrations. Different causes for this phenomenon have been suggested but conclusive data have not been presented. The goals of this study were to measure serum CCK concentrations in dogs after feeding a high-fat meal, to determine whether paradoxical serum CCK results can be observed in healthy dogs, and to determine whether paradoxical serum CCK concentrations correspond to paradoxical serum bile acid concentrations.

Eight healthy client-owned dogs of different breeds were enrolled into this study. The general health of the dogs was evaluated by physical examination, complete blood count, serum chemistry profile, and a heartworm antigen test. Dogs were admitted to the veterinary teaching hospital, fed their regular diet, and then held off food for at least 12 hours. Great care was taken not to expose the dogs to any sight or smell of food. After withholding food for at least 12 hours a baseline blood sample was collected and dogs were fed ¼ can of a high fat canned commercial pet food. Further blood samples were collected at 30, 60, 120, 180, 240, 480, and 720 minutes after feeding. All serum samples were evaluated for serum CCK concentration by use of an in-house immunoassay and for serum total bile acid concentrations using an automated chemistry analyzer. Statistical analyses were performed using a statistical software package (GraphPad Prism 4.00).

Mean serum CCK concentration increased significantly after feeding ($p = 0.0045$). Serum CCK concentrations increased in 6 (75%) of 8 dogs after feeding. In 2 dogs serum CCK concentrations were highest at base-line, thus showing paradoxical results. One of these two dogs with paradoxical serum CCK concentrations in response to feeding also had paradoxical serum bile acid concentrations. However, the second dog showed an increase of serum bile acid concentrations after feeding.

In conclusion, serum CCK concentrations increase in response to feeding. Secondly, paradoxical serum CCK concentrations do occur in response to feeding in some healthy dogs. Additional studies are warranted to further evaluate the relationship of serum CCK and bile acid concentrations in healthy dogs and dogs with gastrointestinal disease.

ABSTRACT #297

ESTABLISHMENT AND CHARACTERIZATION OF A PRIMARY CANINE DUODENAL EPITHELIAL CELL CULTURE. J Golaz[1], IA Burgener[1], N Vonlaufen[2], A Hemphill[2]. [1]Department of Clinical Veterinary Medicine and [2]Institute of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland.

Intestinal epithelial cells (IEC) function as a barrier against viruses, bacteria and parasites present in the intestinal lumen. They act not as a passive barrier, but as active participants in the mucosal immune response, involved in mounting a protective immune response to pathogens whilst maintaining tolerance to harmless environmental antigens such as commensal bacteria and food. The breakdown of this tolerance is a key factor in the

development of chronic intestinal inflammation. The purpose of our study was to establish a primary canine epithelial cell culture that could serve to study the pathogenesis of inflammatory bowel disease and host-pathogen interactions.

The duodenum was selected for tissue harvesting because it is clinically relevant in many forms of inflammatory bowel disease and because of its accessibility by endoscopy. Tissue samples were harvested either by endoscope from adult dogs that were free of clinical and histological signs of gastrointestinal disease or at necropsy from clinically healthy neonatal puppies (supernumerous at a laboratory animal breeding facility) immediately after euthanasia. Following collection, the tissue was washed in ice-cold Dulbecco's Modified Eagle medium (DMEM) supplemented with antibiotics and antimycotics (Primocin, Amaxa) and subsequently disrupted with trypsin for 5 minutes. The surface of the tissue was then scraped gently with a scalpel blade to remove mucus and most of the villi. The tissue was then enzymatically digested with a collagenase/dispase solution and centrifuged on a 2% sorbitol gradient to enrich the crypt cells. The pellet was resuspended with OptiMEM supplemented with Primocin, epidermal growth factor, insulin, hydrocortisone and 10% fetal calf serum (FCS). This suspension was seeded in culture plates coated with Matrigel (BD Bioscience) and placed in an incubator (37°C, 5% CO_2). After 24 hours, the concentration of FCS was reduced to 2.5% and the temperature decreased to 33°C. Thereafter, the medium was renewed every 2–3 days.

Following this procedure, the primary cultures were growing to confluent monolayers within 5–6 days. The IEC were viable and could be maintained and studied for an average of 2 weeks, with the cultures from neonatal tissue mostly outliving the cultures from endoscopic biopsies. The epithelial nature of these cells was confirmed by electron microscopy and by immunofluorescence yielding desmosomes (ZK–31, Sigma), tight junctions (anti-occludin OC-3F10, Zymed) and cytokeratins (C2562, Sigma). In western blots, the cytokeratins 8 and 18 were found to be the most abundant cytokeratins in these cultures.

We conclude that canine duodenal epithelial cells can be cultured in vitro and may serve as a useful model for studying the pathogenesis of inflammatory bowel disease and host-pathogen interactions.

ABSTRACT #298

COMPLICATIONS AND LONG-TERM OUTCOME OF DOGS WITH ESOPHAGEAL FOREIGN BODY - 59 CASES (2000-2005). Krista B. Halling and Stephen Kruth. Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.

A retrospective study was performed to describe the clinical presentation, complications and long-term outcome of dogs with esophageal foreign body (EFB).

Medical records at the Ontario Veterinary College Veterinary Teaching Hospital were searched to identify dogs undergoing endoscopic or surgical intervention for EFB between 2000 and 2005.

Fifty-nine dogs were identified. 37/59 (78%) dogs weighed less than 15 kg. Duration of foreign body lodgment was less than 24 hours (32 dogs), 1 to 4 days (18 dogs), or greater than 4 days (9 dogs). Survey radiographs were diagnostic for EFB in 37/37 cases. The most common EFB was bone (59%). EFB were most commonly lodged at caudal esophagus (47%). Endoscopy alone was successful for EFB retrieval in 49 (83%) dogs: retrieval through mouth (41 dogs) or advancement into stomach (8 dogs). In 2 dogs EFB was advanced into stomach followed by gastrotomy, 2 dogs required gastrotomy approach to caudal esophagus, 1 EFB passed spontaneously into stomach, 4 dogs required esophagotomy, 1 dog required esophageal resection and anastomosis. Early complication rate was 14/59 (24%) with 6 dogs dying: aspiration pneumonia (7 dogs; 2 died), esophageal wall penetration by foreign body (2 dogs; 1 died), esophageal perforation (6 dogs; 4 died), dehiscence of esophageal anastomosis (1 dog; 1 died), iliac thromboembolism (1 dog; 1 died), tracheal compression with cardiorespiratory arrest (1 dog; 0 died). Of 5 dogs that were febrile at presentation, 1 had aspiration pneumonia and 3 had esophageal perforation. 28 dogs were fed slurry or hard food within 48 hours post-retrieval; outcome was normal in 25/28 dogs. 2 dogs had pre-existing esophageal stricture, 1 dog developed apparent secondary esophageal stricture. The late complication rate was 3/34 (9%) dogs and consisted of esophageal stricture (3 dogs; 0 died). Long-term (6 weeks to 62 months) outcome was available for 34 dogs: 28 were normal, 5 required dietary management, 1 required bougienage. Foreign body lodgment for longer than 2 days was associated with increased mortality.

Survey radiography is usually diagnostic for EFB. Patients with an EFB history greater than 24 hours, fever, or radiographic signs of esophageal perforation should be treated on an emergency basis with consideration for esophagotomy. Early diagnosis and endoscopic intervention of EFB within 24 hours minimizes the risk of life-threatening complications.

ABSTRACT #299

SUPPRESSION OF CHYLOMICRON TRIACYLGLYCEROL BUT NOT LIPOPROTEIN LIPASE OR LIPOPROTEIN-CHOLESTEROL DISTRIBUTION DURING WEIGHT LOSS IN BEAGLES FED DIACYLGLYCEROL ENRICHED OIL. Y. Mitsuhashi[1], D. Bandy[1], D. Nagaoka[1], K. Bigley[1], R. Angell[1], T. Umeda[2], K. Otsuji[2] , J. E. Bauer[1]. [1]Comp An Nutr Lab, College of Veterinary Medicine and [3]Faculty of Nurition, Texas A&M University, College Station, TX, USA. [2]Kao Corp., Tokyo, Japan.

Obesity is the most common nutritional disorder in canine veterinary practice. Dietary components to improve adipose tissue mobilization or prevent its accumulation have potential to help manage this important problem. Vegetable oils enriched in diacylglycerol (DAG) are one such component because utilization of DAG favors fatty acid beta-oxidation rather than re-esterification. Thus, compared to traditional triacylglycerol oils (TAG), less lymphatic secretion of chylomicron triglyceride (TG) and less net TG storage may occur. In addition, low glycemic index (LGI) starch may also be beneficial. We previously found that a single meal containing DAG and LGI starch in normal weight beagles resulted in post-prandial TG lowering. The present study investigated effects of DAG and LGI starch containing diets in obese dogs for a longer period. Specifically we addressed whether decreased chylomicron secretion or increased utilization would explain TG lowering effects observed in our earlier study. Twelve obese adult, female beagles with body condition scores of 8.2 ± 0.2 (SEM) out of 9 and % body fat of 40.9 ± 1.9 (SEM) were randomly divided into four groups. Four diets were formulated containing poultry meal, DAG or TAG oil, low- (LGI, high amylose corn) or high- (HGI, waxy corn) glycemic index starch, and vitamin/mineral premix (ca. 4300 kcal/kg DM). The diets were fed mixed with water as a gruel. After acclimation to a similar diet, the dogs were fed one of the experimental diets for 9 wk (n = 3 per group). Dogs were offered the equivalent calories each day to maintain their starting obese body weights, weighed weekly, and food consumption recorded daily. Percent body fat was measured 1 wk prior to the study and at wk 4 and 9 using bioelectrical impedance. Fasting blood was collected at wk 1, 4 and 8 for plasma cholesterol (TC) and lipoprotein profiles. Also on wk 1 and 8, post-heparin blood samples were collected 10 min after iv injection of 100 I.U. Na heparin/kg-body wt, and lipoprotein lipase (LPL) activities determined. Blood was also collected and pooled for each dog at 2 and 3 hr post-prandially on wk 8 after feeding a test meal with cooked chicken breast meat in place of poultry meal in the diet mixture. Chylomicrons were isolated from the 2 and 3 hr pooled samples via ultracentrifugation and TG determined. The dogs consumed 68 ± 4 (SEM)% of the diet amounts offered. All dogs lost body weight and body fat independently of diet type by ANOCVA (kcal consumed as covariate). No significant differences were found in plasma TC, lipoprotein-cholesterol distributions, or LPL activities. However, chylomicron TG concentrations were lower ($p = 0.012$) with DAG vs TAG independent of starch type. This latter finding combined with no differences in LPL activities supports the suppression of chylomicron TG production with DAG rather than increased TG utilization via LPL. Hence, DAG diets may be more beneficial in supporting healthy weight loss while mitigating excessive post-prandial chylomicron TG assembly and secretion during weight reduction compared to TAG diets.

ABSTRACT #300

PLANT-BASED FIBERS RESULT IN REDUCED ENERGY INTAKE IN THE FIRST MEAL FOR DOGS FED TWICE DAILY. LS Rae[1], J Rand[1], J Morton[1], A Litster[1], E Seton[1], E Flickinger[2]. [1]Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Australia. [2]The Iams Company, Ohio, United States of America.

The purpose of this study was to evaluate the effect of fiber type and carnitine on energy intake in young adult dogs to identify a diet that may aid in management and prevention of obesity.

Eighteen neutered (11 males, 7 females) mixed-breed young adult dogs were studied. Dogs were clinically healthy based on physical examination and routine hematological and biochemical analyses. All dogs were sequentially fed five test diets and a control diet, with each dog randomly allocated to dietary sequence using a Latin square design. In 4 of the 5 test diets, whole corn in the control diet was substituted, as is, for 0.5% fructooligosaccharides, 1% high viscosity sodium carboxymethylcellulose, 0.5% plant fiber extract A and 2% plant fiber extract B. The fifth test diet contained 100 ppm L-carnitine compared to 30 ppm L-carnitine in the control diet and the other 4 test diets. Crude fiber on a dry matter (DM) basis was 2.4%–3.0% for all 6 diets. Dogs were fed each diet for 7 consecutive days, with no washout period between diets. The feeding regimen consisted of 2 meals, 4 hours apart, with any uneaten food removed and weighed 1 hour after each meal was offered. To prevent weight gain during the study, on days 1 and 2 of each week, dogs were fed 2 meals comprising 75% and 25% of maintenance

energy requirements (MER), which were calculated based on daily energy intake in the preceding month. On days 3 to 7 of each week, the 2 meals comprised 75% and 150% of MER and only data from these days were analyzed. Food intake was measured for each of the 2 meals on a daily basis. Body weight and body condition score (scale 1–5) were assessed weekly.

At the first meal, energy intake (expressed as % MER) of diets containing 0.5% plant fiber extract A or 2% plant fiber extract B was significantly less than diets containing 100 ppm L-carnitine, 0.5% fructooligosaccharides or the control diet. However, diet type was not associated with total energy intake for the two meals combined. Energy intake at the first meal was negatively associated with energy intake at the second meal.

We conclude that diets containing 0.5% fructooligosaccharides, 1% carboxymethylcellulose, 0.5% plant fiber extract A, 2% plant fiber extract B or 100 ppm L-carnitine do not significantly reduce total energy intake in young adult dogs and therefore would not be expected to result in short-term rapid weight loss. However, it is possible that dogs fed only one meal daily over long periods with supplemental plant fiber extracts might have lower energy intake than those fed diets containing whole corn, which could contribute to maintenance of ideal body weight. This hypothesis needs to be investigated in appropriate long-term studies.

ABSTRACT #301

DIETARY SUPPLEMENTATION WITH RESVERATROL RESULTS IN GREATER BODY WEIGHT GAIN AFTER GONADECTOMY THAN SUPPLEMENTATION WITH L-CARNITINE IN HEALTHY YOUNG ADULT DOGS. LS Rae[1], J Rand[1], J Morton[1], A Litster[1], E Seton[1], E Flickinger[2]. [1]Centre for Companion Animal Health, School of Veterinary Science, The University of Queensland, Australia. [2]The Iams Company, Ohio, United States of America.

It has been well documented that gonadectomy increases the risk of dogs and cats becoming overweight, especially if they are fed ad libitum and lack regular exercise. Studies have shown when estradiol benzoate was given to ovariectomized rats, their bodyweight normalized. However, in a study of post-menopausal women, hormone replacement therapy did not prevent an increase in fat mass. The purpose of this study was to investigate the effects of the dietary supplements, resveratrol and L-carnitine on changes in bodyweight and body condition score following gonadectomy in clinically healthy young adult dogs. Many commercial dog foods include soybeans. Soybeans contain phytoestrogens and phytoestrogens exert both estrogenic and antiestrogenic effects on health in animals and humans. Resveratrol, a phytoalexin found in grapes, wine and peanuts, also may act as a phytoestrogen, having mixed agonistic/antagonistic estrogenic effects. L-carnitine is required for energy production from fat. In a study of obese cats on a weight reducing diet, those supplemented with L-carnitine lost weight more rapidly than cats not receiving L-carnitine. Thirty-five entire (18 male, 17 female) mixed-breed young adult dogs were studied. Dogs were clinically healthy based on physical examination and routine hematological and biochemical analyses. A double-blinded, randomized controlled trial was conducted with blocking based on gender, body weight and body condition score. Dogs within each block were randomly assigned to one of three treatments: resveratrol (60 mg/day), L-carnitine (12 mg/day) and placebo. All 35 dogs underwent gonadectomy two weeks prior to commencing the 16 week study. They received a commercially available canine maintenance diet and supplements or placebo were administered orally using microcrystalline capsules. Dogs were fed once a day at night, and capsules were administered at the time of feeding. Quantities of food in excess of the dogs' expected consumption were fed overnight, to allow free access to food for 14 hours daily. Any excess food was removed and weighed each morning, enabling calculation of daily food intake. Body weight and body condition scores (scale 1–5) were assessed weekly. Mean body weight and body condition increased in all treatment groups during the study. Supplement type (resveratrol, L-carnitine or placebo) was not significantly ($p = 0.09$) associated with change in bodyweight. However, in a pair-wise comparison, supplementation with resveratrol resulted in a significantly greater increase in bodyweight than L-carnitine ($p = 0.034$). Mean increases were 6.2 kg and 2.7 kg for resveratrol and L-carnitine, respectively. Changes in body condition score over the 16 week study did not differ significantly between treatment groups. We conclude that in recently gonadectomized healthy young adult dogs, dietary supplementation with resveratrol results in a greater increase in bodyweight than supplementation with L-carnitine.

ABSTRACT #302

A LOW CARBOHYDRATE, HIGH PROTEIN, MODERATE FAT AND FIBER DIET REDUCES POSTPRANDIAL GLUCOSE CONCENTRATIONS COMPARED WITH A TRADITIONALLY RECOMMENDED CANINE DIABETES DIET AND AN ADULT MAINTENANCE DIET IN HEALTHY DOGS. Kathryn F Elliott[1], Jacquie S Rand[1], Linda M Fleeman[1], John M Morton[1], Annette L Litster[1], Vincent C Biourge[2], Peter J Markwell[3] [1]Centre for Companion Animal Health, the University of Queensland, Australia, [2]Royal Canin, France, [3]WALTHAM Centre for Pet Nutrition, UK.

The objective of this study was to compare the effect of a new low carbohydrate diabetes diet (low CHO diet: carbohydrate 25%ME, protein 43%ME, fat 32%ME, fiber 3 g/100 kcal) with a diet traditionally recommended for canine diabetes (high CHO diet: carbohydrate 55%ME, protein 22%ME, fat 23%ME, fiber 10 g/100 kcal) and a commercially-available maintenance diet (control diet: carbohydrate 45%ME, protein 24%ME, fat 31%ME, fiber 2 g/100 kcal) on postprandial plasma glucose, insulin, triglyceride, and free fatty acid concentrations in healthy dogs.

Twelve (6 male, 6 female) healthy, neutered, mixed breed dogs with ideal body condition weighing 12–23 kg were studied. Dogs were randomly assigned to 1 of 3 diet sequences in a 3 period crossover study. Each diet was fed for 3 weeks, then plasma glucose, insulin, triglyceride, and free fatty acid concentrations were measured at −1, −0.08, 1, 2, 3, 4, 5, 6, 9, and 12 hr after a meal of 50–65 kcal/kg body weight. Analysis of variance (ANOVA) was performed on mean analyte concentrations, area under the curve (AUC) from zero, and AUC/gram of carbohydrate or fat fed/kg body weight. Fisher's Least Significant Difference was calculated from ANOVA to compare the dietary effect.

The low CHO diet resulted in the lowest postprandial glycemic load with $AUC_{glucose}$ significantly lower than for both other diets. Despite the differences in dietary fat content, $AUC_{triglyceride}$ was not significantly different between the low CHO diet (fat 32%ME) and the high CHO diet (fat 23%ME). Although the fat content of the control diet (fat 31%ME) was similar to the low CHO diet, it resulted in significantly higher postprandial triglyceride concentrations compared with both other diets. Mean postprandial free fatty acid concentration was significantly lower between 2–5 hr for the high CHO diet than the other diets. Because there was no difference in $AUC_{free\ fatty\ acid}$ 2–5 hr/gram of fat fed/kg body weight, the difference between diets could be accounted for by the amount of fat ingested in the meal. In contrast, $AUC_{glucose}$ to 12 hr/gram of carbohydrate fed/kg was significantly greater for the low CHO diet compared with both other diets, indicating that the difference in postprandial glucose concentrations between the diets could not be fully accounted for by differences in amount of carbohydrate ingested.. Although $AUC_{insulin}$ to 12 hr was significantly greater for the control diet compared with both other diets, no consistent significant difference in mean insulin concentration was found.

We conclude that a restricted carbohydrate, high protein, moderate fat and fiber diet might be advantageous for diabetic dogs, and warrants further evaluation of clinical benefit in diabetic dogs.

ABSTRACT #303

INDUCTION OF OBESITY IN BEAGLES BY FEEDING DOG FOOD/HUMAN FOOD COMBINATIONS. J. E. Bauer, Y. Mitsuhashi, D. Nagaoka, K. Bigley, D. Bandy, R. Angell. Companion Animal Nutrition Lab, College of Vet Med, Texas A&M Univ., College Station, TX.

Treatment of companion animal obesity is a common nutritional problem in veterinary practice. Most treatment regimens include reduced calorie intake and increased activity but these are fraught with frustration. A better understanding of obesity management in the pet population may come from modeling how obesity is initially induced under more controlled conditions in a colony setting. Knowledge of such key features as the number of calories, length of time, and food types leading to obesity may provide animal owners with better advice regarding nutrition interventions. A study was thus conducted to follow the time course of obesity induction in a beagle colony using commercially available dog foods combined with human foods to mimic at home snacking and diet supplementation behaviors. Adult female, modestly overweight, beagles were individually housed in approved sized kennels with 12 hr light cycles, allowed free access to water and exercise, and fed *ad libitum* during the study ($n = 9$). No effort was made to quantify the amount of exercise of each animal; some variability was observed. Only the amount of total calories and food types offered were changed over time based on starting body wt (10.4–14.3 kg range, 12.0 kg median). The dogs were initially fed 2× their daily calculated number of calories ($2X130BW^{0.75}$) using a commercially available dry type extruded diet plus 40 cc of a 50 : 50 blend of canola and soybean oils. After 3 weeks the animals were fed this mixture along with 5 pecan shortbread cookies for the next 16 weeks (wk 4–19). Total calorie consumption was estimated daily and weekly averages were calculated. Body condition scores (BCS) and body wt were determined weekly. Percent body fat was measured at selected intervals using bioelectrical impedance techniques. Data were analyzed via repeated measures ANOVA with LSD multiple comparisons where

appropriate ($p < 0.05$). At the end of the first three weeks BCS had not changed and body weights were not significantly different. During this time, mean daily calorie intake averaged 1228 ± 48 kcal with ca. 2020 kcal/day offered. Consumption was approximately 50% over basal needs although ca. 140% more than basal calories were offered. The animals self-regulated their intakes during this time. Between wk 4 and 15, after the cookies were added, daily calorie consumption increased to 1820 ± 137 kcal with ca. 2470 calories being offered. At wk 9 body weights were statistically significant from those measured at wk 1–8. They continued to increase until wk 15 after which a plateau was reached. Similarly, BCS were significantly different beginning at wk 9 and thereafter reaching a plateau at wk 14, $p < 0.05$. Percent body fat increased incrementally from 32 ± 2 (SEM) at wk 6 to 40.2 ± 2.3 (SEM) at wk 18, $p < 0.05$. Adding the human snack food (cookies) stimulated palatability of the entire ration overall. During wk 16–19 the average daily calorie consumption dropped to 1410 ± 70 kcal (ca. 25%) even though the kcal offered remained constant (ca. 2470). The animals had thus reached an obese set-point (steady state) whereby they were able to maintain their obese body weights at a lower total caloric intake than that needed to achieve their obese state. It is concluded that human snack foods increase the net calorie consumption of the total ration and provide a calorie dense dietary supplement leading to the rapid induction of an obese steady state that requires fewer calories to maintain.

ABSTRACT #304

DISTRIBUTION OF GLUCOKINASE IN FELINE HEPATOCYTES DETERMINED BY IMMUNO-FLUORESCENCE CONFOCAL MICROSCOPY. O Suwitheechon[1], D Boyle[2], T Schermerhorn[1]. [1]Dept. Clinical Sciences, College of Veterinary Medicine, Kansas State University. [2]Div. Biology, Kansas State University.

In most species, glucokinase (GK) undergoes a nuclear-cytoplasmic translocation cycle that is regulated by carbohydrates. During fasting, GK is sequestered in the hepatocyte nucleus by the GK regulatory protein (GKRP). After feeding, GK dissociates from GKRP and translocates to the cytoplasm where it is metabolically active. Release of the nuclear GK reserve is important for normal hepatic glucose handling. Previously, our lab showed that the feline liver expresses GK but not GKRP, suggesting that GK cycling is not present in feline hepatocytes. The purpose of this study was to investigate the hypothesis that GK does not localize in the nucleus of feline hepatocytes under fasted conditions.

Rat liver was used as a control tissue. Hexokinase 1 (HK1), which does not localize to the nucleus, was used as an additional control. Liver samples were obtained from fasted animals and prepared for microscopy using routine techniques. Liver sections were probed for target proteins using primary antibodies against GK, HK1, or GKRP. Secondary antibodies labeled with either Alexa-Fluor488 or Alexa-Fluor555 were used to label bound primary antibodies. Images were obtained using a Zeiss laser-scanning confocal microscope.

GK was primarily detected in the cytoplasm of feline hepatocytes and in the nucleus of rat hepatocytes. HK1 had a cytoplasmic localization in both cat and rat hepatocytes. In dual label experiments, GK and HK1 were both detected in the cytoplasm of feline hepatocytes but the labels were distinct indicating that the antibody for GK did not cross react with HK 1 in feline hepatocytes. GKRP was abundant in the nucleus of rat hepatocytes but was absent in feline hepatocytes.

The results indicate that GK has a cytoplasmic rather than a nuclear localization in fasted feline hepatocytes. The feline distribution pattern is in contrast to the nuclear localization detected in fasted rat hepatocytes. The cytoplasmic location of GK in feline hepatocytes is consistent with the lack of detectable GKRP expression in the feline liver. The lack of nuclear GK reserve may contribute to the low GK activity reported in feline hepatocytes.

ABSTRACT #305

DEPLETION OF INTRAMUSCULAR TRIGLYCERIDES AND HYPERKETONEMIA IN SLED DOGS DURING PROLONGED EXERCISE. KW Hinchcliff[1], EC McKenzie[2], L. Durocher[1], KK Williamson[3], MS Davis[3]. [1]The Ohio State University, Columbus, OH. [2]Oregon State University, Corvallis, OR. [3]Oklahoma State University, Stillwater, OK.

Alaskan sled dogs run distances of >1600 km in less than 10 days. The dogs eat a high fat diet but do not have cumulative depletion of muscle glycogen despite the low intake of carbohydrate suggesting use of other intramuscular or extramuscular energy substrates. Intramuscular triglyceride is an important source of energy during prolonged exercise, however the effect of the prolonged and repetitive exercise on muscle triglyceride concentrations is not reported. Therefore the purpose of this study was to determine the extent of depletion and recovery of muscle triglyceride concentrations during 4 days of repetitive prolonged submaximal exercise in elite Alaskan sled dogs.

Fifty-four fit sled dogs were used in the study. Six dogs were used as trained non-exercising control animals. The remaining 48 dogs ran 140 km/day for up to 4 days while consuming a diet providing 39% of calories as fat and 33% as carbohydrate. Six dogs were randomly selected before the trial or to be withdrawn after 140, 420 or 560 km. Muscle biopsies were obtained immediately after exercise and 7 h after the same dogs rested and consumed a large meal. Additional single muscle biopsies were performed on dogs that completed 4 days of exercise after 28 (6 dogs), 50 (6 dogs) and i98 (5 dogs) h of recovery. Muscle samples were analyzed for muscle triglyceride concentration (IMTG) and data were analyzed via analysis of variance. Significance was set at $P < 0.05$. Blood samples were collected concurrent with collection of muscle samples. Muscle samples were analyzed for muscle triglyceride concentration (IMTG) and data were analyzed via analysis of variance techniques. Significance was set at $P < 0.05$.

Thirty-eight of 48 dogs completed distances that allowed them to be used in investigative procedures. IMTG in control dogs was 26.3 ± 10.3 (SD) mmol/kg dry weight. IMTG immediately after 140 km was 6.1 ± 4.0 mmol/kg and remained low after 420 and 500 km (9.8 ± 6.8 and 6.7 ± 5.2 mmol/kg, respectively). IMTG increased significantly during the 7 hour rest period (7.5 ± 5.4 to 11.2 ± 4.8 mmol/kg, $P = 0.008$) after each run. IMTG rose progressively over 96 hours of recovery and values at 96 hours were not significantly different to that of control dogs. Serum ketone concentrations increased from 24 ± 10 μmol/L in control dogs to 301 ± 127 μmol/L after 140 km, and then declined ($P < 0.001$). Serum concentrations of non-esterified fatty acids, but not triglyceride, increased significantly during exercise. Serum concentrations of insulin declined and concentrations of glucagon and cortisol increased significantly during exercise.

Prolonged exercise depleted IMTG in sled dogs. This change was associated with ketonemia and evidence of lipolysis (NEFA) in a hormonal milieu that favored lipolysis.

ABSTRACT #306

CLINICAL RESPONSE TO HUMAN ALBUMIN ADMINISTRATION IN HEALTHY DOGS. LA Cohn, ME Kerl, JR Dodam, J Throop. University of Missouri – College of Veterinary Medicine, Columbia, MO.

Veterinarians sometimes administer commercial human albumin products to treat hypoalbuminemic dogs and presumably decrease morbidity and mortality associated with the condition. The purpose of this study was to describe the clinical and clinicopathological reactions of healthy dogs to administration of human albumin.

Nine healthy adult dogs (mean weight 20.3 kg) with normal serum albumin concentrations were studied. Complete physical exam (PE) with blood pressure (BP) and gait evaluation, CBC, chemistry profile, plasma oncotic pressure, and assay of microalbuminuria were completed just prior to treatment. Each dog was to be administered 50 g of a 25% human albumin solution beginning at 0.5 ml/kg/hr. Attitude, temperature, pulse, respiration, and blood pressure were monitored every 15 to 30 minutes and if stable, infusion rate was increased incrementally to a maximum of 4 ml/kg/hr. Infusion was stopped if adverse reaction was observed in which case the dog treated with intravenous crystalloid fluids and diphenhydramine. Identical parameters were checked 1, 2, 4 and 8 hours post infusion. At 24, 48, 72 hours post infusion as well as at 7, 14, and 21 days post infusion, PE with BP, gait evaluation, CBC, and albuminuria assay were repeated. Chemistry profile was repeated at 24 hours and 7 days. In two dogs, albumin infusion was repeated 5 weeks after the first infusion. Either parametric (repeated measures analysis of variance) or non-parametric (Friedman repeated measures analysis of variance on ranks) tests were used to compare clinical and clinicopathological parameters based on normality of distribution with $p < 0.01$ considered significant.

Adverse clinical reactions were observed after both first and second albumin infusions. One dog treated developed anaphylactic shock after administration of no more than 1 ml of albumin but recovered with treatment. This same dog developed severe facial edema and urticaria 6 days following infusion. A second dog did not experience problems during the albumin infusion, but also developed severe facial, limb, and ventral edema as well as urticaria 7 days after infusion. No adverse reactions were observed after initial infusion in the remaining 7 dogs. Two dogs without adverse reaction to initial infusion were administered albumin a second time 5 weeks after the first infusion. Both dogs developed anaphylactic shock after infusion of only 0.2 to 0.3 ml of albumin. Both dogs recovered, and neither developed edema or urticaria. Short-term increases were detected in serum albumin, plasma proteins, and calcium after infusion of albumin. Significant decreases were observed in serum cholesterol as well as platelet and lymphocyte counts.

Administration of human albumin resulted in profound hypersensitivity reactions in 2 of 9 dogs receiving a single infusion, and in 2 of 2 dogs receiving a second infusion 5 weeks after the first. Repeat administration of human albumin to dogs should be avoided, and first administration must be carefully observed so that hypersensitivity reactions can be treated in a timely manner.

ABSTRACT #307

STABILITY OF CARVEDILOL IN AN ORAL LIQUID PREPARATION. SG Gordon,[1] DM Boothe,[2] VA Gaudette[1], MW Miller.[1] [1]Department of Small Animal Clinical Science and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas and [2]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama.

Carvedilol (Coreg ®) is a 3rd generation non-selective beta blocker with alpha$_1$ blocking and antioxidant properties licensed for the treatment of essential hypertension and congestive heart failure (CHF) in humans. Chronic beta blockade has proven useful in ameliorating systolic dysfunction in an experimental model of canine mitral insufficiency. Mitral insufficiency resulting from spontaneous, chronic degenerative valvular disease (CVD) is the leading cause of CHF in the dog. Currently, there is no therapy known to delay progression of asymptomatic CVD to CHF. Yet, the beneficial pharmacologic properties of carvedilol may be useful in this regard. Although the target dose and dosing interval in dogs with asymptomatic CVD have been reported, a gradual up-titration protocol is imperative to minimize potential side effects. A stable suspension would facilitate accurate up-titration and reduce cost.

The purpose of this study was to evaluate the stability of a compounded oral suspension of carvedilol. A suspension of carvedilol was produced by powdering 25 mg tablets, then adding enough deionized water to make a paste, allowing the tablet coating to dissolve. The paste was then suspended in commercially available simple syrup to a concentration of 2 (n = 1) or 10 (n = 1) mg/ml. Suspensions were stored in amber screw top plastic bottles and refrigerated at 22–25°C. Aliquots (1 ml) were then collected and frozen immediately at 0°C on days 1, 7, 14, 21, 30, 60 and 90. The suspension was manually shaken prior to all sample collection to ensure adequate dispersion of the suspension.

Samples were prepared by solid phase extraction using a C8 column (Varian, Inc, # 1210–2059, Palo Alto, CA) and 90% methanol & 10% KH_2PO_4 solution as the elutant. The eluant was subjected to reverse phase HPLC according to previously described methods with slight modifications. Briefly, carvedilol was separated from the eluant using a C18 5µ 250 × 4.6 mm column (Prevail; Alltech #99211). Carvedilol was eluted using a mobile phase comprised of 40% 50 mM KH_2PO_4 adjusted to a pH 2.5 and 60% methanol at a flow rate of 1.0 ml/min. Carvedilol was detected using fluorescence spectrophotometry set for an excitation wavelength (λ) of 47nm and a mission λ of 344 nm. The assay was validated in canine plasma; lower and upper limits of quantitation (LOQ) of were 2.0 and 800 ng/ml, respectively and were based on the lowest and highest concentrations that could be accurately detected within 15%, with the exception of the lowest control for which 20% variability was accepted.

Carvedilol 2 mg/ml and 10 mg/ml was stable in oral suspension in a vehicle of commercially available simple syrup for at least 90 days when stored at 22–25°C and protected from light. This information may facilitate the clinical utility of carvedilol in veterinary medicine.

ABSTRACT #308

EVALUATION OF AN ION SELECTIVE ELECTRODE IN DETERMINATION OF CANINE SERUM BROMIDE CONCENTRATION. WJ Kimber, CL Gaskill. Atlantic Veterinary College, Charlottetown, PEI, Canada.

Potassium bromide (KBr) is a commonly used treatment for canine epilepsy. The recommended therapeutic range for serum bromide concentration is 12.5–31.3 mM (100–250 mg/dl) when combined with phenobarbital, and 12.5–37.5 mM (100–300 mg/dl) when used as monotherapy. The purpose of this study was to evaluate an ion selective electrode technique of measuring canine serum bromide concentration (Radiometer Copenhagen PHM 240 pH/ion meter, Willich-Schiefbahn, Germany). Performance parameters including accuracy, precision, linearity, detection limit, and interference from hemolysis, lipemia, chloride and phenobarbital were determined. Pooled canine serum was spiked with KBr to achieve final bromide concentrations of 1, 2.5, 5, 10, 20, 30, 40, and 50 mM. Measurements of spiked serum samples and a control (0 mM bromide) were performed using the PHM 240 ion selective electrode. Recoveries ranged from 113.0–122.0% (mean = 116.0%, st dev = 3.7), coefficient of variation ranged from 2.0–4.4% for intrassay precision, and 1.1–9.7% for interassay precision, and the linear regression coefficient was 0.99. Hemolysis significantly increased measurements by 9.2–39.9% ($P < 0.001$) in a linear fashion ($r = 0.97$), depending on hemoglobin concentration (2.39–7.44 g/L; corresponding to 1+–4+ hemolysis). Lipemia significantly increased measurements by 4.3–12.5% ($P < 0.001$) in a linear fashion, depending on degree of lipemia. No significant interference occurred from chloride or phenobarbital. We conclude that this ion selective electrode technique is suitable for measurement of bromide concentration in non-hemolyzed, non-lipemic serum samples.

ABSTRACT #309

SERUM CHEMISTRY ALTERATIONS IN ALASKAN SLED DOGS DURING FIVE SUCCESSIVE DAYS OF PROLONGED ENDURANCE EXERCISE. EC McKenzie[1], KW Hinchcliff[2], KK Williamson[3], MS Davis[3]. [1]Oregon State University, Corvallis, OR. [2] The Ohio State University, Columbus, OH. [3]Oklahoma State University, Stillwater, OK.

Alaskan sled dogs commonly run prolonged distances on multiple consecutive days during competition. Such exertion can result in catabolism of energy stores, muscle damage, and potentially dehydration and electrolyte and acid-base aberrations. The purpose of this investigation was to determine the impact of multi-day endurance exercise on serum biochemical variables in elite Alaskan sled dogs via serial blood sampling and analysis.

Sixteen fit Alaskan sled dogs were used in the study. Six dogs served as non-exercising controls and the remaining 10 dogs ran 160 km/day for 5 consecutive days. Serum samples were obtained from all dogs prior to exercise and from running dogs immediately after each exercise segment prior to feeding. Serum electrolyte, mineral and protein concentrations were determined in addition to indicators of liver injury (ALP, ALT, bilirubin) and renal function (urea N, creatinine). Serum cardiac troponin I concentrations and CK and AST activity were measured. Data were analyzed using commercial statistical software using PROC GLM with dog as a blocking variable and time as a covariate. Linear and quadratic contrasts were performed. Significance was set at $P < 0.05$.

Prolonged endurance exercise resulted in significant increases in serum [Cl], [P], urea N, CK, AST, ALT and cardiac troponin I. A progressive decrease was noted in serum [K] and total protein (R^2 0.994). An increase in serum [Cl] was the most prominent electrolyte change (111 ± 2 mEq/L at rest; >120 mEq/L at all other timepoints; R^2 0.869). Serum [K] was 4.8 ± 0.3 mEq/L at rest and decreased linearly to 4.3 ± 0.3 mEq/L after 800 km (R^2 0.621). All dogs were hypoglobulinemic at rest (2.2 ± 0.3 g/L) and displayed progressive depletion of serum globulin. (1.6 ± 0.4 g/L after 800km; R^2 0.882). Serum albumin decreased with exercise (R^2 0.945) but remained within normal range (3.8 ± 0.2 at rest; 3.2 ± 0.3 at 800 km). Serum AST was elevated at rest (32 ± 6 IU/L) and peaked at 480 km (230 ± 167 IU/L). Serum CK (log) was higher than at rest after all distances (R^2 0.843). Exercise had a significant impact on serum ALT (R^2 0.911) which increased above normal range after 480km or more. Serum creatinine increased significantly with exercise (R^2 0.618) but remained within normal range. Serum urea N was greater than normal at all time points excluding rest (R^2 0.663). Serum cardiac troponin I concentrations were elevated above 0.1 mg/dL (R^2 0.102) after all distances run and were greatest after 160 km (0.56 ± 0.3 mg/dL).

Trained Alaskan sled dogs display mild but specific aberrations in serum biochemical variables with repeated prolonged exercise. These findings probably result from alterations in energy metabolism, prolonged muscular activity and changes in glomerular function.

ABSTRACT #310

MUSCLE GLYCOGEN REPLETION DURING PROLONGED REPETITIVE EXERCISE BY ALASKAN SLED DOGS. EC McKenzie[1], KW Hinchcliff[2], SJ Valberg[3], KK Williamson[4], MS Davis[4]. [1]Oregon State University, Corvallis, OR. [2]The Ohio State University, Columbus, OH. [3]University of Minnesota, St Paul, MN. [4]Oklahoma State University, Stillwater, OK.

Elite Alaskan sled dogs have been shown to be capable of running 160 km/day for 5 consecutive days without cumulative depletion of skeletal muscle glycogen even while consuming a relatively low carbohydrate diet. However, it is not known if preservation of muscle glycogen stores in these dogs results from rapid glycogen replenishment during brief recovery

periods, or from progressive dependence on energy sources other than muscle glycogen. Therefore the purpose of this study was to determine the depletion and recovery of muscle glycogen stores during 4 days of repetitive prolonged submaximal exercise in elite Alaskan sled dogs.

Fifty-four fit sled dogs were used in the study. Six dogs were used as trained non-exercising control animals. The remaining 48 dogs were scheduled to run 140 km/day for up to 4 days while consuming a diet providing 39% of calories as fat and 33% as carbohydrate. Six dogs were randomly selected and withdrawn immediately after 140, 420 and 560 km. Muscle biopsies were performed in these dogs immediately after exercise and 7 hours later after rest and a large meal. Additional single muscle biopsies were performed on dogs that completed 4 days of exercise at 28 (6 dogs), 50 (6 dogs) and 98 (5 dogs) hours of recovery. Muscle samples were analyzed for glycogen concentration (MG) and data were analyzed via analysis of variance techniques. Significance was set at $P < 0.05$.

Ten dogs were withdrawn prematurely due to musculoskeletal injury and 38 dogs underwent investigative procedures. MG in control dogs was comparable to previously reported values for elite sled dogs (375 $\pm$ 37 mmol/kg dry weight). MG immediately after 140 km (137 $\pm$ 36 mmol/kg) was significantly lower than before exercise but not different to MG immediately after 420 km (203 $\pm$ 30 mmol/kg). MG in dogs immediately after running 560 km (298 $\pm$ 86 mmol/kg) was significantly greater than in dogs that ran the lesser distances and not significantly different to MG of control dogs. During recovery periods, MG increased significantly, but the rate of replenishment was no different after running 140, 420 or 560 km (13.5 $\pm$ 6.7; 15.5 $\pm$ 5.0; 11.0 $\pm$ 4.5 mmol/kg/hr respectively). MG in single samples after 28 hours of recovery increased to 122% of control values (457 $\pm$ 88 mmol/kg) and remained elevated at 50 and 98 hours of recovery (464 $\pm$ 123 and 458 $\pm$ 41 mmol/kg respectively). MG 50 hours after exercise was significantly greater than control values but no different to concentrations at 28 and 98 hours.

MG progressively increases in sled dogs performing repeated prolonged exercise largely due to decreased glycogen utilization. Supercompensation of MG occurs when prolonged exercise ceases.

ABSTRACT #311

SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR IN DOGS WITH HEMOABDOMEN. Lee V. Herold[1], Douglas H. Thamm[2], EJ Ehrhart[2], Rebecca A. Kirby[1], Eric Stumpp[1]. [1]Animal Emergency Center, Glendale, WI. [2]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO.

Hemangiosarcoma (HSA) is an endothelial neoplasm that can cause hemoabdomen (HA). HSA has an aggressive biologic behavior, and is associated with short survival times. Other etiologies for HA, including trauma, coagulopathy, hematoma, hemangioma, and splenic and liver lobe torsions, may carry a better prognosis compared with HSA. In patients presenting with acute HA, therapeutic decisions must be made rapidly with most patients requiring transfusion and/or surgical intervention prior to a definitive diagnosis. A rapid diagnostic test that can differentiate between those patients with HA caused by HSA versus patients with HA of other etiologies may provide a valuable preoperative prognostic tool. Dysregulated vascular endothelial growth factor (VEGF) expression has been demonstrated in canine HSA and is thought to contribute to its pathogenesis. Serum VEGF levels have been demonstrated to be higher in dogs with HSA compared with normal dogs. The purpose of this study was to compare the serum VEGF levels in dogs with HA caused by HSA versus dogs with HA of other etiologies.

Serum was collected at presentation from dogs presenting to a 24-hour emergency facility with suspected HA. Dogs were included in the study if HA was confirmed and definitive diagnosis was achieved clinically or via necropsy examination. VEGF concentrations were determined using a commercially available ELISA kit.

Sera were collected from 34 dogs. 21 of these were diagnosed with HSA, and 13 were diagnosed with other diseases including gastric dilatation-volvulus, trauma, other neoplasia (carcinoma), or other. The mean VEGF concentration in dogs with HSA was higher than in dogs without HSA (83.6 vs 37.1 pg/mL), however this difference was not significant. When dogs with other neoplasia were excluded from the ''Not HSA'' group, the difference in mean VEGF concentration was significant (83.6 vs. 14.5 pg/mL, $p = 0.049$). Only dogs with neoplasia had VEGF concentrations greater than 53 pg/mL (8 of 21 HSA, 1 of 2 with other tumors), however 5 of 21 dogs with HSA had undetectable VEGF concentrations.

These data suggest that increased serum VEGF concentration may be a specific, but not sensitive marker for distinguishing between dogs with HA from HSA and dogs with HA of non-neoplastic etiology. An expanded cohort of patients with other non-HSA diseases should be examined to confirm these results.

ABSTRACT #312

INHIBITION OF ENDOTOXIN RESPONSES IN EQUINE MONOCYTES BY E5564. MD Figueiredo, JN Moore, MJ Fant, NA Norton, TF Murray. College of Veterinary Medicine, University of Georgia, Athens, GA.

Endotoxin (lipopolyssachride, LPS), a structural component of Gram-negative bacteria, gains access to the circulation in acute gastrointestinal diseases in horses and in septicemia in neonatal foals. LPS binds to the CD14/TLR4/MD2 complex expressed on the surface of mononuclear phagocytes, thereby initiating the production of a plethora of inflammatory cytokines and chemokines that lead to septic shock. E5564 is a synthetic endotoxin antagonist that prevents the effects of LPS in humans and laboratory animals. We hypothesized that E5564 would inhibit the responses of equine monocytes to endotoxin.

Blood monocytes isolated from six horses were incubated for 6 h in either media (non-stimulated control), media containing *E. coli* 0111:B4 LPS (100 pg/ml), media containing E5564 (0.1 nM to 1000 nM), or media containing E5564 + *E. coli* 0111:B4 LPS (100 pg/ml); media contained 1% heat-inactivated fetal bovine serum as a source of LPS-binding protein. Cell lysates and supernatants were assayed for procoagulant activity (PCA) and TNF-α, respectively. To test for possible agonist effects of E5564, whole blood samples from 3 horses were incubated with saline, *E. coli* 0111:B4 LPS (100 pg/ml) or E5564 (10 nM to 1000 nM), and then assayed for TNF-α production. Similarly, monocytes from 6 horses were incubated for 6 h with the vehicle for E5564 and then assayed for PCA.

Monocytes and whole blood from all horses had increased production of PCA and TNF-α in response to *E. coli* 0111:B4 LPS. Neither the non-stimulated control monocytes nor the monocytes incubated with E5564 or the vehicle produced PCA or TNF-α. Similarly, E5564 did not increase TNF-α concentrations in the whole blood. E5564 inhibited LPS-induced TNF-α production by monocytes in a concentration-dependent manner [0.1 nM (8%), 1 nM (14%), 10 nM (72%), 100 nM (97%), and 1000 nM (100%)]. The calculated IC50 was 4.6 nM. E5564 also inhibited LPS-induced PCA expression [0.1 nM (0%), 1 nM (30%), 10 nM (52%), 100 nM (88%), 1000 nM (92%). The calculated IC50 was 1.1 nM. The results of these studies indicate that E5564 is a potent antagonist of LPS in equine monocytes, and, in contrast to other LPS antagonists appears to lack agonist activity in equine whole blood. Furthermore, the calculated IC50 values were similar to those reported for cells from humans and mice. In conclusion, E5564 appears to have potential as an effective therapeutic agent in endotoxemia in horses.

ABSTRACT #313

ENDOTHELIN-1 IMMUNOREACTIVITY, INSULIN, GLUCOSE AND PLATELET-NEUTROPHIL AGGREGATES IN HORSES ADMINISTERED CARBOHYDRATE OVERLOAD. Susan C. Eades[1], Ashley M. Stokes[1], Philip J. Johnson[2], Casey J. LeBlanc[1], V. K. Ganjam[2], Preston R. Buff[2], Rustin M. Moore[1]. [1]Equine Health Studies Program, Louisiana State University and [2]Biomedical Sciences, College of Veterinary Medicine, University of Missouri.

These studies were performed to evaluate serial changes in venous blood endothelin-1 (ET-1) immunoreactivity, nitric oxide (NO), glucose, insulin, and platelet-neutrophil aggregates during carbohydrate overload (CHO)-induced laminitis. Endothelin-like immunoreactivity was measured by use of an ELISA kit and NO was measured using an ISO-NO Mark II NO sensor. The effects of treatment and the random effects of horse on each variable were analyzed by use of a repeated measures model. Carbohydrate overload caused a significant increase in the concentration of ET-like immunoreactivity in the digital blood above baseline at 11 hours after CHO. Endothelin-like immunoreactivity in the digital blood was significantly greater than that in the jugular venous blood at 8, 9, 11 and 12 hours after CHO. The concentration of glucose in digital and jugular venous blood increased significantly at 3, 4 and 5 hours after CHO. The insulin concentration increased significantly at 5 hours after CHO. The number of platelet neutrophil aggregates in digital and jugular venous blood increased significantly at 12 hours after CHO. The present study documents increased concentrations of digital venous ET-1 concentrations after CHO. Concurrent increases in ET-1, insulin, glucose, and platelet-neutrophil aggregates support a role of endothelial dysfunction in the pathogenesis of laminitis.

ABSTRACT #314

EVALUATION OF MICROBIAL COMMUNITY DIVERSITY IN THE EQUINE GASTROINTESTINAL TRACT USING PCR-DENATUR-

ING GRADIENT GEL ELECTROPHORESIS. MS Phelps[1], AJ Fascetti[2], BA Byrne[3]. [1]College of Veterinary Medicine, University of Georgia, Athens, GA. [2]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA. [3]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA.

A culture-independent method of bacterial evaluation, PCR amplification of 16S ribosomal genes and product separation with denaturing gradient gel electrophoresis (PCR-DGGE), was used to evaluate the microbial community structure in the gastrointestinal (GI) tract of adult horses.

Two adult horses, that presented to the Veterinary Medical Teaching Hospital at the University of California, Davis for elective euthanasia due to neurologic disease, were evaluated. Horses consumed a mixed diet of forage and processed grain before euthanasia was performed with an intravenous overdose of pentobarbital. Immediately following euthanasia, GI tract samples were collected from the rectum, small colon, transverse colon, large colon, cecum, small intestine and stomach. Individual anatomic sections were isolated using umbilical tape, the intestine was incised with a #10 blade, and GI tract contents were collected into sterile Whirl Pak bags. Samples were placed on ice, and within 2 hours of collection, were frozen at −70°C until analysis. Bacterial DNA was extracted from feces using a commercial DNA extraction kit (QIAamp® DNA Stool Mini Kit, Qiagen, Valencia, CA), which produced the best yield of PCR product compared to other fecal DNA extraction methods tested. Total genomic DNA served as a template for PCR amplification of the V3 region of the eubacterial 16S rDNA. PCR product yield was evaluated with a 1% agarose gel electrophoresis. The denaturing gradient gel electrophoresis was performed using a DCode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA) with a 10% polyacrylamide gel with a 25% to 60% formamide urea gradient.

DGGE band patterns were identified in all anatomic regions that were evaluated. The location and intensity of band patterns varied across different anatomic locations. The greatest microbial diversity was identified in GI contents from the cecum and large colon. Less microbial diversity was noted in stomach, small intestine, and fecal contents. Fecal band patterns showed some similarities to those identified in the large colon and cecum. Gastrointestinal tract band patterns differed between horses.

PCR-DGGE can be used to describe the diverse fecal microbial community at all anatomic locations of the equine GI tract. The GI tract microbial community varies between horses, possibly due to differences in diet. Analysis of PCR-DGGE from fecal samples is a culture-independent, non-invasive diagnostic tool that can be used to improve the description of the microbial community in the GI tract of horses.

ABSTRACT #315

FALL PANICUM (*PANICUM DICHOTOMIFLORUM*) HEPATOTOXICITY IN HORSES AND SHEEP. AL Johnson[1], TJ Divers[1], SP McDonough[1], ML Freckleton[2], HC McKenzie[3], E Mitchell[3], JM Cullen[4]. [1]Cornell University College of Veterinary Medicine, Ithaca, NY. [2]Haymarket Veterinary Service, Haymarket, VA. [3]Marion DuPont Scott Equine Medical Center, Leesburg, VA. [4]North Carolina State University College of Veterinary Medicine, Raleigh, NC.

Fourteen horses at a boarding stable in Virginia were diagnosed with hepatic disease and locally grown hay was implicated as the cause. Hay analysis and feeding trials with horses and sheep were undertaken to confirm the toxicity of the hay. Serial hematology and liver biopsy results from the spontaneous cases and research animals were compared.

Hay analysis revealed that the predominant species was fall panicum (*Panicum dichotomiflorum*). Two research horses fed only the fall panicum hay for 12 days developed hepatic disease with marked elevations in AST (aspartate aminotransferase), SDH (sorbitol dehydrogenase), GGT (gamma glutamyl transferase), and ALP (alkaline phosphatase). These enzyme elevations were similar to those seen in the naturally occurring cases. Liver biopsy specimens from both the research and spontaneous cases revealed mild non-suppurative inflammation, mild vacuolar change, prominently clumped chromatin, and marked individual hepatocyte necrosis. Five Virginia horses were euthanized because of persistent clinical deterioration; one research horse was euthanized and the other recovered after cessation of the feeding trial. The fall panicum hay was also fed to two sheep for 12 days, and the sheep displayed biochemical and histologic abnormalities similar to the horses.

The rapidity and severity of signs of hepatotoxicity following fall panicum hay exposure were noteworthy. Pathologic liver changes were dominated by patchy hepatocyte necrosis, implicating apoptosis as the mechanism of hepatotoxicity. The absence of significant fibrosis in the research cases indicates that immediate cessation of feeding fall panicum hay should allow all but very severely affected cases to recover.

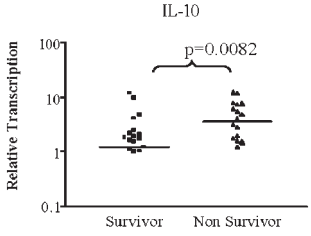
ABSTRACT #316

EVALUATION OF THE DIAGNOSTIC AND PROGNOSTIC VALUE OF SELECTED MOLECULAR MARKERS IN THE BLOOD OF EQUINE NEONATES WITH SEPSIS USING REAL-TIME TAQMAN PCR. N. Pusterla, K.G. Magdesian, S. Mapes, C.M. Leutenegger. Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California.

The goal of this study was to determine the gene expression of selected molecular markers (tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, IL-10, procalcitonin (PCT), transforming growth factor (TGF)-β) in the peripheral blood of healthy and sick foals using real-time TaqMan PCR. The study material consisted of peripheral blood collected from 28 sick, non septic foals (sepsis score < 11 and negative blood culture), 21 septic foals (sepsis score ≥ 11 with or without positive blood culture) and 21 healthy foals. Total RNA was extracted from Tempus™ RNA tubes using a nucleic acid preparation station and converted into complementary DNA (cDNA). Gene expression was measured for TNF-α, IL-1β, IL-6, IL-8, IL-10, PCT, TGF-β and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by real-time TaqMan PCR and final quantitation was done using the comparative C_T method

All foals showed levels of transcription for TNF-α, IL-1β, IL-6, IL-8, IL-10, PCT and TGF-β. TNF-α and TGF-β were significantly downregulated in the sick, non septic and the septic group when compared to the healthy group. IL-8 was significantly upregulated in the patient groups when compared to the healthy group. IL-10 was the only cytokine that showed a trend towards significance in relative transcription between sick, non-septic and septic foals. No significant difference in gene expression of IL-1β, IL-6 and PCT was found between the healthy and the two patient groups. When comparing the outcome of foals, IL-10 was significantly upregulated in the non-survival group compared to the survival group.

Sick foals have a downregulation of pro-inflammatory cytokines TNF-α and tissue factor TGF-β and an upregulation of chemokines IL-8. Interestingly anti-inflammatory cytokine IL-10 showed a trend towards upregulation in septic foals. Thus, the cytokine profile determined in septic foals may be characterized by an immunosuppressive state. IL-10 expression was a good marker for identification of patients with a guarded prognosis.



ABSTRACT #317

AGE-RELATED DIFFERENCES IN THE EXPRESSION OF CYTOKINE mRNA BY PERIPHERAL BLOOD MONONUCLEAR CELLS. BA Sponseller[1,2], DM Wong[1], SK Clark[2], DE Jones[3]. [1]Department of Veterinary Clinical Sciences, [2]Department of Veterinary Microbiology and Preventive Medicine, [3]Department of Veterinary Pathology, Iowa State University, Ames, IA.

It has been well documented across species that the early postnatal period is characterized by an increased susceptibility to infectious diseases. This vulnerability is especially apparent for diseases caused by *intracellular pathogens*, suggesting a functional deficit in *cell-mediated immunity*. We speculate that age-related differences in antigen presenting cell function may help explain foal susceptibility to disease in general, and the intracellular pathogen, *Rhodococcus equi*. While immature T-cell responses may contribute to immunologic immaturity, it is also likely that functional deficits in antigen-cell presentation contribute to reduced cell mediated immunity during the juvenile period, and polarize the immune response toward a humoral, Th2-type of response.

IL-12 is a cytokine elaborated by antigen presenting cells (APC) that is important in generation of a T helper type 1 (Th1) response. In contrast, IL-10 is a cytokine elaborated by APC that drives a Th2 (humoral) immune response. Th1 responses are critical for effective cell-mediated immunity to intracellular pathogens and are characterized by high IL-12 and low IL-10. Thus, detection of the mRNA of these two cytokines present in peripheral blood mononuclear cells (PBMC) is a useful approach in characterizing the type of immune response that would be expected to be elicited. We therefore carried out preliminary experiments with PBMC derived from young foals and adult horses to determine if there were quantitative differences in IL-12 and IL-10-production that were related to age. Expression of IL-10, IL-12p35, and IL-12p40 mRNA in peripheral blood mononuclear cells was determined by relative quantitative fluorogenic one-step real-time RT-PCR using Primer Express v.2.0-designed 6FAM probes at 150 nM and primers at 1000 nm. All target signals were normalized to equine GA3PDH. Cells were stimulated (or not) with LPS (10 μg/ml) and IFN-γ (250 ng/ml).

Our results suggest that, under identical cell culture conditions and stimuli, IL-10/IL-12 ratios of stimulated equine PBMC are higher in young foals than adult horses. High IL-10/IL-12 ratios are inhibitory to effective Th1 responses. These results are consistent with data obtained for human children and adults where IL-10/IL-12 ratios are consistently higher among children. Our data indicate that PBMC derived from juvenile horses are not as efficient as PBMC derived from adult horses in generating T helper 1-polarizing cytokines.

ABSTRACT #318

EVALUATION OF NASOPHARYNGEAL SWABS AND FECES AS POTENTIALLY USEFUL DIAGNOSTIC SPECIMENS FOR *RHODOCOCCUS EQUI* PNEUMONIA IN FOALS USING REAL-TIME TAQMAN PCR. N. Pusterla, W.D. Wilson, S. Mapes, C.M. Leutenegger. Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California.

Bacterial culture or PCR amplification combined with cytological examination of tracheal wash (TW) fluid are necessary to make a definitive diagnosis of *Rhodococcus equi* pneumonia in foals.

Thirty-one foals with suspected bacterial pneumonia were evaluated for this study. *R. equi* was found to be the causative agent in 12 foals on the basis of final interpretation of all available information about each foal (history, clinical signs and diagnostic test results). We hypothesized that nasopharyngeal swabs (NS) and/or feces collected from foals with possible *R. equi* pneumonia could represent alternative specimens to TW fluid for molecular detection of *R. equi*.

Conventional culture recognized 82% of the *R. equi* cases, while real-time TaqMan PCR targeting the VapA gene of *R. equi* was positive in all the TW fluid samples tested. PCR of NS yielded positive results in only 1 of 12 affected foals (8%), while feces tested positive in 9 of 12 foals (75%). Fecal PCR had a similar diagnostic accuracy as that of culture of TW fluid samples. False negative fecal PCR results were associated with prior use of antimicrobials.

Foal	TW fluid culture	TW fluid PCR	NS PCR	Feces PCR	Antimicrobial† prior referral (days)
1	*E. coli, Streptococcus sp.*	+	+	+	None
2	*S. zooepidemicus*	+	-	-	Doxycycline (22)
3	*R. equi*	+	-	+	TMS (1)
4	*R. equi*	+	-	+	None
5	*R. equi*	+	-	+ (15)‡	Ceftiofur (10)
6*	ND	ND	-	+	Azithromycin (10)
7	*R. equi*	+	-	-	Azithromycin (30)
8	*R. equi*	+	-	+ (4)‡	Azithromycin (1)
9	*R. equi*	+	-	+	None
10	*R. equi*	+	-	-	Azithromycin (10)
11	*R. equi*	+	-	+	Azithromycin (7)
12	*R. equi, S. zooepidemicus*	+	-	+ (7)‡	Amikacin, PPG (1)
Total	9/11 (82%)	11/11 (100%)	1/12 (8%)	9/12 (75%)	

*Foal without TW fluid collection due to severe respiratory distress. *R. equi* cultured *post-mortem* from lungs. †TMS = trimethoprim-sulfamethoxazole, PPG = penicillin procaine G. ‡Days of *R. equi* PCR positive fecal samples after initiation of antimicrobial treatment at the VMTH

Although PCR amplification of NS is poorly sensitive for the diagnosis of *R. equi* pneumonia, the use of fecal samples for the molecular detection of *R. equi* may be very helpful in a situation where the severity of the clinical signs does not allow TW sampling. However, as with any diagnostic technique, molecular results should always be interpreted in light of the individual foal's history, clinical signs, and radiographic and laboratory results

ABSTRACT #319

VALIDATION OF A REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR RAPID IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* DIRECTLY FROM NASAL SWABS IN HORSES. MEC Anderson, JS Weese. Ontario Veterinary College, Guelph, ON, Canada.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial and community-associated pathogen in human medicine. Outbreaks of MRSA infection and colonization in horses have recently been reported in numerous locales worldwide. Zoonotic transmission and human-to-horse transmission of MRSA have been documented. One of the major obstacles to control of equine MRSA is timely identification of infected and colonized horses. Typically identification of MRSA from clinical specimens or screening swabs takes from 3–7 days, a period during which the bacterium may be widely disseminated within a facility if isolation precautions are not in use. Molecular identification techniques such as real-time polymerase chain reaction (RT-PCR) have recently been employed in human medicine to reduce this turn-around time to as little as 1–2 hours. The purpose of this study was to compare a RT-PCR assay for rapid identification of MRSA directly from nasal swabs in horses to standard culture techniques.

Nasal swabs collected from 294 clinically normal horses from Ontario and Kentucky were processed using a commercial RT-PCR assay previously validated for use with human specimens (IDI-MRSA, GeneOhm Sciences, San Diego, CA) according to the manufacturer's directions. Following broth enrichment, the swabs were also cultured on mannitol salt agar containing 2 μg/mL oxacillin, and MRSA colonies were identified using biochemical and latex agglutination tests as per standard protocols.

From the results of the first run of PCR, 2/177 and 168/177 samples were positive and negative, respectively, by both PCR and culture. Seven of 177 samples were positive by PCR and negative by culture, whereas 0/177 samples were negative by PCR and positive by culture. The *kappa* statistic was 0.33, which represents poor agreement between the two tests. Of the remaining 117 samples, on the first run of PCR 12 failed the external control and were excluded, while 105 samples were reported as "unresolved". Following one freeze-thaw cycle of the lysates, the recommended technique to resolve such samples, 61/110 (55%) samples remained unresolved, which represents 21% of all samples tested.

In this study, the IDI-MRSA assay was not a clinically practical screening test for horses harboring MRSA in the nose. Its agreement with culture, the current accepted standard, was poor, although the lower than anticipated sample prevalence of MRSA colonization may have impacted the results. Regardless, the unresolved rate was also very high (37%) on initial testing of samples, which significantly decreases the clinical utility of the test. The reason for the high unresolved rate compared to that found with human specimens is unknown. Modification of the technique for sample processing may help decrease the unresolved rate, but this requires further investigation.

ABSTRACT #320

INTERACTION OF DI-TRI-OCTAHEDRAL SMECTITE WITH EQUINE COLOSTRAL ANTIBODIES IN VITRO. J Boggs Lawler, DM Hassel, JL Traub-Dargatz, C Hirota, DR Hyatt, PM McCue. Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, CO.

Di-tri-octahedral (DTO) smectite has prophylactically been administered to foals in the early neonatal period for life-threatening clostridiosis; however, its effect on colostral immunoglobulins has not been evaluated.

Colostrum was harvested from 9 healthy broodmares at foaling and stored at −70°C. Baseline IgG levels were evaluated by single radial immunodiffusion (SRID). DTO smectite was added to colostral samples in serial concentrations ranging from 1 : 4 to 1 : 32. Colostral samples were incubated with DTO smectite at 37°C for 1 hour and evaluated by SRID.

This range of concentrations mimics the estimated ratio of DTO smectite to colostrum and gastric contents in a foal's GI tract following administration of the recommended dosage of DTO smectite (3TB in 30 mls water). The 1 : 4 concentrations represent approximately 1 hour of colostral ingestion, while the 1 : 32 concentrations mimic 6–12 hours of ingestion of good quality colostrum.

Results of this *in vitro* study revealed that co-incubation of equine colostrum with DTO smectite decreases SRID detection of IgG in a dose dependent manner. While the effect was negligible in samples representing 6–12 hours of colostral ingestion, there was a consistent decrease in SRID in the 1 : 4 concentrations. It is hypothesized that DTO smectite is absorbing IgG by nonspecific binding and may decrease the bioavailability of colostral antibodies. Based on extrapolation from these *in vitro* results, caution should be exercised during prophylactic administration of DTO smectite to healthy foals prior to consumption of an adequate quantity of good quality colostrum until further *in vivo* studies can be conducted.

ABSTRACT #321

EFFECTS OF DEXAMETHASONE ON GLUCOSE DYNAMICS AND INSULIN SENSITIVITY IN STANDARDBRED HORSES. H. A. Tiley, R. J. Geor, L. J. McCutcheon. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

Increased risk of laminitis has been associated with exogenously administered glucocorticoids, perhaps by potentiating vascular dysfunction secondary to an increase in insulin resistance. The purpose of this study was to characterize the effects of dexamethasone (DEX) on insulin sensitivity and glucose homeostasis in healthy horses. In a randomized crossover design, 6 Standardbred horses received either DEX (0.08 mg/kg bwt) or the equivalent volume of saline (CON) IV every 48 h for 21 days (11 doses), with a 3-wk washout period between treatments. Pre-feeding blood samples were collected 2 days before and after day 1, 7, 14, and 21 days of treatment for measurement of plasma glucose and serum insulin and cortisol concentrations. At the end of DEX and CON, an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) was administered. A series of 32 blood samples was collected from −45 to 180 min, with an IV bolus of glucose (0.3 g/kg bwt) given at 0 min and an insulin bolus (30 μU/kg bwt IV) injected at 20 min. All samples were analyzed for glucose and insulin concentrations, and the minimal model of glucose and insulin dynamics was used to determine insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), and the disposition index (DI). Repeated measures ANOVA or paired Student's *t* tests, where appropriate, were used for statistical analysis. The null hypothesis was rejected at $P < 0.05$. Data are reported as mean ± SEM.

Dexamethasone treatment resulted in marked suppression of serum cortisol concentrations, with values significantly lower in DEX than in CON on day 7, 14 and 21 of treatment. Baseline serum insulin concentrations were higher ($P < 0.05$) in DEX (32.4 ± 6.8 mU/L) than in CON (12.7 ± 2.2 mU/L) on day 7, and remained significantly elevated on days 14 and 21. Baseline plasma glucose concentration was higher in DEX (115.4 ± 8.6 mg/dl) than in CON (95.9 ± 4.3 mg/dl) on day 14 only. Dexamethasone induced profound insulin resistance, with mean Si 79% lower ($P < 0.001$) in DEX ($0.53 \pm 0.13 \times 10^{-4}$ min^{-1}·(mU/L)$^{-1}$) than in CON ($2.59 \pm 0.46 \times 10^{-4}$ min^{-1}·(mU/L)$^{-1}$). The AIRg was higher ($P < 0.01$) in DEX (398.2 ± 36.6 mU min L^{-1}) than in CON (153.8 ± 38.9 mU min L^{-1}), while DI tended ($P = 0.08$) to be lower in DEX than in CON. Mean Sg did not differ between DEX and CON.

This study has demonstrated that a 3-week period of dexamethasone treatment (0.08 mg/kg IV q 48h) results in marked disturbances to glucose homeostasis in horses. Interference with insulin action, reflected by the marked hyperinsulinemia and decrease in Si following dexamethasone treatment, is consistent with a link between glucocorticoid treatment and the development of laminitis.

ABSTRACT #322

EQUINE SKELETAL MUSCLE INSULIN SIGNALING, GLUCOSE METABOLISM AND GLUT4 EXPRESSION IN A DEXAMETHASONE MODEL OF INSULIN RESISTANCE. H. A. Tiley, R. J. Geor, K. Ho, L. Stewart-Hunt, L. J. McCutcheon. Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

In rats, the molecular mechanisms by which glucocorticoid treatment alters insulin-stimulated glucose uptake include decreased GLUT-4 translocation, reduced activation of PI3-K and decreased phosphorylation of PKB (Akt) or IRS-1 expression. In horses however, no study has investigated the mechanisms responsible for the onset of insulin resistance after glucocorticoid treatment. The purpose of this study was to examine the effects of dexamethasone (DEX) treatment on selected aspects of skeletal muscle glucose metabolism in equine skeletal muscle obtained before and after a 2-hour period of sustained hyperinsulinemia. In a randomized crossover design, 6 Standardbred horses received either DEX (0.08 mg/kg bwt) or equivalent volume of saline (CON) IV every 48 h for 21 days (11 doses) with a 3-wk washout period between treatments. Following DEX and CON, a 2-h euglycemic-hyperinsulinemic clamp (EHC) was administered, initiated with an IV priming dose of insulin (18 μU/kg) followed by a concurrent variable rate IV glucose infusion to maintain blood glucose at 5 mM and constant rate infusion of insulin (3 μU/kg/min) over a 2-hr period. Blood samples for measurement of plasma glucose were collected at 5 min intervals and at 15 min intervals for analysis of serum insulin concentration. Pre and post EHC muscle biopsies were obtained to assess glycogen synthase (GS) and hexokinase (HK) activity, muscle glycogen concentration (GLY), and Western blot analysis for GSK-3 and Akt phosphorylation state and GLUT-4 total protein content. Data are reported as means ± SE.

EHC glucose infusion rate (mg/kg/min) was significantly lower ($P < 0.001$) in DEX (2.15 ± 0.44) when compared to CON (7.73 ± 1.05) with a higher serum insulin concentration (μU/ml) in DEX than CON (409.5 ± 37.9 vs. 332.7 ± 21.2). In DEX, basal GS activity (0 mol G6P; nmol/mg/h) was decreased ($P < 0.05$) following hyperinsulinemia (CON: 100.9 ± 12.7 vs DEX: 40.6 ± 7.5) and HK activity (nmol/μg protein/h) was increased ($P < 0.05$) in pre-and post EHC samples (Pre: 12.34 ± 1.09; Post: 12.89 ± 1.47) when compared to CON (Pre: 7.45 ± 0.96; Post: 9.02 ± 0.97). There was no difference in GLY between treatments. There also was no difference in total Akt content or phosphorylated form between treatments or insulin state. However, in DEX total GSK-3β and GSK-3α & β phosphorylation were decreased ($P < 0.05$) in pre and post EHC samples. Total GLUT-4 protein content was not different within or between treatments.

These results indicate that, as demonstrated in other species, dexamethasone treatment can induce hyperinsulinemia, reduce insulin-stimulated glucose uptake and decrease glycogen synthase activity. The observed increase in insulin resistance in skeletal muscle most likely reflects the decrease in GSK-3 phosphorylation and subsequent reduction in the ability of insulin to dephosphorylate (through GSK-3) and activate glycogen synthase.

ABSTRACT #323

EFFECTS OF DIETARY ENERGY SOURCE AND EXERCISE ON INSULIN SENSITIVITY AND SKELETAL MUSCLE GLUCOSE METABOLISM IN STANDARDBRED HORSES. L. Stewart-Hunt, R. J. Geor, S. E. Pratt, L. J. McCutcheon Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

The purpose of this study was to characterize the effects of dietary energy source and physical conditioning on insulin sensitivity (Si) and glucose metabolism in skeletal muscle. Fourteen mature Standardbred horses, paddock rested for at least 3 mo, were randomly assigned to 2 equal groups. In an initial sedentary 3 wk period, all horses received forage cubes only, at a rate of 2.5% of body weight (bwt) (Phase 1). All horses remained sedentary for an additional 6 wk (Phase 2) in which groups received a 1:1 ratio of forage to concentrate diet, with each component fed at a rate of 1% bwt. One group received a concentrate rich in soluble carbohydrate (HiCHO) while the other group was fed an isocaloric concentrate higher in fat and lower in soluble carbohydrate content (LoCHO). Diets for each group were maintained during a subsequent 7 wk period of exercise training (Phase 3). To assess Si and disposition index (DI), a frequently sampled intravenous glucose tolerance test (FSIGT) for minimal model analysis of blood glucose and serum insulin concentrations and a euglycemic hyperinsulinemic clamp (EHC) were administered 48 and 72 h, respectively, after the end of each phase. Middle gluteal muscle biopsies to assess glycogen content (GLY), hexokinase (HEX) and glycogen synthase activity (GS) and Glut-4 transporter protein expression (Glut4) were obtained prior to and following each EHC.

In HiCHO, Si was decreased at the end of Phase 2 when compared to Phase 1 but was unchanged in LoCHO. At the end of Phase 3, Si and DI were increased in both groups when compared to Phase 1 and 2 ($P < 0.05$). In Phases 2 and 3, GS fractional velocity, calculated as active GS divided by total GS, was increased in post EHC samples in HiCHO and LoCHO when compared to pre EHC samples. HEX was unchanged in both groups after Phase 2 but was increased ($P < 0.05$) in HiCHO at the end of Phase 3. In both groups, GLY was unchanged in all phases following insulin stimulation whereas GLY was decreased in LoCHO after Phase 2 but had returned to Phase 1 values by the end of Phase 3. In HiCHO, pre and post EHC GLY was unchanged after Phase 2 but was increased significantly ($P < 0.05$) at the end of Phase 3 when compared to prior phases. There was no change in Glut4 throughout the study in LoCHO. In contrast, in HiCHO Glut4 was significantly increased after Phases 2 and 3 when compared to Phase 1 ($P < 0.05$) such that there was a significant difference in Glut4 expression between HiCHO and LoCHO ($P = 0.002$) following physical conditioning.

This study demonstrated moderate physical conditioning may modulate changes in insulin sensitivity resulting from a diet high in soluble carbohydrate. Increased hexokinase and glycogen synthase activity, enzymes key to the regulation of glucose disposal, as well as an enhancement in glucose transport may have contributed to an improvement in insulin sensitivity evident following a period of regular physical conditioning.

ABSTRACT #324

EFFECT OF DIETARY FRUCTAN CARBOHYDRATES ON PLASMA INSULIN LEVELS IN LAMINITIS-PRONE PONIES. S.R. Bailey[1] and P.A. Harris[2]. [1]Royal Veterinary College, London, UK. [2]Waltham Centre for Pet Nutrition, Leicestershire, UK.

Acute laminitis in equines on 'lush' pasture is thought to be associated with consumption of rapidly fermentable carbohydrate, in particular fructans. However, some animals seem to be predisposed to this condition, while others on the same pasture remain unaffected. An association between laminitis and insulin resistance has been demonstrated previously, with obesity also contributing to an increased risk of laminitis in ponies. However, the mechanism of this link remains unknown. The purpose of this study was to assess the insulin responses to dietary fructan consumption in non-obese ponies predisposed to laminitis, compared with unaffected controls.

Eleven adult non-obese mixed native breed ponies (4 mares and 7 geldings) were used in this study. Six of the ponies had one or more episodes of acute laminitis in the previous two years, but none had shown clinical signs in the 3 months leading up to the study (laminitis-prone group); the remaining five had not shown any signs of laminitis in at least the previous 3 years (normals). The ponies were fed hay *ad-libitum* for two weeks, before inulin (a commercially available form of fructan carbohydrate; 3 g/kg) and dried grass (Readigrass®; replacing one third of forage ration by weight) were included, split into three meals. The amount of inulin in the diet was safely below the amount expected to cause laminitis, based on published data from other groups using raftilose, and caused a small but significant decrease in faecal pH (from 6.8 ± 0.1 to 6.3 ± 0.1 pH units); no clinical signs were observed. Blood samples were taken (3 hrs post-prandial) for insulin, glucose and triglyceride analysis, before and 1, 2 and 5 days after the inclusion of inulin.

No significant changes in plasma glucose or triglyceride levels were observed in either group following the addition of fructan carbohydrates to the diet. In the normal group of ponies, insulin concentrations increased by 2.0 ± 0.3 fold between diets (median on hay diet 14.4 μIU/ml (range 9.5–31.2, with one outlier at 127.0) vs. 37.9 (23.1–38.4 plus one individual at 174.0) μIU/ml on hay plus inulin, 48 hrs (no significant difference). Normal range 5.5–36.0 μIU/ml). However, in the ponies predisposed to laminitis, although their insulin levels were not significantly different from the normal ponies while on the basal hay diet (44.7 (8.9–152) μIU/ml; 3 within normal range), insulin concentrations increased dramatically and significantly (5.5 ± 2.2 fold increase) following the addition of fructan carbohydrates (peak of 137.0 (52.1–576.0) μIU/ml at 48 hrs; $p < 0.03$).

We conclude from these findings that the ponies predisposed to laminitis exhibit a compensated insulin resistance, which may not be apparent when plasma insulin concentrations are measured during the feeding of diets containing low amounts of rapidly fermentable carbohydrate. However, in these individuals, dietary fructans are capable of unmasking an exaggerated insulin response. This effect may be useful in the identification of animals suspected of being insulin-resistant and therefore predisposed to laminitis. It also has implications for the feeding and management of such animals.

ABSTRACT #325

INITIAL CLINICAL IMPRESSIONS OF THE UC DAVIS LARGE ANIMAL LIFT AND ITS USE IN RECUMBENT EQUINE PATIENTS. N. Pusterla[1], J.E. Madigan[1], G.L. Ferraro[2]. [1]Department of Medicine and Epidemiology and [2]Center of Equine Health, School of Veterinary Medicine, University of California, Davis, California.

The goal of this study was to evaluate the use of a new lightweight sling on recumbent horses in the field and hospital setting. The UC Davis Large Animal Lift (LAL) consists of a counterbalance bar and two sling components made out of nylon straps. The LAL application and lifting attempts were recorded for 16 recumbent horses (12 at the hospital, 4 in the field) in order to assess their ability to stand and provide important diagnostic and prognostic information, as well as to elevate them for easier application of a more permanent sling. For every procedure, the patient's history, sedation, ease and time of LAL application and hoisting, standing ability post-hoisting, LAL tolerance and outcome were recorded.

The 16 horses ranged in age from 1 to 20 years (mean ± SD = 10.6 ± 7.5 years) and weighed 363 to 1,000 kg (559 ± 189 kg). The 16 patients were diagnosed with a variety of neurologic, muscular and musculoskeletal diseases. The time from recumbency to application of the LAL was less than 1 hour in 6 hospital patients (i.e. recumbency occurred at the hospital), 4 to 17 hours in 6 referred cases and 8 hours to 14 days in 4 field cases. The LAL was easily and safely applied in all horses in less than five minutes and the procedure was well tolerated with minimal to no sedation. Hoisting was performed without any problem in all cases with an electric hoist (12 horses) in the hospital, and a backhoe (3) or a manual hoist in the field (1). Eight horses were able to stand and bear weight once lifted. Two horses were able to stand with assistance, while 6 horses were unable to support their weight. Ten hospitalized horses (5 standing, 2 standing with support, 3 unable to support weight) were transitioned into the Anderson Sling Support Devise (ASSD) to allow better distribution of support and long-term slinging, while two hospital patients were euthanized at the request of the owner due to inability to bear weight in the LAL. Three out of the four horses lifted in the field were able to stand without support, while one horse was euthanized because he could not bear weight in the LAL. The three horses that were unable to stand and were switched to the ASSD never regained the ability to stand despite intensive medical treatment and supportive care and were euthanized shortly thereafter. All the horses able to bear weight after being lifted survived and were discharged.

In conclusion, the LAL has proven to be a valuable aid in lifting recumbent horses with a variety of debilitating problems. The application of the LAL required little to no sedation, which is of benefit when evaluating the standing ability of recumbent horses once lifted. The unique feature of this device makes it easy and safe to use in the clinic and field setting and is well tolerated by the recumbent horse. The initial standing ability is an important prognostic indicator, since all horses unable to bear weight were eventually destroyed. For horses that need long-term support, it was very easy to transition these horses to the ASSD while standing.

ABSTRACT #326

BLACK LOCUST (*ROBINIA PSEUDOACACIA*) POISONING IN 18 PONIES AND A MULE. N. Metzger, M. Wehrli Eser, J. Auer. Equine Clinic, Vetsuisse Faculty, University of Zurich, Switzerland.

The purpose of this report is to describe the clinical features, treatments, clinical courses, and outcomes in 18 ponies and 1 mule with accidental black locust (*Robinia pseudoacacia*) poisoning.

Eight Welsh ponies, 8 Shetland ponies, 2 Miniature horses, and one mule were accidentally fed black locust twigs with leaves. Two to twelve hours after the feeding they appeared depressed, showed signs of mild colic, and had bouts of diarrhea. Other clinical signs – in order of decreasing incidence – included tachycardia, reduced or absent abdominal sounds, hyperthermia, congested mucous membranes, tachypnea, profuse sweating, prolonged capillary refill time, hypothermia, trembling, and muscle spasms. Abnormal hematologic and serum biochemical values included leukocytosis; decreased serum magnesium, calcium, phosphate, chloride, sodium, and protein concentrations; hyperglycemia; decreased BUN; and increased activities of GLDH, ALP, and CK. A presumptive diagnosis of black locust poisoning was made based on the identification of black locust twigs and the temporal association between feeding and onset of clinical signs.

Emergency treatment consisted of intravenous crystalloid fluids to promote diuresis, gastric lavage, and administration of either mineral oil or activated charcoal. Electrolyte abnormalities were treated with supplementation of a commercial electrolyte concentrate. Food was withheld. Nine animals normalized after emergency treatment. In 10 ponies progression with two distinct courses of disease was observed. Six of these ponies displayed central nervous signs. They were apathetic and trembled, then developed bilateral mydriasis, absent menace response, nystagmus, head pressing, and ataxia, and eventually progressed to lateral recumbency, became unresponsive to stimuli, and developed increased muscle tone, spastic limbs, trismus, seizures, and irregular breathing with severe dyspnea and high respiratory rates. Treatment of this group consisted of flunixin meglumine, dimethyl sulfoxide, dexamethasone or methyl-prednisolone sodium succinate, diazepam or climazolam, and intranasal oxygen. None of the ponies improved. One pony died; the other five were euthanized for humane reasons. The four remaining ponies displayed continued signs of colic up to 28 hours after ingesting black locust. Rectal examinations and gastric lavages were unremarkable. Treatment consisted of metamizole, flunixin meglumine, and butyl-scopolamine as needed. Clinical signs resolved within 1–5 hours. One pony in this group had a temporary accelerated idioventricular rhythm which resolved after 48 hours. Thirteen animals were dismissed after complete resolution of clinical signs three days after admission to the clinic. No further health problems have been reported since.

The toxic agents found in black locust are lectins, glycosides, and alkaloids. The bark contains the highest concentrations. Toxins show seasonal fluctuations with a peak in fall. There is no antidote to the toxin and treatment is purely supportive. Our case series shows that black locust poisoning is a serious condition with a survival rate as low as 68%. Rapid removal of the toxic agent, decontamination, and initiation of supportive therapy is considered critical for survival.

ABSTRACT #327

EVALUATION OF PHENYLBUTAZONE AND FLUNIXIN MEGLUMINE IN COMBINATION IN HORSES WITH NAVICULAR SYNDROME USING FORCE PLATE ANALYSIS. RS Erkert, CG MacAllister, R Royse, ME Payton. Oklahoma State University Center for Veterinary Health Sciences, Stillwater, OK.

The objective of this study was to compare the analgesic efficacy of single-dose, intravenous injections of phenylbutazone and flunixin meglumine alone and in combination in horses with chronic navicular syndrome utilizing force plate analysis. It was hypothesized that the maximal analgesic effect of the drugs in combination would be similar to that of the individual drugs.

Ten (10) horses diagnosed with chronic navicular syndrome were used. Horses were randomly assigned to one of five treatment groups that consisted of phenylbutazone (4.4 mg/kg IV); flunixin meglumine (1.1 mg/kg IV); high dose combination of phenylbutazone (4.4 mg/kg IV) and flunixin meglumine (1.1 mg/kg IV); low dose combination of phenylbutazone (2.2 mg/kg IV) and flunixin meglumine (0.55 mg/kg IV); and physiologic saline (1 mL/45 kg BWT IV). Each horse received each treatment with a two week washout between treatments. Horse were evaluated objectively on the force plate just prior to treatment (time 0) and 6, 12, 24, and 30 hours post-treatment. Data were analyzed by use of orthogonal contrasts and effects considered significant at $P < 0.05$.

For the force plate data, at 6 and 12 hours there was significant improvement in mean peak vertical force for all treatments compared to saline control, but no significant difference between any of the treatments. At 24 and 30 hours, both combination treatments were significantly different from the individual treatments and saline; however, there was no significant improvement in the individual treatments compared to saline.

These results suggest that, under the conditions of this study, there is no difference in maximal analgesic efficacy between administration of phenylbutazone and flunixin meglumine in combination compared to the individual drugs. However, by administering the drugs in combination, there was an increased duration of effect compared to the individual drugs. This indicates that there is little advantage to administering the two drugs in combination and that doing so may lead to increased risks of toxicosis.

ABSTRACT #328

PULMONARY THROMBOEMBOLISM IN 5 HORSES. TE Norman[1], MK Chaffin[1], EE Perris[2], JF Edwards[1], JB David[3]. [1]Texas A&M College of Veterinary Medicine, College Station, TX. [2]Perris Equine Veterinary Associates, Hopewell, NJ. [3]Blue Ridge Equine Clinic, Free Union, VA.

This case series presents 5 horses that developed pulmonary thromboembolism (PTE) secondary to serious medical and/or surgical primary diseases. PTE is a serious and important complication in critically ill humans, accounting for as many as 5–10% of hospital deaths. However, to the authors' knowledge, this disorder has never been described in the horse.

The records of 5 horses, all of which had pulmonary thromboembolism at necropsy, were reviewed. Information collected from the records included signalment, primary disease process, results of diagnostic testing, treatments administered, and complications of treatment.

The horses ranged in age from 6 to 16 years. Primary disease processes included small intestinal ischemia, cecal impaction, uterine torsion, laminitis, and clostridial myonecrosis. All horses were administered intravenous therapies via an indwelling intravenous catheter; 4/5 horses developed thrombophlebitis. All horses exhibited tachypnea and dyspnea prior to death or euthanasia.

The data obtained from this study suggest that pulmonary thromboembolisn is a potentially fatal complication of severe illness in horses.

ABSTRACT #329

CONCHAL NECROSIS IN HORSES. A Cehak, M von Borstel, H Gehlen, B Ohnesorge. Equine Clinic, University of Veterinary Medicine Hannover, Germany.

Conchal necrosis is a rare condition in horses, and published data on this disease is limited. The present study analyses clinical signs, diagnosis, therapy, etiology, and outcome in eleven horses with conchal necrosis presented to the Equine Clinic, University of Hannover in 1999 to 2004.

Seven warmbloods, one Trakehner Horse, one Friesian Horse, and one Shetland Pony, seven mares, three geldings, and one stallion (minimum age of 4 years, maximum age of 17 years) were presented with a history of unilateral, mucopurulent, malodorous nasal discharge. One horse had epistaxis temporarily, three horses exhibited a nasal stridor. One horse had a history of a successfully treated fractured second right upper molar tooth, but nasal discharge reoccurred. In two horses, the owner described a piece of necrotic tissue that had been fallen out of the nostrils. All hoses underwent a clinical, endoscopic and radiographic examination. Tissue samples were taken transendoscopically for microbiology in four horses and for pathohistology in three horses.

In eight horses, an apparent ongoing conchal necrosis was diagnosed. In three horses, the endoscopic examination revealed only residues of the concha without any acute inflammation. An underlying, primary disease was not found in any of the horses. In three horses with a dorsal conchal necrosis, most parts of the affected concha did not exist anymore at the time of examination allowing an endoscopic view into the sinus via the nasal passages. Microbiology revealed an infection with Streptococcus equi zooepidemicus, Fusobacterium necrophorum, Pseudomonas species, Pantoeas species and gram-negative anaerobic bacteria. In one case, a fungal infection with Aspergillus species was diagnosed. Pathohistologically, fibrinous purulent inflammation, epithelialized, well vascularised tissue with central necrosis and hemorrhage, multifocal cellular dedritus, coccoid bacteria and fungi were found.

For treatment, the affected tissue was removed transendoscopically using a polypectomy snare in eight horse. In six of these horses, the surgical therapy was complemented with a local treatment with mild disinfectant solutions. In all horses, the affected concha healed without any complications after removal of all necrotic tissue. In all cases, the owners reported that the horses were free of any clinical signs 4–6 weeks after surgery. In five horses, the healing process was confirmed by an endoscopic follow-up examination. One horse exhibited unilateral, malodorous nasal discharge from the contralateral nostril five months later due to a dorsal conchal necrosis on this side. The transendoscopic surgical removal of the necrotic tissue resulted in a healing process without any complications.

In literature, conchal necrosis is described to develop secondarily to bacterial or fungal infection of the paranasal sinuses and nasal passages. However in this study, a primary disease was not evident in any horse. The etiologic meaning of the identified bacteria and fungi remains debatable considering the widespread occurrence of these organism. In published data, the removal of the affected concha via frontonasal flap approach under general anesthesia with a risk of intraoperative hemorrhage is the therapy of choice. In the present study, all affected tissue was removed in the standing sedated horse. Transendoscopic instruments, i.e. a polypectomy snare, allow comfortable handling and sufficient removal of the necrosis. The uncomplicated postoperative healing process suggest that the crucial part in therapy is the complete surgical removal of the affected tissue.

ABSTRACT #330

THE EFFECT OF HYPERBARIC OXYGEN ON FULL-THICKNESS SHEET GRAFTS APPLIED TO WOUNDS OF HORSES. T. Holder, J. Schumacher, R. Rohrbach, R. Donnell, M. Mallicotte, S. Adair. College of Veterinary Medicine, University of Tennessee, Knoxville, TN.

The purpose of the study was to determine the effects of hyperbaric oxygen therapy (HBOT) on full–thickness sheet grafts applied to fresh and granulating wounds of horses.

Six horses were used. On day zero, with the horses anesthetized, two, 4-cm diameter, circular sections of full-thickness skin were removed from each of two randomly selected limbs (one metatarsal, one metacarpal) of each horse, and two, 4-cm diameter, circular skin grafts were harvested from the pectoral region. A graft was applied to one randomly selected wound on each limb, leaving the two non-grafted wounds to heal by second intention. On day 7, two grafts were harvested from the pectoral region with the horses standing and applied to the granulating wounds created on day zero, and wounds grafted on day zero were biopsied. On day 14, one wound was created on each of the two unwounded limbs, with the horses anesthetized, and the wounds grafted on day 7 were biopsied. All four wounds, two fresh and two with one-week-old granulation beds, were grafted. The horses were then subjected to HBOT for one hour daily at 23.0 PSI for 7 days. On day 21, the grafts applied on day 14 were biopsied.

Histological examination of biopsies revealed that grafts treated with HBOT developed less granulation tissue, edema, inflammation, and neovascularization and were superficially less viable than those not treated with HBOT.

The use of HBOT after full thickness skin grafting of fresh and granulating wounds of horses appears to be contra-indicated.

ABSTRACT #331

RIB OSTEOMYELITIS IN THREE SEPTIC FOALS. C Cesarini, S Macieira, C Girard, R Drolet, MA D'Anjou, D Jean. Faculty of Veterinary Medicine, Université de Montréal, St-Hyacinthe, Canada.

Osteomyelitis is a frequent complication of septicemia in foals, most commonly affecting growth plates and surrounding bony tissues. Despite there are several reports of rib osteomyelitis in human infants, this condition is not well described in foals. In this report we describe the clinical, radiological, ultrasonographic, bacteriological and histopathological findings of rib osteomyelitis in three septic foals.

Three foals, aged 5 to 15 days, were admitted at the Veterinary Teaching Hospital and a septicemia was diagnosed, caused by *E. coli*, *K. pneumoniae* and *S. aureus*, respectively. In all foals infection involved more than one body system: rib osteomyelitis (3 foals), polyarthritis (3 foals), omphaloarteritis (1 foal) and pneumonia (1 foal). Two foals presented concomitant fracture affecting several ribs. All foals were euthanized because of the poor prognosis of the severe septic process.

In all cases, rib infection was localized at the costo-chondral junction (CCJ). Radiological findings included focal areas of bone expansion and lysis, with increased opacity of the CCJ area. Ultrasonographic examination revealed an increased size of the CCJs and several irregular hyperechoic spots. Macroscopically, the infected CCJs were found swollen and a cavity filled with purulent material was observed on section. Bacteriologic culture of affected CCJs revealed respectively *E. coli* and *S. aureus* in two of the foals despite a broad spectrum antimicrobial therapy. Microscopically, neutrophils and macrophages infiltrated the cavity, where bony structure was replaced by fibrin and fibroblasts. Neovascularization, osteoclastic activity and bone neoformation were predominant in the periphery of the lesion.

In conclusion, rib osteomyelitis should be considered as a possible complication of septicemia in foals. Thoracic radiography and ultrasound are useful diagnostic tools to identify affected ribs.

ABSTRACT #332
EFFECT OF FUROSEMIDE ADMINISTRATION ON THE RATIO OF URINE DEOXYPYRIDINOLINE TO PYRIDINOLINE IN HORSES WITH HYPERELASTOSIS CUTIS (A.K.A. HEREDITARY EQUINE REGIONAL DERMAL AESTHENIA, HERDA).

T Buchheit[1], M Pasquali[2,3], Lis Schwarz[2], Eleanor Cooke[1], AM Rashmir-Raven[1], CE Swiderski[1]; [1]College of Veterinary Medicine, Mississippi State University, Mississippi State, MS. [2]Department of Pathology, University of Utah, Salt Lake City, UT; [3]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

Hereditary Equine Regional Dermal Aesthenia (HERDA), an inherited disorder of increasing prevalence in Quarter Horses, is characterized by areas of fragile, hyperextensible skin. Normally innocuous contact, such as saddling, leads to subcutaneous hematomas and wounds that heal poorly. Affected horses generally cannot be ridden; most are humanely destroyed. Pedigree analysis supports an autosomal recessive mode of inheritance and has identified affected bloodlines. Horses with HERDA have a statistically significant increase in the ratio of deoxypyridinoline (DPD) to pyridinoline (PYD) in their urine. DPD and PYD are the intermolecular crosslinks of Type I collagen. Elevation of the urinary DPD to PYD ratio is also diagnostic of human Ehlers Danlos Syndrome Type VIA. The purpose of this study was to determine the effect of furosemide administration on the ratio of DPD:PYD in urine. Urine was collected from four horses with HERDA and four control horses via urethral catheterization. Following evacuation of the bladder, furosemide (0.5 mg/kg IV) was administered and a voided urine sample was collected 15 minutes following the administration of furosemide. Despite dilution of the urine, as supported by a significant decrease in the urine creatinine and specific gravity, there was not a statistically significant change in the DPD: PYD ratio following furosemide injection. We conclude that furosemide administration does not significantly alter the DPD:PYD ratio.